Monsanto

94-257-01P

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September 7, 1994

Mr. Michael A. Lidsky, Esq.
Deputy Director, BBEP, APHIS, USDA
6505 Belcrest Road
Federal Building
Hyattsville, MD 20782

Subject: Petition for Determination of Nonregulated Status: Potatoes Producing the Colorado Potato Beetle Control Protein of *Bacillus thuringiensis* subsp. *tenebrionis*
Monsanto# 94-143U

Dear Mr. Lidsky:

The Agricultural Group of Monsanto Company is submitting a Petition for Determination of Nonregulated Status to the Animal and Plant Health Inspection Service (APHIS) regarding potatoes producing the Colorado potato beetle (CPB) control protein of *Bacillus thuringiensis* subsp. *tenebrionis*. This petition requests a determination from APHIS that Colorado potato beetle resistant potato lines; BT6, BT10, BT12, BT16, BT17, BT18 and BT23 no longer be considered a regulated article under regulations in 7 CFR part 340. These CPB resistant potato lines have been field tested at multiple locations since 1991 (USDA# 91-011-04, 91-050-02, 91-360-01, 92-002-01, 92-002-02, 92-262-02, 92-363-05, 93-004-01 and 93-056-03). Copies of the final reports for these field release permits are included in the petition.

Please feel free to contact either Dr. Dickerson (202-783-2460) or myself (314-537-6547) if you need any additional information.

Sincerely,

Terry B. Stone
Regulatory Affairs Manager

cc: Dr. C. T. Dickerson, Jr.

94-257-01P

PETITION FOR DETERMINATION OF NONREGULATED STATUS

POTATOES PRODUCING

THE COLORADO POTATO BEETLE CONTROL PROTEIN

OF

Bacillus thuringiensis subsp. *tenebrionis*

Submitted by

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PETITION FOR DETERMINATION OF NONREGULATED STATUS FOR POTATOES PRODUCING THE COLORADO POTATO BEETLE CONTROL PROTEIN OF *Bacillus thuringiensis* subsp. *tenebrionis*

SUMMARY

The Agricultural Group of Monsanto Company submits this petition under 7 CFR part 340.6 to request a determination from the Animal and Plant Health Inspection Service (APHIS) for seven Russet Burbank potato variety lines (BT6, BT10, BT12, BT16, BT17, BT18 and BT23) transformed with the plasmid vector PV-STBT02, which confers resistance to the Colorado potato beetle (CPB), should no longer be considered regulated articles under 7 CFR part 340.

Monsanto's CPB resistant potato lines exhibit no plant pathogenic properties; are no more likely to become a weed than their non-modified parental variety; are unlikely to increase the weediness potential of any cultivated plant or native wild species; and exhibit no toxicity to non-target organisms, including those organisms that are beneficial to agriculture.

Monsanto has worked for over a decade to develop potato plants that control the Colorado potato beetle (CPB, *Leptinotarsa decemlineata*), the most damaging pest of the United States potato crop. These genetically modified potatoes produce an insect control protein derived from the common soil bacterium *Bacillus thuringiensis* subsp. *tenebrionis* (*B.t.t.*). Microbial formulations containing the insecticidal protein, have been commercially available for CPB control since 1988.

The protein produced by CPB resistant potatoes is identical to that found in nature and in commercial *B.t.t.* formulations registered as pesticides with the Environmental Protection Agency (EPA). This protein is highly selective in controlling the CPB and is expressed at a consistently effective level in the potato foliage throughout the growing season.

Results from field experiments conducted throughout the primary potato growing regions under USDA permits have demonstrated that these potatoes are protected season long from all CPB life stages. The above CPB resistant potato lines were evaluated at four locations in 1991 (USDA# 91-011-04 and 91-050-02); nine locations in 1992 (USDA# 91-360-01, 92-002-01, 92-002-02 and 92-262-02) and at 21 locations in 1993 (USDA# 92-363-05, 93-004-01 and 93-056-03). In addition, it was demonstrated that beneficial insects were not adversely affected and actually increased in number. Growers planting CPB resistant potatoes will not require insecticide applications to control CPB. This substantial reduction in the use of chemical insecticide will enhance biological control and the implementation of other integrated pest management strategies for controlling potato pests such as aphids and leafhoppers and the diseases these insects transmit.

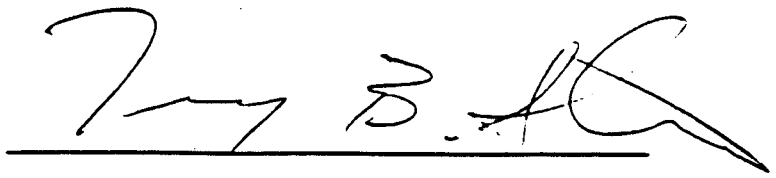
Agronomic evaluations of these CPB resistant potato lines consisting of plant vigor, growth habit characteristics, and general disease susceptibility, have shown these lines to be equivalent to the parental Russet Burbank potatoes. In addition, there is no

indication that these potatoes are more likely to become a weed than the non-modified parental variety or that they will increase the weediness potential of any other cultivated plant or native wild species. The lack of compatible wild species and the clonal propagation system used in potatoes leads to the conclusion that no opportunity exists within the U.S. for the escape of introduced genes from cultivated types to wild relatives of the potato. The nutritional components and quality characteristics of tubers from the seven CPB resistant lines are substantially equivalent to the tubers produced by the parental variety.

Based on this information, these potato lines show no plant pest characteristics and pose no environmental concerns. Therefore, we request a determination of non-regulated status be granted for these seven CPB resistant Russet Burbank potato lines (BT6, BT10, BT12, BT16, BT17, BT18, BT23).

CERTIFICATION

The undersigned certifies, that to the best knowledge and belief of the undersigned, this petition includes all information and views on which to base a determination, and that it includes relevant data and information known to the petitioner which are unfavorable to the petition.



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ABBREVIATIONS

B.t.t.	*Bacillus thuringiensis* subsp. *tenebrionis*
bp	Base pair
CPB	Colorado potato beetle, *Leptinotarsa decemlineata* (Say)
CV	Coefficient of variability
cryIIIA	Class III (Coleoptera-specific) parasporal crystal protein gene from *B.t.t.*
°C, °F	Degree Centigrade, degree Fahrenheit
DNA, T-DNA	Deoxyribonucleic acid, Transferred-DNA
E. coli	*Escherichia coli*
ELISA	Enzyme linked immunosorbent assay
IPM	Integrated pest management
Kg, g, mg, µg, ng, pg	Kilogram, gram, milligram, microgram, nanogram, picogram
kD	Kilodalton
LB, RB	Left border, Right border
LSD	Least significant difference
nptII	Neomycin phosphotransferase type II gene
OD	Optical density
R. B.	Russet Burbank
SE	Standard error

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A. Need for Colorado Potato Beetle Resistant Potatoes

Potatoes are produced to some extent in all fifty states and the United States is currently ranked fourth among potato producing countries (National Potato Council, 1992). The Colorado potato beetle (CPB, *Leptinotarsa decemlineata*) is the most damaging pest of the 2.3 billion dollar U.S. potato crop (Krieg *et al.*, 1983; Casagrande, 1987; National Potato Council, 1992) and approximately one-third of the 2.8 million pounds of chemical insecticides annually applied to potatoes are targeted for its control (USDA, 1993). CPB damage is particularly severe in the eastern and north central potato production areas and is becoming an increasing problem in the northwest. Both larval and adult stages feed on potato foliage and, if not controlled, can undergo population growth rates exceeding 40 fold per generation (two and potentially three generations per year are possible in many areas) and a potential overwintering survival rate of more than 60% (Groden and Casagrande, 1986; Harcourt, 1971). If poorly managed, the CPB is capable of completely defoliating potato plants, resulting in yield reductions of as much as 85%, which is sufficient to prevent potato production in some areas (Roush, 1993; Hare, 1980; Ferro *et al.*, 1983; Shields and Wyman, 1984). Loss of revenue due to the CPB in Michigan alone was estimated at more than 15 million dollars in a state where total potato production in 1991 was valued at 70 million dollars (Potato Growers of Michigan, Inc. and the Michigan Potato Industry Commission, 1992; Olkowski *et al.*, 1992).

Current control of CPB relies heavily upon the use of chemical insecticides that are variably effective due to environmental factors or insect sensitivity. These insecticides are also expensive with costs that can exceed $200 per acre per season (Ferro and Boiteau, 1992). Additional management options for CPB include, crop rotation, vacuum suction (Boiteau *et al.*, 1992), propane flaming (Moyer, 1992; Moyer *et al.*, 1991), polyethylene-lined trenches (Roush, 1993; Wyman, 1993) and trap plots (Roush, 1993; Roush and Tingey, 1992). These options are not often practical, effective, economical nor easily implemented throughout the season (Roush, 1993; Wyman, 1993).

Microbial *Bacillus thuringiensis* subsp. *tenebrionis* (*B.t.t.*) formulations containing the insecticidal proteins have been commercially available for CPB control since the late 1980's (Zehnder and Gelernter, 1989). These formulations are variably effective due to poor spray timing, inadequate plant coverage, short residual activity, and an inability to control large larvae and adults (Ferro and Lyon, 1991; Ferro and Gelernter, 1989; Zehnder and Gelernter, 1989). In contrast, CPB resistant potatoes produce the *B.t.t.* protein throughout the potato foliage and at a level high enough to control all CPB life stages throughout the growing season (Perlak, *et al.*, 1993; Appendix 1, page 98). Such consistently sustained control of CPB is not possible with currently available microbial, chemical, or physical control methods. In addition, the specificity of the *B.t.t.* protein to CPB permits the populations of predaceous and parasitic insects to increase unhindered by the application of broad spectrum chemical insecticides as no additional applications to control CPB are required (Appendix 1, page 98). These beneficial insects can then aid

in the control of non-target potato insect pests such as aphids and leafhoppers and the diseases they transmit. The combination of CPB resistant potatoes and beneficial insects provides a safe and an environmentally compatible foundation for the implementation of other potato pest management practices.

B. Risk Reduction Due to the Introduction of CPB Resistant Potatoes

Reducing the amount of chemical insecticides applied to CPB resistant potatoes will not only enhance potato pest management but will also reduce the potential for farm worker and environmental exposure. A wide range of chemistry has been employed for CPB control with the organophosphates, carbamates and pyrethroids currently predominating (Casagrande, 1987; Tette and Heinmiller, 1992). When applied according to the label, these insecticides are not expected to pose any unacceptable risks to workers and the environment. However, the potential for accidental release or exposure during shipping, storage, mixing and loading, application and container disposal does exist. CPB resistant potatoes would reduce chemical pesticide use in potato production and the associated risks of accidental exposure.

The integrity of groundwater resources is of critical concern in potato production since potatoes are frequently grown on irrigated, coarse textured soils which are highly vulnerable to pesticide leaching (Wyman, 1993). In the 1970's and 1980's systemic carbamate insecticides were widely used by potato growers because of their effectiveness in CPB control. As a result, contamination of groundwater resulted in several areas (Zaki *et al.*, 1982; Rothschild *et al.*, 1982) and carbamate systemics were withdrawn from use and replaced with intensive foliar spray programs. CPB resistant potatoes will offer growers a selective, long lasting control alternative to systemic chemical insecticides without risk of groundwater contamination and will provide an alternative to intensive foliar spray programs.

C. Economic Factors Due to the Introduction of CPB Resistant Potatoes

The costs associated with potato production are considerable. The USDA (1988) estimates that as much as $1,000 per acre is spent for production with as much as 35% utilized to control the CPB (Wyman, 1993). This financial burden could be significantly alleviated by the planting of CPB resistant potatoes. While the cost of the seed potatoes has not yet been determined, growers can expect a savings when the cost of the potatoes is deducted from the present CPB insecticide costs. Just as important, however, will be the reduction in yield losses resulting from the superior CPB control provided by these genetically modified plants. Even the best currently available CPB management programs result in some yield loss due to CPB defoliation (Appendix 2, page 137; EPA, 1992). CPB resistant potatoes are essentially immune to CPB feeding, consequently, the full yield potential of potatoes without CPB damage can be realized. This decrease in yield loss will result in increased grower profits. In addition, CPB resistant potatoes will benefit equally both large and small growers. The technology will be equally accessible and available to all growers, as no additional labor, planning, or machinery will be required for implementation (Appendix 2, pages 137-138).

For the consumer, more potatoes produced at less cost may result in lower prices for

potato products (Appendix 2, page 138; Hill and Florkowski, 1991). The ability to successfully control CPB will also positively impact the potato processing industry. Potato processing facilities are located primarily in areas of extensive potato production. In several of these areas potato production is seriously threatened by the increasing inability to manage CPB (Hare, 1980; Ferro *et al.*, 1983). The ability of CPB resistant potatoes to successfully control this serious pest could contribute to the stability of the potato industry in these areas.

CPB resistant potatoes utilizing the *B.t.t.* CPB control protein will have a more positive impact on the environment than the use of chemical insecticides to control CPB. The *B.t.t.* protein produced by these potatoes breaks down rapidly in the soil, cannot volatilize or drift, and is safe to nontarget organisms such as fish, birds, humans and other mammals. The superior CPB control offered by these potatoes will enable growers to significantly reduce the amount of chemical insecticide now applied to their crop. As a result, they will be able to utilize a host of IPM practices that cannot be currently implemented because of their current dependence on chemical insecticides to control this pest. An increase in the biological and cultural control of non-target potato pests and a more judicious use of chemical insecticides will also result in safer work conditions for farm employees and reduce the potential for pesticidal drift, groundwater contamination and accidental spills. In addition, the costs associated with the use of insecticide handling, storage and disposal will also decrease.

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II. THE POTATO FAMILY

The Potential for Gene Escape from Cultivated Transgenic Potatoes Within the U.S.

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A. Summary

Potato genetic engineering has caused concern that exotic genes will escape into wild relatives of potato and develop the potential for ecological disruption. In some situations this could happen. Over nine hundred species of *Solanum* have been identified, most near the centers of origin in Central and South America and many cross freely with the cultivated potato (*S. tuberosum*). However, within the borders of the U.S., only two species of tuberizing *Solanum*, *S. fendleri* and *S. jamesii*, have been confirmed to exist. Neither species hybridizes with *S. tuberosum* due to differences in ploidy level, differences in endosperm balance number (EBN) or a combination of the two. Both species are found in high elevation, arid climates and are seldom geographically adjacent to potato production areas. Several species of *Solanum* are considered weeds in cultivated fields, including several species of nightshade. None of these species are closely related and none will hybridize with potatoes. The lack of compatible wild species and the clonal propagation system used in potatoes leads to the conclusion that within the borders of the U.S. no opportunity exists for the escape of introduced genes from cultivated types to wild relatives of potato.

B. History and Geography of Potato Production and Use

The potato (*Solanum tuberosum*) is native to the western hemisphere and occurs in abundance from the tropical highlands of Mexico, southward throughout western South America. Around 1570, South American cultivated potatoes were introduced into Europe. Descendants of these early European potatoes were permanently introduced into the U.S. in 1719 by Irish immigrants when they established a colony in New Hampshire (Stevenson, 1951). As Europeans settled in North America, potato production spread throughout the geographical area currently controlled by the U.S.

Potatoes are currently produced to some extent in all fifty states and the U.S. ranks fourth in world production (National Potato Council, 1992). The major commercial production areas are located in the northernmost states of the continental United States and include Maine, New York, Michigan, Wisconsin, Idaho, Oregon and Washington. Exceptions are substantial potato acreages in California and Florida, and minor, but significant acreage in Alabama, Arizona, Louisiana, Nevada, New Mexico, North Carolina, Texas and Virginia (National Potato Council, 1992).

Per capita consumption of potatoes in the United States is ca. 130 pounds or more than one 150 g potato each per day (USDA, 1991). In 1990, 85% of the crop was used for

human consumption (either processed or as tablestock), 6% was planted as seed, and less than 1% was used for animal feed. The feed use is limited by region, by season and is confined to a few integrated potato grower/processors or individual farmers. Shrinkage, loss, and home use represented the remaining 8% (National Potato Council, 1992).

C. Modern Potato Production and Potato Life Cycle

Cultivated potatoes are a clonally propagated crop, grown as an annual, with tubers from the previous year's crop serving as propagules. In the U.S., potato acreage is rotated with other crops on a cycle of two to five years. In most potato growing regions of the U.S., winters are severe enough to freeze and destroy tubers left after the harvest season, eliminating the possibility of escapes. However, in areas with heavy snow cover or mild winters, clonally generated volunteer potatoes are common and may persist for several years. The number of volunteers is reduced, but not eliminated by cultivation and herbicide usage in subsequent crops. Small grains are a common rotation crop and herbicides used in small grains are effective for reducing the number of volunteers. The rate of decline for volunteers has not been well documented but is highly dependent on the severity of the environment. Volunteers from true seed following berry production by fertile varieties will germinate for up to eleven years following seed production with a 40-50% reduction in emergence each year (Lawson, 1983). However, in the long term, potatoes are not competitive with other cultivated crop species and are even less competitive in noncultivated areas. There has been no documented case of cultivated potatoes (*S. tuberosum*) becoming a persistent weed outside of cultivated areas.

In wild species, the predominant method of propagation is also clonal (Hawkes, 1978). Sexual reproduction occurs readily, but is not obligatory and only occasionally results in viable hybrids populations. Nearly all potato species are at least partial outcrossers and require insects, in particular bumblebees, for pollination. Insects rarely visit flowers of cultivated species because they lack nectaries (Pavek, pers. comm.). This results in very limited pollen dissemination. In the only definitive study completed to date, Tynan *et al.* (1990) found that dispersal of pollen from transgenic plants did not occur outside a range of five meters.

D. Taxonomy of Genus *Solanum*

Potatoes belong to the family Solanaceae and the genus *Solanum*. This family comprises 2000 species and includes tomatoes, peppers, eggplant, tobacco, petunia and several forms of the weed commonly called nightshade (Benson, 1959). The genus *Solanum* contains more than 900 species (Correll, 1962; Hawkes, 1990). All potatoes cultivated in the U.S. belong to a single species, *Solanum tuberosum*. Native cultivated potatoes in South America are taxonomically divided among several species including *S. ajanhuiri*, *S. curtilobum*, *S. goniocalyx*, *S. x chaucha*, *S. phureja*, *S. tuberosum*, *S. stenotomum*, and *S. juzepczukii* (Bavyko, 1978). Most can be hybridized with *S. tuberosum*. Native cultivated types are found in Peru, Columbia, Ecuador, Bolivia, and Argentina with *S. tuberosum* ssp. *tuberosum* limited to Chile (Hanneman and Bamberg, 1986).

Only two close relatives of potato, *S. fendleri* and *S. jamesii*, occur naturally within the

borders of the U.S. (Hawkes, 1990). They are considered close relatives because both are tuber bearing *Solanum* (section petota) with at least some possibility of producing hybrids with *S. tuberosum*. *S. fendleri* belongs to the series longipedicellata, is tetraploid, and has been found in Arizona, Colorado, New Mexico and Texas. It resides in dry forests at altitudes of 5,000 to 10,000 feet. *S. jamesii* belongs to the series pinnatisecta, is a diploid, and has been found in Arizona, Colorado, New Mexico, Texas and Utah. It resides in environments similar to those where *S. fendleri* is found.

Several other *Solanum* species are either native or introduced weeds in the U.S. including bitter nightshade (*S. dulcamara*), silverleaf nightshade (*S. elaeagnifolium*), black nightshade (*S. nigrum*), hairy nightshade (*S. sarrachoides*), cutleaf nightshade (*S. triflorum*), buffalobur (*S. rostratum*), and turkeyberry (*S. torvum*) (Whitson *et al.*, 1991). All of these are non-tuber bearing and will not hybridize with tuberizing *Solanum* species.

E. Genetics of Potato

The genetic structure, and crossability of potato species are important considerations in understanding the flow of genes from cultivated to wild species. A brief description follows.

1. Genetic Structure:

A basic chromosome number of 12 was established by Smith (1927) for the genus *Solanum*. Polyploidy is common in both wild and cultivated potatoes. Most species are diploid (73%), or tetraploid (15%), but triploids (4%), pentaploids (2%) and hexaploids (6%) have also been documented (Hawkes, 1990).

The production of numerically unreduced gametes is common in many diploid cultivated and wild species (Camadro and Peloquin, 1980; Yerk and Peloquin, 1990). The result is a production of tetraploid progeny from diploid x tetraploid, tetraploid x diploid, or diploid x diploid crosses with a resultant transfer of genes from the diploid into the tetraploid population. Triploid potatoes are occasionally partially female fertile, producing a limited number of both n and 2n eggs. Triploids may also be crossed as pollen parents with cultivated tetraploids (Brown, 1988; Brown and Adiwilaga, 1990). These may act as 'triploid bridges', serving to allow gene flow in both directions (Jackson *et al.*, 1978). In nature, this is probably a rare event. Crosses of either tetraploids or 2n egg producing diploids with hexaploid species are usually easily made.

2. Crossability:

Three major factors influence the crossability of species. The ploidy level, the endosperm balance number (EBN), and cross incompatibility. The ploidy level, as has been discussed above, restricts the frequency of interspecies hybrids and the direction of gene flow, but by and large, does not prevent such events.

EBN is a term given to the ratio of maternal to paternal genomes in the endosperm of a species. Crosses of species with unequal EBN's result in a nonviable endosperm, causing the embryo to abort. The result is a very effective hybridization barrier between many

Solanum species. Most South American diploid species and nearly all tetraploid species, including *S. fendleri*, have an EBN of 2. *Solanum tuberosum*, a tetraploid, is an exception with an EBN of 4. Most Mexican diploids have an EBN of 1, including *S. jamesii* (Hanneman and Bamberg, 1986). The production of 2n gametes in 2 EBN diploids effectively doubles the EBN, allowing hybridization with *S. tuberosum* to occur. EBN is an important guideline for determination of crossability, however, many exceptions have been noted.

Most diploid species are self-incompatible due to the presence of S-alleles (Howard, 1970). Many closely related species are also cross incompatible because they share identical S-alleles. For reasons not completely understood, cultivated tetraploids and tetraploids derived from self-incompatible diploids show a weakened effect of the S-alleles and are usually self-compatible.

Hawkes (1990) cites evidence from a number of studies that hybrids between wild and cultivated, or between two wild species occur frequently in nature. However, the adaptability of the hybrids is poor and they rarely survive more than one or two seasons. Crosses of *S. tuberosum* with intrageneric species outside the section petota, such as with many types of nightshade, have been attempted, but no fertile progeny have been recovered (Dale *et al.*, 1992 and Rick, 1979).

F. Hybridization of Potato With Wild Relatives

Within the borders of the 50 United States, no opportunity exists for gene flow from cultivated potatoes to wild species. None of the solanaceous weedy species growing in and around potato fields will hybridize with cultivated potatoes. *S. jamesii* and *S. fendleri* are the only closely related species that are endemic to the U.S. Both are very difficult to hybridize with *S. tuberosum* due to incompatible EBN's. The only documented hybrids have been created under carefully controlled conditions in a laboratory situation (Adiwilaga and Brown, 1991; Novy and Hanneman, 1991). *S. fendleri* is the most likely of the two to produce hybrids with *S. tuberosum* because the development of a 2n gamete will produce a compatible EBN. However, no 2n gametes have been reported for *S. fendleri*. Any resulting progeny would be hexaploid with an EBN of 4 and would not be compatible parents for further hybridization with *S. fendleri*.

In addition to genetic incompatibility, the possibility of outcrossing is diminished due to geographical separation. Both *S. fendleri* and *S. jamesii* are found in high elevation, dry forest environments, isolated from all potato production areas. In the event an unlikely hybridization event does occur, the progeny probably will not be adapted to either environment and will not survive.

G. Hybridization of Potato With Other Cultivated Varieties

Other than the common occurrence of sterility, there is no genetic mechanism to prevent the hybridization of two cultivated varieties within the U.S. However, due to production methods, it is unlikely that gene transfer will occur in this manner. Pollen transfer occurs infrequently and over short distances. Tynan and his coworkers (1990) demonstrated no pollen dispersal in a field interplanted with genetically engineered and control potatoes beyond 4 - 5 meters and Dale *et al.* (1992) in a similar study, reported

no pollen transfer beyond 10 meters. Hybrid seed that does occur is not used for further propagation and will remain in the field. If this seed germinates, long term propagation and survival of the resulting seedlings is not expected due to standard cultivation practices, and in fact has never been documented. In the event of self-pollination within a fertile variety containing the *B.t.t.* gene, germination of the resulting seed will present no more concern than clonal volunteers (J. Pavek, pers. comm.).

H. Escape of Transgenic Plant Materials

Escape of plant materials will take the form of lost tubers. Other plant parts are not suitable for propagation. Once in commerce, tubers containing the *B.t.t.* gene can and will be lost during all phases of the growing and marketing operations. The major recipient locations of lost tubers will be fields where the crop is grown, roadsides, and areas around buildings where the potatoes are stored and shipped. Given the non-competitive nature of potatoes in these locations, escape will be inconsistent and temporary. No unusual steps need be taken to control escape through vegetative plant parts.

I. Ecological Impact of Gene Escape

If the *B.t.t.* gene escapes into the environment in a persistent manner it is most likely to do so in Central or South American where appropriate wild species are present. Even there, gene movement into a diploid wild species is unlikely due to the infrequent flow of genes from tetraploids into diploids via triploid bridges, an event never documented in nature. Hybrids are more likely with tetraploid and hexaploid species, but in a native situation will likely be noncompetitive. If hybrids do survive, the predominance of clonal propagation will limit spread. In the event the gene does become established in a wild population, it will provide no competitive advantage because Colorado potato beetle resistance already occurs frequently among wild species (Hanneman and Bamberg, 1986). Also, the Colorado potato beetle is either not present or is not a pest problem in Central and South America, (Bill Cantelo, personal communication; C.A.B. International, 1991) and will not provide the selection pressure that may create an ecological advantage. The *B.t.t.* insecticidal protein does not have known activity against non-target insects and is non-toxic to other animals (Hermstadt *et al.*, 1986; MacIntosh *et al.*, 1990). It is environmentally safe and should have no ecological consequences.

J. Conclusion

Potato production in the United States provides a closed system for the production of transgenic varieties. No likely avenue exists for uncontrolled introduction of the *B.t.t.* gene into the environment either through loss of plant material or gene flow to related species. In the unlikely event the gene does escape, it will probably provide no competitive advantage and is nontoxic to other insect and animal systems.

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III. DESCRIPTION OF TRANSFORMATION SYSTEM AND PLASMID UTILIZED

The seven Russet Burbank potato lines for which this determination is requested (BT6, BT10, BT12, BT16, BT17, BT18 and BT23) contain the *cryIIIA* gene from *Bacillus thuringiensis* subsp. *tenebrionis* which encodes the CPB active protein and the *nptII* gene from the prokaryotic transposon Tn5 which codes for the enzyme neomycin phosphotransferase II. These genes were stably transferred into the genome of potato plants using *Agrobacterium tumefaciens* mediated transformation utilizing a binary double border plant expression vector, PV-STBT02 (Perlak *et al.*, 1993).

A. *Agrobacterium tumefaciens* Transformation System

The *Agrobacterium tumefaciens* transformation method has been reviewed by Klee and Rogers (1989). The transformation vector contains well-characterized DNA segments required for selection and replication of the plasmid vector in bacteria and transfer of the T-DNA into plant cells. The plant expression vector was assembled in *Escherichia coli* and mated into the ABI *Agrobacterium* strain. The ABI strain contains the disarmed pTi58 plasmid pMP90RK which does not carry the T-DNA phytohormone genes (Koncz and Schell, 1986). Therefore, the *Agrobacterium* is unable to cause crown gall disease and is no longer considered a threat as a plant pest (Huttner *et al.*, 1992). The pMP90RK plasmid was engineered to provide the *trfA* gene functions required for autonomous replication of the plasmid vector after conjugation into the ABI strain. The ABI *Agrobacterium* strain containing the binary vector was added to potato stem sections (Newell *et al.*, 1991) in tissue culture dishes. The T-DNA, which includes the *cryIIIA* and *nptII* genes, was transferred into the genome of individual potato cells thereby allowing selection in kanamycin. After a few days, the residual *Agrobacterium* cells were killed using different antibiotics. Subsequently, the potato tissues were treated to stimulate regeneration of transgenic cells into shoots and ultimately plantlets were grown in soil and assayed for CPB resistance.

B. Properties of the Non-transformed Cultivar Russet Burbank

Russet Burbank is the dominant potato cultivar produced in the U.S. and is estimated by the USDA to represent 50.9% of total Fall potato production of 1.14 million planted acres. This potato variety is grown primarily in the northern tier of the United States in the following states: Colorado, Idaho, Maine, Michigan, Minnesota, North Dakota, Oregon, Washington, and Wisconsin (National Potato Council, 1993).

Multiple end uses make Russet Burbank unique among the major cultivars with good consumer quality for boiling, and excellent for baking and french fry processing. Russet Burbank is classified as a table and processing variety. Principal markets include the fresh market and processing trades for the manufacture of french fries. It is the standard of french fry quality on the North American continent. A smaller percentage of Russet Burbank production, mostly that which does not meet the quality standards for the fresh market and processing, is utilized for dehydration and cattle feed.

1. Parentage: Parentage goes back to the variety Burbank. Luther Burbank was the breeder of Burbank variety which was released in 1874 (from seed ball from cv. Early Rose). Lon D. Sweet selected the russeted mutation (Barkley and Schrage, 1993).

2. Description: Russet Burbank tubers are long with numerous well distributed shallow eyes. It has a russeted and heavily netted skin with white flesh. Russet Burbank plants are medium size and spreading with few white flowers.

3. Characteristics: Russet Burbank is a male sterile potato variety. It is a high yielding, high specific gravity potato with a very late maturity. It is resistant to common scab and *Fusarium* storage rot, and is highly resistant to blackleg. It is highly susceptible to Potato Virus-Y and Potato Leaf Roll Virus (which causes tuber net necrosis). It produces a heavy set of tubers and is normally spaced at 12 to 14 inches when planted for seed and 14 to 16 inches when planted for table and processing. Russet Burbank production requires a regular moisture supply to avoid second growth (Barkley and Schrage, 1993).

C. Construction of the Plasmid Vector, PV-STBT02, Utilized for Transformation

The plasmid vector, PV-STBT02, is a double border binary transformation vector. It contains well-characterized DNA segments required for selection and replication of the plasmid in bacteria as well as right and left borders for delineating the region of DNA (T-DNA) transferred into the plant genomic DNA. The host for all DNA cloning and vector construction was the *E. coli* MM-294, a derivative of the common laboratory *E. coli* K-12 strain. The vector is composed of the following genetic components. The first segment is the 0.45 Kb fragment from the pTi15955 octopine Ti plasmid (a *Cla*I to *Dra*I restriction fragment) which contains the T-DNA left border region (Barker *et al.*, 1983). This is joined to the 1.3 Kb origin of replication (*oriV*) region derived from the broad-host range plasmid RK2 (Stalker *et al.*, 1981). The next segment is a 1.8 Kb segment of pBR322 which provides the origin of replication for maintenance in *E. coli* and the *bom* site for the conjugational transfer into the *Agrobacterium tumafaciens* cells (Bolivar *et al.*, 1977; Sutcliffe, 1978). This is fused to the 0.93 Kb fragment isolated from transposon Tn7 which encodes the 0.79 Kb *aad* gene that allows for bacterial selection on spectinomycin or streptomycin (Fling *et al.*, 1985) which is fused to a 0.36 Kb *Pvu*I to *Bcl*I restriction fragment from the pTiT37 plasmid containing the nopaline-type T-DNA right border region (Depicker *et al.*, 1982).

Two chimeric genes, with signals for plant expression, were introduced between the right and left border regions of the plant transformation vector. The chimeric gene for selection on kanamycin (35S/*nptII*/NOS 3') consists of the cauliflower mosaic virus 35S promoter, the neomycin phosphotransferase type II (*nptII*) gene and the nontranslated region of the 3' region of the nopaline synthase gene referred to as NOS 3' (Rogers *et al.*, 1985). The chimeric gene responsible for the efficacious control of CPB (E35S/*cryIIIA*/E9 3') consists of the enhanced 35S promoter (Kay *et al.*, 1987; Odell *et al.*, 1985), the *cryIIIA* gene which encodes the *B.t.t.* protein (McPherson *et al.*, 1988; Perlak *et al.*, 1993) and the nontranslated region of the small subunit of ribulose-1,5-bisphosphate carboxylase (RUBISCO) referred to as E9 3' (Coruzzi *et al.*, 1984).

The plasmid map of PV-STBT02 is shown in Figure III.1. A summary of the specific DNA

components in vector PV-STBT02 are listed in Table III.1.

D. Genetic Elements Contained in PV-STBT02 but Absent from the CPB Resistant Russet Burbank Lines

The scientific literature supports that only the T-DNA is transferred and integrated into the plant genome (Zambryski, 1992) in an irreversible manner (Huttner *et al.*, 1992). The border sequences themselves are partially transferred during the process of insertion of the T-DNA into the plant genome (Bakkeren *et al.*, 1989). Consequently, the inserted DNA is no longer a functional T-DNA; *i.e.*, once integrated, it cannot be remobilized into the genome of another plant even if acted on again by *vir* genes (genes involved in T-DNA excision and transfer). The following elements are present on the plasmid PV-STBT02, but are not transferred and hence not present in the genome of CPB resistant Russet Burbank lines BT6, BT10, BT12, BT16, BT17, BT18 and BT23:

1. *aad* – A 0.79 Kb gene isolated from transposon Tn7 (Fling *et al.*, 1985) is located just outside the right border of PV-STBT02 (see Figure III.1). This gene encodes for the enzyme streptomycin adenylyltransferase that allows for bacterial selection on spectinomycin or streptomycin. Southern analysis using a PCR generated probe specific for the *aad* gene demonstrated that this gene is not present in any of the CPB resistant Russet Burbank lines. A detailed discussion of the genetic analysis is contained in Section V of this application.

2. *oriV* – A 1.3 Kb origin of replication segment derived from the broad-host range plasmid RK2 (Stalker *et al.*, 1981) is located just outside the left border of PV-STBT02 (see Figure III.1). Southern analysis, using a PCR generated probe specific for the *oriV*-region, demonstrated that this segment is not present in any of the CPB resistant Russet Burbank lines. A detailed discussion of the genetic analysis is contained in Section V of this application.

3. *ori322* – A 1.8 Kb segment of pBR322 which provides the origin of replication for maintenance of the PV-STBT02 plasmid in *E. coli* and the *bom* site for the conjugational transfer into the *Agrobacterium tumafaciens* cells (Bolivar *et al.*, 1977; Sutcliffe, 1978) is located between the *aad* gene and the *oriV* segment. The absence of this genetic element in the seven CPB resistant Russet Burbank lines is substantiated by the absence of the *aad* gene and *oriV* segment, the two regions which flank the *ori322* genetic element.

Southern analyses (see Section V) confirmed that the above genetic elements are not present in the genome of the seven CPB resistant potato plants. Only the region of DNA, from plasmid PV-STBT02, within 550 bp outside the right and left borders is present in the seven lines of CPB resistant Russet Burbank potatoes.

Table III.1 Summary of DNA Components in PV-STBT02.

Genetic Element	Size, Kb	Function
RB	0.36	A restriction fragment from the pTiT37 plasmid containing the 24 bp nopaline-type T-DNA right border used to initiate the T-DNA transfer from *Agrobacterium tumefaciens* to the plant genome (Depicker *et al.*, 1982).
P-E35S	0.62	The cauliflower mosaic virus (CaMV) promoter (Odell *et al.*, 1985) with the duplicated enhancer region (Kay *et al.*, 1987).
cryIIIA	1.8	The gene which confers resistance to CPB. The gene encodes an amino acid sequence identical to the CPB control protein (referred to as the *B.t.t.* Band 3 protein) found in *B.t.t.* as described by Perlak *et al.* (1993).
E9 3'	0.63	A 3' nontranslated region of the pea ribulose-1,5-bisphosphate carboxylase, small subunit (rbcS) E9 gene (Coruzzi *et al.*, 1984), which functions to terminate transcription and direct polyadenylation of the *cryIIIA* mRNA.
P-35S	0.32	The 35S promoter region of the cauliflower mosaic virus (CaMV) (Gardner *et al.*, 1981; Sanders *et al.*, 1987).
nptII	0.79	The gene isolated from Tn5 (Beck *et al.*, 1982) which encodes for neomycin phosphotransferase type II. Expression of this gene in plant cells confers resistance to kanamycin and serves as a selectable marker for transformation (Fraley *et al.*, 1983).
NOS 3'	0.26	A 3' nontranslated region of the nopaline synthase gene which functions to terminate transcription and direct polyadenylation of the *nptII* mRNA (Depicker *et al.*, 1982; Bevan *et al.*, 1983).
ori V	1.3	Origin of replication segment for ABI *Agrobacterium* derived from the broad-host range plasmid RK2 (Stalker *et al.*, 1981).
ori-322/rop	1.8	A segment of pBR322 which provides the origin of replication for maintenance of the PV-STBT02 plasmid in *E. coli*, the replication of primer (*rop*) region and the *bom* site for the conjugational transfer into the *Agrobacterium tumefaciens* cells (Bolivar *et al.*, 1977; Sutcliffe, 1978).
aad	0.79	The gene for the enzyme streptomycin adenylyltransferase that allows for bacterial selection on spectinomycin or streptomycin (Fling *et al.*, 1985).
LB	0.45	A restriction fragment from the octopine Ti plasmid, pTi15955, containing the 24 bp T-DNA left border used to terminate the transfer of the T-DNA from *Agrobacterium tumefaciens* to the plant genome (Barker *et al.*, 1983).

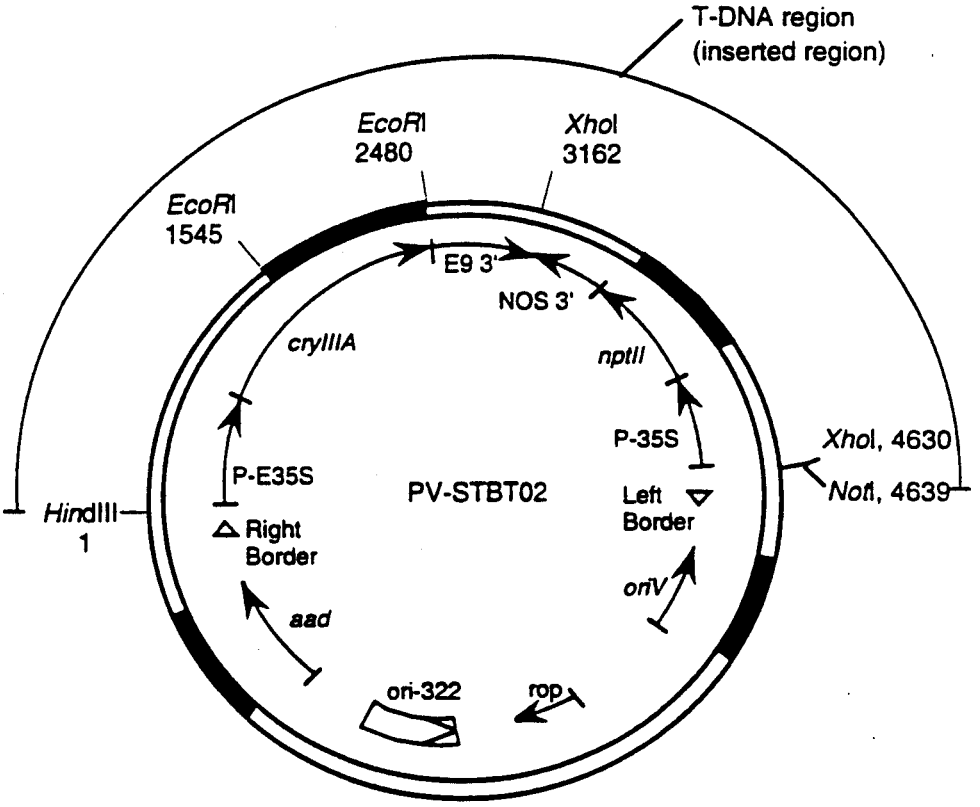

Figure III.1. Plasmid map of PV-STBT02.
Restriction sites, and their locations in base pairs, utilized during Southern analyses are shown. The region which served as the T-DNA is marked and its delineating right and left borders are denoted by open arrows. The blackened regions denote the positions of homology for PCR probes used during Southern analyses as described in Section V.A. Cleavage sites for *Hin*dIII, *Eco*RI, *Xho*I and *Not*I restriction endonucleases are shown. A description of the genetic elements appears in Table III.1.

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IV. THE DONOR GENES TO BE CONSIDERED FOR NON-REGULATED STATUS

A. *cryIIIA* Gene

The gene used to produce the seven lines of CPB resistant Russet Burbank potato plants, designated *cryIIIA* (Höfte and Whitely, 1989), was isolated from the DNA of *B. thuringiensis* subsp. *tenebrionis*, strain BI 256-82 (Krieg *et al.*, 1983). A full length clone and the complete nucleotide sequence has been reported for this gene (McPherson *et al.*, 1988; Perlak *et al.* 1993). The *cryIIIA* gene encodes a protein of 644 amino acids with a molecular weight of 73 kD which is produced by the bacterium during sporulation. This protein has insecticidal properties with selective activity against a narrow spectrum of Coleoptera (MacIntosh *et al.*, 1990). Upon ingestion by susceptible species, feeding is inhibited with disruption of the gut epithelium, which results in the eventual death of the insect (Slaney *et al.*, 1992). In addition to the full-length protein, the *B.t.t.* bacterium also produces a smaller form of this protein called *B.t.t.* band 3. The *B.t.t.* band 3 protein has a molecular weight of 68 kD (597 amino acids) which results from an internal translational initiation event within the same gene starting at amino acid 48 (McPherson *et al.*, 1988; Perlak *et al.* 1993). This protein has been shown to possess the same insecticidal potency and selectivity to CPB larvae as the full-length protein (McPherson *et al.*, 1988). The gene encoding the *B.t.t.* band 3 protein, modified with plant preferred codons for increased plant expression, was introduced into potato plants. The modification changed 399 out of 1791 nucleotides within the gene which codes for the *B.t.t.* band 3 protein, without altering any of the encoded amino acids. This modified gene encodes the nature identical amino acid sequence of band 3 protein as produced by the *B. thuringiensis* subsp. *tenebrionis* microbe (Perlak *et al.*, 1993).

The *cryIIIA* gene sequence, as introduced in the seven lines of CPB resistant potato plants, is shown in Appendix 3, page 143. The corresponding amino acid sequence is shown in Appendix 4A, page 150.

B. *nptII* Gene

This gene functions as a dominant selectable marker in the initial, laboratory stages of plant cell selection following transformation (Horsch *et al.*, 1984; DeBlock *et al.*, 1984). The NPTII enzyme uses ATP to phosphorylate neomycin and the related kanamycin, thereby inactivating these aminoglycoside antibiotics and preventing them from killing the cells producing NPTII. The coding sequence for the *nptII* gene is derived from the prokaryotic transposon Tn5 (Beck *et al.*, 1982). The sole purpose of inserting the *nptII* gene into potato cells with the *cryIIIA* gene is to have an effective method of selecting cells that contain the insecticidal gene. In general, the frequency of cells that are transformed is often as low as 1 in 10,000 or 1 in 100,000 of the cells treated (Fraley *et. al.*, 1984). Therefore, to facilitate this process, a selectable marker gene, *nptII*, and selective agent, kanamycin, is used. Consequently, cells selected for plant generation contain the *nptII* and *cryIIIA* genes.

The *nptII* gene sequence, as introduced in the seven lines of CPB resistant potato plants,

is shown in Appendix 3, page 143. The corresponding amino acid sequence is shown in Appendix 4B, page 150.

C. References

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V. GENETIC ANALYSIS, AGRONOMIC PERFORMANCE, AND COMPOSITIONAL ANALYSIS OF THE CPB RESISTANT RUSSET BURBANK LINES.

A. Genetic Analysis

As described in Section III, the CPB resistant potato plants lines were generated by *Agrobacterium tumefaciens* mediated transformation with plasmid PV-STBT02. DNA analyses were performed on the seven lines, for which this determination is requested, to characterize the inserted T-DNA in terms of insert number (number of genetic loci), copy number (number of T-DNA copies at a particular genetic loci) and insert integrity (EPA MRID NO. 42932201). The characterizations were performed by Southern blot analyses (Southern, 1975) on isolated genomic DNA treated with restriction endonucleases. All Southern blots were hybridized with ^{32}P-labelled PV-STBT02 plasmid, unless otherwise indicated.

1. Insert and Copy Number

To obtain information on the number of T-DNA copies transferred into the potato genome, the isolated DNA was cut with the endonuclease *Eco*RI (refer to Figure III.1, for restriction site locations). For a single copy/single insertion event, this restriction enzyme was expected to yield a 935 bp fragment (part of the *cryIIIA* gene) and two other fragments containing T-DNA joined to the plant genomic DNA, referred to as border fragments. The analysis demonstrated that five lines (BT6, BT12, BT17, BT18 and BT23) contained single copies of the inserted T-DNA at a single site within the potato genome. Two lines contained two copies of the inserted T-DNA; BT10 contained the two copies in-tandem while BT16 contained two copies at separate genetic loci. The Southern blot analysis is shown in Figure V.1. Background hybridization (*eg.*, the approximately 4600, 3100 and 700 bp bands) was observed with digested DNA from the Russet Burbank control and all CPB resistant lines.

CPB Resistant Russet Burbank Lines BT6, 12, 17, 18 and 23 – Based on the known *Eco*RI sites within PV-STBT02, (see Figures III.1 and V.1C), digestion of genomic DNA was expected to yield a 935 bp fragment (part of the *cryIIIA* gene) and two other fragments containing T-DNA joined to the plant genomic DNA, referred to as border fragments. The Southern blot in Figure V.1A shows two border fragments and a 935 bp band for lines BT6, BT12, BT17, BT18 and BT23 (Figure V.1A, Lanes 4, 6, 8, 9 and 10, respectively). For line BT17 (Figure V.1A, Lane 8), one of the border fragments co-migrates with the background band at approximately 3100 bp. These results indicated that the T-DNA was inserted only once and at only one site within the potato genome for lines BT6, BT12, BT17, BT18 and BT23. A schematic illustration of the *Eco*RI fragmentation observed from the single copy/single insertion event of the T-DNA in lines BT6, BT12, BT17, BT18 and BT23 is shown in Figure V.2A.

CPB Resistant Russet Burbank Lines BT10 and 16 – The Southern blot analysis of the *Eco*RI restriction digest for lines BT10 and BT16 indicated that two copies of the T-DNA inserted into the potato genome. The *Eco*RI digest for BT10 (Figure V.1, Lane 5) showed one band at approximately 5500 bp and a wide and relatively intense band at 3800 bp plus the 935 bp band. The wide band at 3800 bp was interpreted as two fragments of

DNA closely migrating together. This overall pattern was indicative of two borders and one internal fragment (in addition to the 935 bp band), establishing that two copies of T-DNA inserted within the genome in an in-tandem arrangement. The four possible ways the T-DNA could have inserted in an in-tandem arrangement are:

1. right border (RB) to left border (LB) followed by left border to right border (RB-LB/LB-RB),
2. left border to right border followed by right border to left border (LB-RB/RB-LB),
3. right border to left border followed by right border to left border (RB-LB/RB-LB), and
4. left border to right border followed by left border to right border (LB-RB/LB-RB).

The first two possibilities are inverted repeats and the second two are direct repeats. The last two possibilities are indistinguishable without reference to a landmark within the potato genome and therefore can be considered identical. The fragment sizes generated from the *Eco*RI digestion is consistent with a RB-LB/RB-LB direct repeat. Figure V.2B illustrates the sizes of the DNA fragments observed in-tandem, direct repeat insertion of the T-DNA with line BT10.

Four border fragments and the 935 bp band were observed in the Southern analysis of the *Eco*RI restriction digest for line BT16 (Figure V.1, Lane 7). This shows that two copies were inserted at separate sites within the genome for line BT16. A schematic illustration of the sizes of the DNA fragments resulting from two copy insertion event as observed with Line BT16 is shown in Figure V.2C.

2. Insert Integrity

Information on the insert integrity was obtained by performing two separate digestion experiments. In the first experiment, DNA from each of the seven CPB resistant lines and the control line was digested with a combination of *Hin*dIII and *Xho*I endonucleases. In the second experiment, DNA from each of these lines was digested with a combination of *Hin*dIII and *Not*I endonucleases.

a. *HindIII and XhoI digestions:*

The *Hin*dIII and one of the two *Xho*I sites are located near the right and left borders, respectively (see Figure III.1). This combination of restriction enzymes was expected to release two internal fragments of predicted sizes 3162 and 1468 bp within the inserted T-DNA for a single copy/single insertion event. The resulting DNA fragments were then evaluated and based on the size of the released fragments, an assessment of the T-DNA integrity was made. The Southern blot in Figure V.3 shows the results from digesting the seven CPB resistant lines and the Russet Burbank control with *Hin*dIII and *Xho*I endonucleases. Background hybridization (*eg.*, the 8000 bp band) was observed with digested DNA from the Russet Burbank control and from all the CPB resistant lines.

CPB Resistant Russet Burbank Lines BT6, 17 and 23 – Digestion of DNA from lines BT6, 17 and 23 with a combination of *Hin*dIII and *Xho*I released the two expected internal

fragments, 3162 and 1468 bp (Figure V.3, Lanes 2, 6 and 8, respectively). The sizes of the fragments generated are schematically illustrated in Figure V.4A. Since the entire plasmid was used as the probe, it was concluded that no detectable DNA fragment from the PV-STBT02 plasmid outside the right or left border sequences were transferred into the plant genome.

CPB Resistant Russet Burbank Lines BT12 and 18 – Digestion of DNA from line BT12 simultaneously with *Hin*dIII and *Xho*I resulted in 1468 bp and 8500 bp fragments; the 3162 bp fragment was not observed (Figure V.3, Lane 4). Based on this result it was concluded that for line BT12, the *Hin*dIII site at the right border was missing and that the DNA fragment of approximately 8500 bp resulted from cleavage of part of the T-DNA (nearest to the right border) joined with the plant genomic DNA. In contrast, when DNA from line BT18 was subjected to restriction digestion with *Hin*dIII and *Xho*I, the 3162 bp fragment was observed, but the 1468 bp fragment was not; instead, a DNA fragment of approximately 1800 bp band was observed (Figure V.3, Lane 7). Based on this result, it was concluded that line BT18 was missing the *Xho*I site (nearest to the left border) and that the 1800 bp fragment resulted from cleavage of part of the T-DNA joined with the plant genomic DNA. The sizes of the fragments generated from lines BT12 and 18 are schematically illustrated in Figure V.4A.

CPB Resistant Russet Burbank Lines BT10 and 16 – Digestion of DNA from line BT10 with *Hin*dIII and *Xho*I generated the two expected fragments, 3162 bp and 1468 bp, as well as a third fragment of approximately 4260 bp (Figure V.3, Lane 3). Thus, of the two copies which inserted into line BT10, one copy contained the two *Xho*I sites and *Hin*dIII site. The other copy contained the two *Xho*I sites but was missing the *Hin*dIII site. This conclusion was based on the comparative intensities of the 1468 bp and 3162 bp bands generated from the other lines. The sizes of the bands generated from line BT10 is schematically illustrated in Figure V.4B.

Digestion of DNA from line BT16 with *Hin*dIII and *Xho*I yielded the expected 3162 bp and 1468 bp fragments as well as a fragment of approximately 4500 bp (Figure V.3, Lane 5; the faint band at approximately 10000 bp was attributed to incomplete digestion). Thus, of the two copies which inserted into line BT16, one copy contained the two *Xho*I sites and one *Hin*dIII site. The other copy contained the two *Xho*I sites but was missing the *Hin*dIII site based on the comparative intensities of the 1468 bp and 3162 bp bands generated from the other lines. The sizes of the fragments generated from line BT16 is schematically illustrated in Figure V.4C.

b. *HindIII/NotI digestions:*

The endonucleases *Hin*dIII and *Not*I have single restriction sites immediately inside the right and left borders, respectively, of PV-STBT02 (see Figure III.1). This combination of restriction enzymes was expected to release most of the inserted T-DNA (of 4639 bp) for a single copy/single insertion event. The resulting DNA fragment, was evaluated and based on the size of the released fragment an assessment of the T-DNA integrity was made. The Southern blot analysis in Figure V.5 shows the results of digesting the seven CPB resistant lines and a Russet Burbank control using *Hin*dIII and *Not*I endonucleases. Background hybridization (*e.g.*, the 8000 bp band) was observed with digested DNA from Russet Burbank control and from all CPB resistant lines.

CPB Resistant Russet Burbank Lines BT6, 17 and 23 – Digestion of DNA from lines BT6, 17 and 23 with *Hin*dIII and *Not*I released the predicted 4639 bp fragment (Figure V.5, Lanes 2, 6 and 8). No other fragments were observed for these lines. Since the entire plasmid was used as the probe, it was concluded that no detectable DNA fragment from the PV-STBT02 plasmid, outside the right or left border sequences, was transferred into the plant genome. The sizes of the bands generated from line BT6, 17 and 23 are schematically illustrated in Figure V.6A.

CPB Resistant Russet Burbank Lines BT12 and 18 – Digestion of DNA from lines BT12 and 18 with *Hin*dIII and *Not*I yielded DNA fragments of approximately 10000 and 5000 bp, respectively, (Figure V.5, Lanes 4 and 7) rather than a 4639 bp band, which is predicted if both the *Hin*dIII and *Not*I sites were present. These results support the findings from the *Hin*dIII/*Xho*I digests shown in Figure V.3, which indicate that lines BT12 and 18 were missing *Hin*dIII and *Xho*I sites, respectively. The sizes of the fragments generated from line BT12 and 18 are schematically illustrated in Figure V.6A. Some degree of variability in the amount of T-DNA transferred near the border sequences is known to occur (Zambryski, 1992); therefore, absence of restriction sites near the right and left borders is not unexpected.

CPB Resistant Russet Burbank Lines BT10 and 16 – Treatment of DNA from line BT10 with *Hin*dIII and *Not*I generated the expected 4639 bp fragment and a second fragment of approximately 5800 bp (Figure V.5, Lane 3). Based on this result, it was concluded that one of the two copies of T-DNA within line BT10 was missing a *Hin*dIII site and that the 5800 bp fragment resulted from part of the T-DNA (nearest to the right border) joined with the plant genomic DNA. The sizes of the fragments generated from line BT10 is schematically illustrated in Figure V.6B. The results from the digestions with *Hin*dIII/*Xho*I and *Hin*dIII/*Not*I are consistent with the conclusion that for line BT10, one copy had all the predicted sites and one copy did not have the *Hin*dIII site.

Digestion of DNA from line BT16 with a combination of *Hin*dIII and *Not*I yielded the expected 4639 bp fragment along with a fragment of approximately 9500 bp (Figure V.5, Lane 5). Based on this result, it was concluded that one of the *Hin*dIII sites was missing and that the 9500 bp fragment resulted from part of the T-DNA (nearest to the right border) joined with the plant genomic DNA. The sizes of the fragments generated for line BT16 is schematically illustrated in Figure V.6C. This result, in combination with the results from the *Hin*dIII/*Xho*I digestion showed that for the two copies of T-DNA within line BT16, one copy had all the predicted sites and one copy did not have the *Hin*dIII site.

To demonstrate that the *nptII* and *cryIIIA* genes were linked, the Southern blot of the *Hin*dIII and *Not*I digestion, which released most of the inserted T-DNA as a single fragment (Figure V.5), was stripped and probed separately with probes with homology to the *nptII* and *cryIIIA* genes. The respective autoradiograms are shown in Figures V.8A and V.8B. As expected, the two probes yielded identical banding patterns establishing that the *cryIIIA* and *nptII* genes are located on the same fragment of T-DNA that was transferred into the potato plant genome.

3. Absence of DNA Outside the Borders

To further verify that DNA fragments outside the borders of PV-STBT02 plasmid did not transfer into the potato lines, the Southern blot membrane shown in Figure V.1 (*Eco*RI digestions) and Figure V.3 (*Hin*dIII/*Xho*I digestions) was treated to remove the entire plasmid probe. Figures V.1 and V.3 are presented separately but were analyzed on the same Southern blot. The resulting membrane was then probed in succession with PCR generated probes specific for the *aad* gene and *oriV* regions (see Figure V.7), which are located within 550 bp just outside the right or left border (see Figure III.1), respectively. In both sets of hybridizations, none of the seven CPB resistant lines showed hybridization to either probe. The absence of hybridization strongly suggests that the left and right borders are the endpoints of the transferred DNA.

In summary, genetic analysis established that a single T-DNA was inserted into a single genetic locus for CPB resistant potato lines BT6, 12, 17, 18 and 23. Two lines, BT10 and 16, were shown to contain two inserted T-DNA copies. Line BT10 was shown to contain two T-DNA copies inserted in-tandem at a single site and BT16 was shown to contain two single T-DNA copies inserted at separate genetic loci. No detectable rearrangements or insertions were observed indicating that the T-DNA maintained its integrity during the transfer event. No region of DNA, within 550 bp outside the plasmid borders, was detected for any of the seven CPB resistant lines evaluated.

B. Disease and Pest Characteristics

CPB insect resistant plant lines BT6, BT10, BT12, BT16, BT17, BT18 and BT23 have been tested in the United States in 1991 (USDA# 91-011-04 and 91-050-02), 1992 (USDA# 91-360-01, 92-002-01, 92-002-02, and 92-262-02) and 1993 (USDA# 92-363-05 and 93-004-01) at a combined total of 33 locations. At nearly all of these locations the plant lines were evaluated in replicated trials. Detailed monitoring for the disease and insect susceptibility of these lines versus Russet Burbank control plants was performed one or more times per season at the sites listed in Table V.1. No differences in disease or insect infestation or severity other than CPB control were detected between the CPB resistant plant lines and Russet Burbank control plants. Appendix 5 (pages152 - 276) contains the USDA final reports for the trials conducted in 1991, 1992 and 1993. Example monitoring forms are provided in Appendix 6 (pages 277 - 285). These observations were obtained by private growers, university and USDA researchers and potato seed certification experts who compared the general vigor and disease and insect susceptibility of control and the CPB resistant plant lines. These observations are typical of those taken by potato crop consultants, agronomists, seed producers, and seed certifiers in detecting the presence and magnitude of a disease or insect infestation. On the basis of these critical evaluations, the CPB resistant plant lines were entered for certification in U.S. seed potato certification programs in 1993 and were granted certification for current season and post harvest evaluation (Appendix 7, pages 286-299). Common diseases evaluated included, but were not limited to: early blight, late blight, *Verticillium*, potato leaf roll virus and potato virus Y. The primary insect pests monitored were aphids, potato leafhoppers, Colorado potato beetles, and cutworms. Agronomic observations included plant vigor, growth, color, leaflet shape and flowering. As stated above, no differences in agronomic quality, disease or insect

susceptibility, other than to the CPB, were detected in 1991, 1992 and 1993 among Russet Burbank control plants and CPB resistant plant lines BT6, BT10, BT12, BT16, BT17, BT18 and BT23.

In 1992 and 1993, both control Russet Burbank potatoes and several CPB resistant potato lines were affected by heat stress at several locations. These plants exhibited abnormal foliar growth typified by small, wrinkled and chlorotic leaves, which did not expand normally. The symptoms were evident when the Russet Burbank control and CPB resistant plants first emerged and then declined, typically within two weeks. Evaluation for virus infection and herbicide carry over were negative. Mid-season growth, tuber development, and yields were normal and equal to unaffected control plants. Both the control and transgenic lines grew out of this condition within two weeks. The symptoms of heat stress expressed by potatoes are described in the article by Dr. Gary Pelter of Washington State University and letters by Dr. Dane Hane of Oregon State University and potato seed producers (these documents are included in Appendix 8, page 300).

C. Yield and Quality Characteristics

The seven CPB resistant plant lines that are the subject of this Determination for Non-regulated Status, BT6, BT10, BT12, BT16, BT17, BT18 and BT23, are still in development. These lines have exhibited quality and yield characteristics comparable to control Russet Burbank. Only those lines with commercially acceptable yield and quality characteristics will enter the marketplace.

D. Expression Levels of the *B.t.t.* and NPTII Proteins

The levels of *B.t.t.* and NPTII proteins expressed in the seven CPB resistant Russet Burbank lines were determined in the leaf, tuber and whole plant tissue (entire plant, excluding tubers) by validated enzyme linked immunosorbent assay (ELISA) (EPA MRID NO. 42932202). Tissues were collected during the 1992 season at seven field locations: Hancock, WI; Hermiston, OR; Riverhead, NY; Othello, WA; Aberdeen, ID; Ashland, ME; and Antigo, WI. Two field sites (Othello, WA and Aberdeen, ID) employed a six-replicate randomized complete block design for each line; the other five field sites employed a non-replicated arrangement. A summary of the methods employed and the descriptive features of the ELISAs developed to detect and quantitate the *B.t.t.* and NPTII proteins in the various potato tissues are summarized in Appendix 15 (pages 368 - 380).

1. Leaf Expression

Leaf expression results for *B.t.t.* and NPTII proteins for the seven CPB resistant lines collected at approximately nine weeks post-planting are shown in Tables V.2 and V.3. The mean leaf expression level across the seven field sites for all seven lines was 19.1 µg *B.t.t.* protein/g tissue (fresh weight) and 2.64 µg NPTII protein/g tissue (fresh weight). The *B.t.t.* and NPTII protein levels correspond to 0.12% and 0.02% of the total leaf protein, respectively (based on total protein level in foliage of 1.6% of fresh weight). The *B.t.t.* protein expression level in leaf tissue was also determined at two additional time points during the plant's growing period; approximately, twelve and

fifteen weeks post-planting. The *B.t.t.* protein expression level remained relatively constant with a slight decrease at late season. The mean leaf expression levels for the *B.t.t.* protein across the seven field sites for all seven lines at twelve and fifteen weeks post-planting were 16.41 µg and 15.55 µg *B.t.t.* protein/g tissue (fresh weight).

2. Whole Plant Expression

The expression level of *B.t.t.* and NPTII proteins in whole plant tissue (leaves, stems and roots, excluding tubers) collected at the onset of senescence for the seven CPB resistant lines are shown in Tables V.4 and V.5. The mean whole plant expression level across the seven field sites for all seven lines was 6.64 µg *B.t.t.* protein/g tissue (fresh weight) and 0.53 µg NPTII protein/g tissue (fresh weight). The *B.t.t.* and NPTII protein levels correspond to 0.04% and 0.003% of the total whole plant protein, respectively (based on total protein level in foliage of 1.6% of fresh weight). The *B.t.t.* protein expression level was also determined in whole plant tissue collected early in the season (approximately, six weeks post-planting). The early season mean whole plant expression level across the seven field sites for all seven lines was 5.41 µg *B.t.t.* protein/g tissue (fresh weight).

3. Tuber Expression

The expression level of *B.t.t.* and NPTII proteins in tubers collected at harvest for the seven CPB resistant lines are shown in Tables V.6 and V.7. The mean tuber expression level across the seven field sites for all seven lines was 1.01 µg *B.t.t.* protein/g tissue (fresh weight) and 0.50 µg NPTII protein/g tissue (fresh weight). The *B.t.t.* and NPTII protein levels correspond to 0.005% and 0.0025% of the total tuber protein, respectively (based on total protein level in tuber of 2.0% of fresh weight).

E. Compositional Analyses of CPB Resistant Potatoes

The potato has been long valued as a staple starch in the United States, contributing carbohydrate calories to the diet. In recent years it has been also valued for selected vitamins, minerals and dietary fiber. In terms of domestic usage, typically, 93% is grown for human consumption both as processed foodstuffs and fresh use, 6% is planted for seed production and 1% is used for animal feed (National Potato Council, 1993).

A study was carried out to compare the nutritional constituents and quality attributes of potato tubers from the seven CPB resistant Russet Burbank plant lines with tubers obtained from control plants. The study demonstrated that the tubers produced by the seven CPB resistant plant lines are substantially equivalent to tubers produced by Russet Burbank plants.

The results of the most important potato constituents (total solids, dextrose, sucrose, vitamin C, total protein and natural glycoalkaloid toxicants) are summarized in Table V.8. The summary represents data from tubers collected at field trials carried out in 1992 at six locations: Hancock, WI; Hermiston, OR; Othello, WA; Aberdeen, ID; Ashland, ME; and Antigo, WI. Two field sites (Othello, WA and Aberdeen, ID) employed a six-replicate randomized complete block design for each line; the other four field sites

employed a non-replicated arrangement.

Quality analyses included blackspot bruise, internal tuber attributes (hollow heart, brown center, internal brown spot and vascular discoloration) and french fry color. Analyses were carried out on tubers from the seven CPB resistant plant lines and the Russet Burbank control plants collected in 1992 at the two replicated field sites: Othello, WA and Aberdeen, ID. The results of the blackspot bruise and internal quality analyses are summarized in Table V.9. The results of the french fry color analyses are summarized on Table V.10.

As noted in Tables V.8 and V.9, statistically significant differences in dextrose and glycoalkaloids composition and hollow heart and brown center quality characteristics were observed between tubers from some CPB resistant lines and control plant. These are actually minor differences that are common among potato varieties (Burton, 1989). For all parameters analyzed, the components and quality characteristics of tubers from all seven CPB resistant lines were either equivalent to control tubers or were well within the range of levels reported historically for Russet Burbank potatoes. The reported range for nutritional components in Russet Burbank potatoes is shown in Table V.8. No material differences were observed in french fry characteristics among tubers from seven CPB resistant plant lines and the Russet Burbank control line.

In addition, potato tubers were analyzed for proximate composition (total protein, fat, carbohydrate, total dietary fiber, calories and ash), and the following minor constituents: thiamine (vitamin B_1), pyridoxine (vitamin B_6), folic acid, niacin (vitamin B_3), riboflavin (vitamin B_2) and minerals (calcium, copper, iodine, iron, magnesium, phosphorous, sodium and potassium). The analyses were performed on freeze-dried potato powder composite subsamples from tubers collected in 1992 at the two replicated field sites (Othello, WA and Aberdeen, ID). Composite subsamples for the seven CPB resistant lines and control line were prepared by mixing 20 g of freeze-dried powder from each of the six replicate plots. The results of the proximate analysis, summarized as an average of the data obtained from the composite subsamples from the two replicated field sites, are presented in Table V.11. No significant differences were observed among the seven CPB resistant lines and the Russet Burbank control plant in proximate composition or in any of the analyzed minor constituents. The level of these components are well within the reported ranges for Russet Burbank potatoes.

Overall, the composition and quality results demonstrate that the tubers produced by the seven CPB resistant plant lines are substantially equivalent to tubers produced by Russet Burbank plants.

Table V.1 Disease and Insect Susceptibility of CPB Resistant Plant Lines in Comparison to Russet Burbank Control Plants.

Year/Site	Difference in Susceptibility Versus Control Russet Burbank Disease	Insect[1]
1991		
Ashland, ME	No	No
Hermiston, OR	No	No
Moxee, WA	No	No
Hancock, WI	No	No
1992		
Homestead, FL	No	No
Aberdeen, ID	No	No
Ashland, ME	No	No
Johannesburg, MI	No	No
Riverhead, NY	No	No
Hermiston, OR	No	No
Othello, WA	No	No
Antigo, WI	No	No
Hancock, WI	No	No
1993		
Ashton, ID	No	No
Caldwell, ID	No	No
Grace, ID	No	No
Queenstown, MD	No	No
St. Agatha, ME	No	No
St. David, ME	No	No
Island Falls, ME	No	No
Monticello, ME	No	No
Stanton, MI	No	No
Manhattan, MT	No	No
Ronan, MT	No	No
Beach, ND	No	No
Grand Forks, ND	No	No
Lisbon, ND	No	No
Rollette, ND	No	No
Wooster, OH	No	No
Echo, OR	No	No
Antigo, WI	No	No
Coloma, WI	No	No
Hancock, WI	No	No
Harrison, WI	No	No

1. Susceptibility to insects other than the Colorado potato beetle.

Table V.2 Early-Season *B.t.t.* Protein Expression Levels in Leaf Tissue.

Line	Field Test	Number of Sites (reps.)	Expression Level,[1] µg *B.t.t.* protein/g fresh weight: Least Square Mean	Standard Error	Range[2]
BT6	Othello, WA	1 (6)	14.37	0.994	11.88 - 18.11
	Aberdeen, ID	1 (6)	18.50	0.899	15.71 - 20.92
	Combined[2]	7 (1 & 6)	16.36	1.158	11.88 - 20.82
BT10	Othello, WA	1 (6)	18.12	0.994	13.01 - 21.52
	Aberdeen, ID	1 (6)	27.72	0.899	20.44 - 30.80
	Combined[2]	7 (1 & 6)	23.12	1.158	13.01 - 30.80
BT12	Othello, WA	1 (6)	17.45	0.994	14.92 - 19.94
	Aberdeen, ID	1 (6)	22.39	0.899	19.85 - 25.51
	Combined[2]	7 (1 & 6)	19.63	1.158	14.35 - 25.51
BT16	Othello, WA	1 (6)	14.42	0.994	13.76 - 20.10
	Aberdeen, ID	1 (6)	21.68	0.899	18.96 - 24.84
	Combined[2]	7 (1 & 6)	18.42	1.158	13.18 - 24.84
BT17	Othello, WA	1 (6)	15.56	0.994	13.40 - 19.29
	Aberdeen, ID	1 (6)	21.42	0.899	17.73 - 25.40
	Combined[2]	7 (1 & 6)	17.39	1.158	9.97 - 25.40
BT18	Othello, WA	1 (6)	15.91	1.085	0.20 - 20.04
	Aberdeen, ID	1 (6)	23.26	0.899	22.08 - 24.18
	Combined[2]	7 (1 & 6)	19.76	1.171	0.20 - 24.18
BT23	Othello, WA	1 (6)	16.61	0.994	12.22 - 20.70
	Aberdeen, ID	1 (6)	23.26	0.899	20.65 - 27.41
	Combined[2]	7 (1 & 6)	18.99	1.158	12.22 - 27.41

1. Tissue collection and analytical methods are described in Appendix 15, p. 368. Statistical analyses and combined least square means were obtained using the mixed linear model procedure (PROC MIXED) of the SAS system, version 6.07. For the combined sites analyses, the location, replicate within location, location x line interaction and the residual error were all treated as random effects.
2. Combined data from tubers collected in 1992 at two replicated field trials (Aberdeen, ID and Othello, WA) and five non-replicated field trials (Hancock, WI; Hermiston, OR; Ashland, ME, Riverhead, NY and Antigo, WI)).
3. "Range" denotes the highest and lowest individual assay for each line.

Table V.3 Early-Season NPTII Protein Expression Levels in Leaf Tissue.

Line	Field Test	Number of Sites (reps.)	Expression Level,[1] µg NPTII protein/g fresh weight: Least Square Mean	Standard Error	Range[3]
BT6	Othello, WA	1 (6)	1.94	0.178	0.92 - 2.30
	Aberdeen, ID	1 (6)	2.06	0.240	1.65 - 2.37
	Combined[2]	7 (1 & 6)	2.06	0.198	0.92 - 2.83
BT10	Othello, WA	1 (6)	3.18	0.178	1.25 - 4.18
	Aberdeen, ID	1 (6)	5.04	0.240	3.94 - 6.69
	Combined[2]	7 (1 & 6)	3.90	0.198	1.25 - 6.69
BT12	Othello, WA	1 (6)	1.67	0.178	0.98 - 2.19
	Aberdeen, ID	1 (6)	2.10	0.240	1.83 - 2.39
	Combined[2]	7 (1 & 6)	1.64	0.198	0.95 - 2.39
BT16	Othello, WA	1 (6)	3.04	0.178	2.42 - 3.73
	Aberdeen, ID	1 (6)	3.57	0.240	2.92 - 4.24
	Combined[2]	7 (1 & 6)	3.01	0.198	1.79 - 5.07
BT17	Othello, WA	1 (6)	2.80	0.178	2.24 - 3.51
	Aberdeen, ID	1 (6)	3.85	0.240	3.35 - 4.24
	Combined[2]	7 (1 & 6)	2.93	0.198	1.75 - 4.28
BT18	Othello, WA	1 (6)	2.08	0.178	1.12 - 3.03
	Aberdeen, ID	1 (6)	3.26	0.240	2.45 - 4.50
	Combined[2]	7 (1 & 6)	2.58	0.198	1.12 - 4.50
BT23	Othello, WA	1 (6)	2.23	0.178	1.60 - 3.04
	Aberdeen, ID	1 (6)	2.72	0.240	2.45 - 3.11
	Combined[2]	7 (1 & 6)	2.34	0.198	1.31 - 3.41

1. Tissue collection and analytical methods are described in Appendix 15, p. 368. Statistical analyses and combined least square means were obtained using the mixed linear model procedure (PROC MIXED) of the SAS system, version 6.07. For the combined sites analyses, the location, replicate within location, location x line interaction and the residual error were all treated as random effects.
2. Combined data from tubers collected in 1992 at two replicated field trials (Aberdeen, ID and Othello, WA) and five non-replicated field trials (Hancock, WI; Hermiston, OR; Ashland, ME, Riverhead, NY and Antigo, WI)).
3. "Range" denotes the highest and lowest individual assay for each line.

Table V.4 Late-Season *B.t.t.* Protein Expression Levels in Whole Plant Tissue.[1]

Line	Field Test	Number of Sites (reps.)	Expression Level,[1] µg *B.t.t.* protein/g fresh weight: Least Square Mean	Standard Error	Range[3]
BT6	Othello, WA	1 (6)	4.37	0.522	2.88 - 5.34
	Aberdeen, ID	1 (6)	4.88	0.719	2.44 - 7.17
	Combined[2]	7 (1 & 6)	4.30	0.619	0.53 - 7.17
BT10	Othello, WA	1 (6)	8.71	0.522	6.83 - 10.77
	Aberdeen, ID	1 (6)	9.60	0.719	7.98 - 11.24
	Combined[2]	7 (1 & 6)	8.08	0.619	3.83 - 11.24
BT12	Othello, WA	1 (6)	7.97	0.522	6.93 - 9.00
	Aberdeen, ID	1 (6)	8.77	0.719	3.56 - 11.71
	Combined[2]	7 (1 & 6)	7.88	0.619	3.56 - 11.71
BT16	Othello, WA	1 (6)	7.82	0.522	6.39 - 9.48
	Aberdeen, ID	1 (6)	6.85	0.719	5.83 - 8.64
	Combined[2]	7 (1 & 6)	6.57	0.619	0.91 - 9.48
BT17	Othello, WA	1 (6)	5.52	0.522	4.14 - 6.63
	Aberdeen, ID	1 (6)	6.28	0.719	2.55 - 8.74
	Combined[2]	7 (1 & 6)	5.49	0.619	0.82 - 8.74
BT18	Othello, WA	1 (6)	8.00	0.522	6.54 - 12.24
	Aberdeen, ID	1 (6)	9.46	0.719	6.55 - 12.83
	Combined[2]	7 (1 & 6)	7.91	0.619	4.12 - 12.83
BT23	Othello, WA	1 (6)	4.73	0.522	3.70 - 5.60
	Aberdeen, ID	1 (6)	8.97	0.719	6.29 - 11.48
	Combined[2]	7 (1 & 6)	6.27	0.619	2.67 - 11.48

1. Tissue collection and analytical methods are described in Appendix 15, p. 368. Statistical analyses and combined least square means were obtained using the mixed linear model procedure (PROC MIXED) of the SAS system, version 6.07. For the combined sites analyses, the location, replicate within location, location x line interaction and the residual error were all treated as random effects.
2. Combined data from tubers collected in 1992 at two replicated field trials (Aberdeen, ID and Othello, WA) and five non-replicated field trials (Hancock, WI; Hermiston, OR; Ashland, ME, Riverhead, NY and Antigo, WI)).
3. "Range" denotes the highest and lowest individual assay for each line.

Table V.5 Late-Season NPTII Protein Expression Levels in Whole Plant Tissue.

Line	Field Test	Number of Sites (reps.)	Expression Level,[1] µg NPTII protein/g fresh weight: Least Square Mean	Standard Error	Range[3]
BT6	Othello, WA	1 (6)	0.37	0.091	0.07 - 0.71
	Aberdeen, ID	1 (6)	0.14	0.064	ND[4] - 0.43
	Combined[2]	7 (1 & 6)	0.45	0.087	ND - 0.86
BT10	Othello, WA	1 (6)	0.66	0.091	0.12 - 1.32
	Aberdeen, ID	1 (6)	0.16	0.064	0.05 - 0.38
	Combined[2]	7 (1 & 6)	0.69	0.087	0.05 - 1.79
BT12	Othello, WA	1 (6)	0.35	0.091	0.08 - 0.56
	Aberdeen, ID	1 (6)	0.28	0.064	0.08 - 0.69
	Combined[2]	7 (1 & 6)	0.39	0.087	0.08 - 0.89
BT16	Othello, WA	1 (6)	0.50	0.091	0.37 - 0.99
	Aberdeen, ID	1 (6)	0.35	0.064	0.04 - 0.64
	Combined[2]	7 (1 & 6)	0.51	0.087	0.04 - 1.26
BT17	Othello, WA	1 (6)	0.40	0.091	0.11 - 0.61
	Aberdeen, ID	1 (6)	0.04	0.064	ND - 0.09
	Combined[2]	7 (1 & 6)	0.51	0.087	ND - 0.97
BT18	Othello, WA	1 (6)	0.75	0.091	0.19 - 1.41
	Aberdeen, ID	1 (6)	0.15	0.064	0.03 - 0.28
	Combined[2]	7 (1 & 6)	0.82	0.087	0.03 - 1.84
BT23	Othello, WA	1 (6)	0.31	0.091	0.05 - 0.63
	Aberdeen, ID	1 (6)	0.09	0.064	0.03 - 0.14
	Combined[2]	7 (1 & 6)	0.32	0.087	0.03 - 0.72

1. Tissue collection and analytical methods are described in Appendix 15, p. 368. Statistical analyses and combined least square means were obtained using the mixed linear model procedure (PROC MIXED) of the SAS system, version 6.07. For the combined analyses, the location, replicate within location, location x line interaction and the residual error were all treated as random effects.
2. Combined data from tubers collected in 1992 at two replicated field trials (Aberdeen, ID and Othello, WA) and five non-replicated field trials (Hancock, WI; Hermiston, OR; Ashland, ME, Riverhead, NY and Antigo, WI)).
3. "Range" denotes the highest and lowest individual assay for each line.
4. "ND" denotes no detection.

Table V.6 *B.t.t.* Protein Expression Levels in Tuber Tissue.

Line	Field Test	Number of Sites (reps.)	Expression Level,[1] µg *B.t.t.* protein/g fresh weight: Least Square Mean	Standard Error	Range[3]
BT6	Othello, WA	1 (6)	0.71	0.096	0.55 - 0.84
	Aberdeen, ID	1 (6)	0.53	0.121	0.28 - 0.68
	Combined[2]	7 (1 & 6)	0.66	0.075	0.28 - 0.95
BT10	Othello, WA	1 (6)	1.13	0.096	0.88 - 1.42
	Aberdeen, ID	1 (6)	0.76	0.121	0.36 - 1.25
	Combined[2]	7 (1 & 6)	0.98	0.075	0.36 - 1.29
BT12	Othello, WA	1 (6)	1.13	0.096	0.35 - 1.72
	Aberdeen, ID	1 (6)	1.06	0.121	0.50 - 1.54
	Combined[2]	7 (1 & 6)	1.17	0.075	0.50 - 1.54
BT16	Othello, WA	1 (6)	1.20	0.096	0.75 - 1.41
	Aberdeen, ID	1 (6)	1.04	0.121	0.55 - 1.90
	Combined[2]	7 (1 & 6)	1.19	0.075	0.55 - 1.90
BT17	Othello, WA	1 (6)	0.94	0.096	0.43 - 1.23
	Aberdeen, ID	1 (6)	0.56	0.121	0.48 - 0.88
	Combined[2]	7 (1 & 6)	1.00	0.075	0.43 - 2.00
BT18	Othello, WA	1 (6)	1.34	0.096	1.17 - 1.44
	Aberdeen, ID	1 (6)	0.79	0.121	0.39 - 0.96
	Combined[2]	7 (1 & 6)	1.09	0.075	0.39 - 1.66
BT23	Othello, WA	1 (6)	1.04	0.096	0.73 - 1.26
	Aberdeen, ID	1 (6)	0.73	0.121	0.49 - 0.97
	Combined[2]	7 (1 & 6)	0.98	0.075	0.49 - 1.29

1. Tissue collection and analytical methods are described in Appendix 15, p. 368.. Statistical analyses and combined least square means were obtained using the mixed linear model procedure (PROC MIXED) of the SAS system, version 6.07. For the combined sites analyses, the location, replicate within location, location x line interaction and the residual error were all treated as random effects.
2. Combined data from tubers collected in 1992 at two replicated field trials (Aberdeen, ID and Othello, WA) and five non-replicated field trials (Hancock, WI; Hermiston, OR; Ashland, ME, Riverhead, NY and Antigo, WI)).
3. "Range" denotes the highest and lowest individual assay for each line.

Table V.7 NPTII Protein Expression Levels in Tuber Tissue.

Line	Field Test	Number of Sites (reps.)	Expression Level,[1] µg NPTII protein/g fresh weight: Least Square Mean	Standard Error	Range[3]
BT6	Othello, WA	1 (6)	0.44	0.052	0.31 - 0.57
	Aberdeen, ID	1 (6)	0.21	0.065	0.14 - 0.28
	Combined[2]	7 (1 & 6)	0.35	0.049	0.14 - 0.60
BT10	Othello, WA	1 (6)	0.88	0.052	0.74 - 1.11
	Aberdeen, ID	1 (6)	0.47	0.065	0.25 - 0.77
	Combined[2]	7 (1 & 6)	0.68	0.049	0.21 - 1.11
BT12	Othello, WA	1 (6)	0.36	0.052	0.27 - 0.49
	Aberdeen, ID	1 (6)	0.28	0.065	0.19 - 0.45
	Combined[2]	7 (1 & 6)	0.36	0.049	0.19 - 0.64
BT16	Othello, WA	1 (6)	0.50	0.052	0.31 - 0.84
	Aberdeen, ID	1 (6)	0.40	0.065	0.26 - 0.58
	Combined[2]	7 (1 & 6)	0.48	0.049	0.26 - 0.84
BT17	Othello, WA	1 (6)	0.66	0.052	0.53 - 0.80
	Aberdeen, ID	1 (6)	0.63	0.065	0.29 - 1.22
	Combined[2]	7 (1 & 6)	0.54	0.049	0.29 - 1.22
BT18	Othello, WA	1 (6)	0.77	0.052	0.50 - 1.05
	Aberdeen, ID	1 (6)	0.52	0.065	0.40 - 0.68
	Combined[2]	7 (1 & 6)	0.62	0.049	0.37 - 1.05
BT23	Othello, WA	1 (6)	0.56	0.052	0.44 - 0.62
	Aberdeen, ID	1 (6)	0.38	0.065	0.28 - 0.55
	Combined[2]	7 (1 & 6)	0.49	0.049	0.28 - 0.72

1. Tissue collection and analytical methods are described in Appendix 15, p. 368.. Statistical analyses and combined least square means were obtained using the mixed linear model procedure (PROC MIXED) of the SAS system, version 6.07. For the combined sites analyses, the location, replicate within location, location x line interaction and the residual error were all treated as random effects.
2. Combined data from tubers collected in 1992 at two replicated field trials (Aberdeen, ID and Othello, WA) and five non-replicated field trials (Hancock, WI; Hermiston, OR; Ashland, ME, Riverhead, NY and Antigo, WI)).
3. "Range" denotes the highest and lowest individual assay for each line.

Table V.8 Total Solids, Sugar, Vitamin C, Protein and Glycoalkaloid Content of Tubers From CPB Resistant Plant Lines and Russet Burbank Control Plants.[1,2]

Line	Total Solids %	Sugars Dextrose % FW	Sugars Sucrose % FW	Vitamin C mg/100g FW	Protein %DW	Total Glycoalkaloids mg/100g FW
BT6	20.5	.18	.13	8.8	4.9	6.6
BT10	20.4	.20	.11	9.4	5.0	5.5
BT12	20.5	.18	.13	9.6	4.7	6.8
BT16	19.9	.18	.11	9.0	4.7	6.1
BT17	19.8	.21	.11	8.5	4.6	6.8
BT18	19.5	.21	.11	8.3	4.7	9.4
BT23	20.1	.20	.11	9.1	5.0	5.4
R.B. Control	19.9	.15	.12	9.0	5.2	4.5
SE (mean)	0.97	.019	.017	1.06	0.43	1.39
SE (difference)	0.60	.016	.011	0.58	0.30	0.95
R.B. Normal Range	16.8 - 24.5[3]	0.04 - 0.52[4]	0.10 - 0.88[4]	10.3 - 22.0[4]	3.4 - 7.3[5]	3.1 - 16.1[6]

1. Compositional analysis methods are summarized in Appendix 15, p. 368. Results are combined data from tubers collected in 1992 at two replicated field trials (Aberdeen, ID and Othello, WA) and four non-replicated field trials (Hancock, WI; Hermiston, OR; Ashland, ME and Antigo, WI). 2. Statistical analyses and combined least square means were obtained using the mixed linear model procedure (PROC MIXED) of the SAS system, version 6.07. For the combined analyses, the location, replicate within location, location x line interaction and the residual error were all treated as random effects. Underlined values indicate means that are significantly different from the R.B. control at the 5% level. In PROC MIXED, these statistical significances were determined by t-tests using the mixed model difference standard errors and 70 degrees of freedom.

3. Results from a conversion of specific gravity made for Russet Burbank potatoes. Specific gravity measurements were taken from the western regional trial (1980 - 1992) and includes means from 132 location/year.

4. Taken from tubers grown in Aberdeen, ID and the results are averages from eleven individual trials. Sugar content is strongly dependent on storage conditions and varies widely within a single season.

5. Taken from analysis of tubers grown at Aberdeen, ID and results are the averages from eight individual trials.

6. Taken from analysis of tubers grown in Aberdeen, ID and results are the averages from six individual trials. Sinden and Webb reported a range for Russet Burbank of 3 - 39 mg/100g FW (*Amer. Pot. J.*, 49:334-338).

Table V.9 Internal Defects and Blackspot Bruise Characteristics of Tubers From CPB Resistant Plant Lines and Russet Burbank Control Plants.[1,2]

Line	Hollow Heart and Brown Center[3] %	Internal Brown Spot[3] %	Vascular Discoloration[3] %	Total Internal Defects[3] %	Blackspot Bruise[4]
BT6	42	0	5	47	3.4
BT10	28	0	4	32	3.0
BT12	33	0	2	34	3.2
BT16	35	0	3	38	3.3
BT17	33	1	3	37	3.3
BT18	13	1	6	19	3.4
BT23	39	1	1	41	3.3
R.B. Control	25	1	4	30	3.4
LSD (.05)	12	NS[5]	NS	12	NS

1. Quality assessment methods are summarized in Appendix 15, p. 368. Results are combined data from tubers collected in 1992 from field trials conducted at Aberdeen, ID and Othello, WA. Both field trials employed a six replicate randomized complete block design for each line.
2. Underlined values are significantly ($P \leq 0.05$) different from the control.
3. Internal defects were evaluated by visual inspection of ten tubers from each replicate plot (>10oz) cut longitudinally. Values represent percentage of tubers exhibiting internal defects.
4. Blackspot was evaluated using the abrasive peel method of Pavek (*Amer. Potato J.*, 1985, 62: 511-517). Rated 1-5 with 5=worst.
5. "NS" denotes no significant difference.

Table V.10 French Fry Quality of Tubers from CPB Resistant Plant Lines and Russet Burbank Control Plant.[1]

Line	Fry Color 40°F[2]	Fry Color 45°F[2]	Sugar-end Incidence,[3] %	Sugar-end Severity[3]
BT6	3.9	2.1	60	1.6
BT10	4.0	2.2	67	1.9
BT12	3.9	2.3	58	1.9
BT16	4.0	2.2	62	2.0
BT17	4.0	2.2	57	1.8
BT18	4.0	2.3	66	2.2
BT23	4.0	2.2	54	2.0
R.B. Control	4.0	2.1	38	1.6
LSD (.05)	NS[4]	NS	NS	NS

1. French-fry quality assessment methods are summarized in Appendix 15, p. 368. Results are combined data from tubers collected in 1992 at two replicated field trials (Aberdeen, ID and Othello, WA).
2. French fry color determined after 2 months storage at 40 or 45°F. Rated using USDA fry chart on a 0-4 scale with smaller numbers indicating lighter color.
3. Sugar-end incidence and severity were evaluated only from 45°F storage. Incidence is the percentage of tubers with the defect. Severity is a measure of the color difference between the light and dark end of the fry and is reported in USDA fry chart units. Higher numbers indicate more severe expression of sugar-ends.
4. "NS" denotes no significant difference.

Table V.11 Proximate Composition of Tubers from CPB Resistant Plant Lines and Russet Burbank Control Plants.[1]

Line	Protein, g/100g	Fat, g/100g	Ash, g/100g	Total Dietary Fiber, g/100g	Carbohy-drate, g/100g	Calories, kcal/100g
BT6	10.62	0.30	4.78	7.08	79.72	364
BT10	10.88	0.28	4.82	7.55	79.95	366
BT12	10.75	0.25	5.00	7.55	81.35	370
BT16	10.65	0.25	4.75	7.20	80.45	367
BT17	10.52	0.28	4.62	7.15	79.82	364
BT18	10.60	0.30	4.75	7.38	79.70	364
BT23	10.54	0.25	4.88	7.28	79.92	364
R.B. Control	10.40	0.28	4.80	6.65	80.28	366
Published Range[2]	7.1 -14.6	0.2 - 0.8	2.2 - 9.5	5 - 13	84.5 (ave. value)	350 (ave. value)

1. Analytical methods employed in the determination of proximate composition are summarized in Appendix 15, p. 368. Values reported are based on dry weight matter and are the average of composite samples from tubers collected in 1992 at two replicated field trials (Aberdeen, ID and Othello, WA). Composite samples were prepared by mixing 20 g of dry tuber powder from six plots for each field site.
2. Published range are approximate values compiled from Kadam *et al.* (1991) and Scherz *et al.* (1989). Some values were converted from fresh weight to dry matter basis on assumption that dry matter was 20% of the fresh weight.

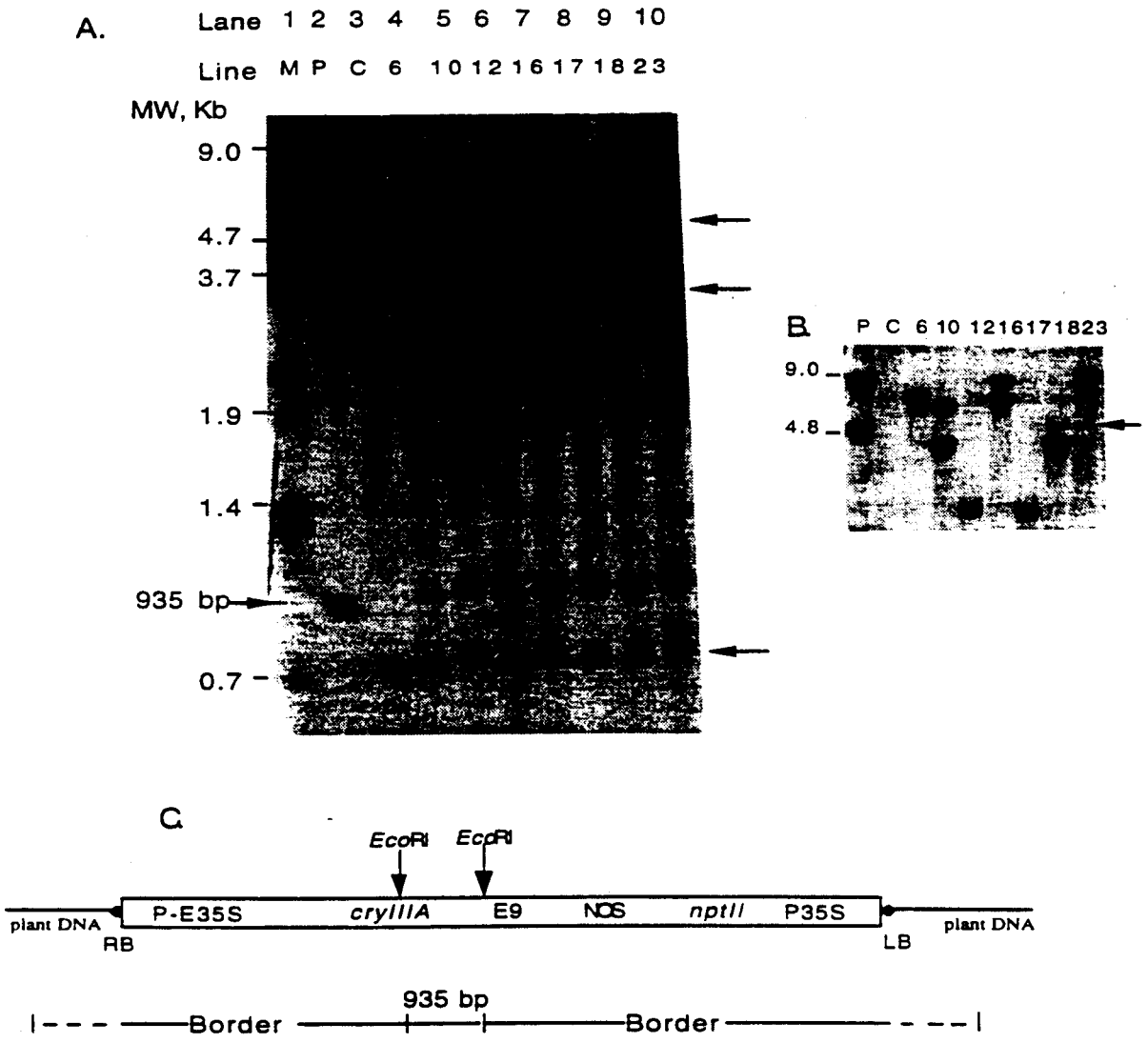
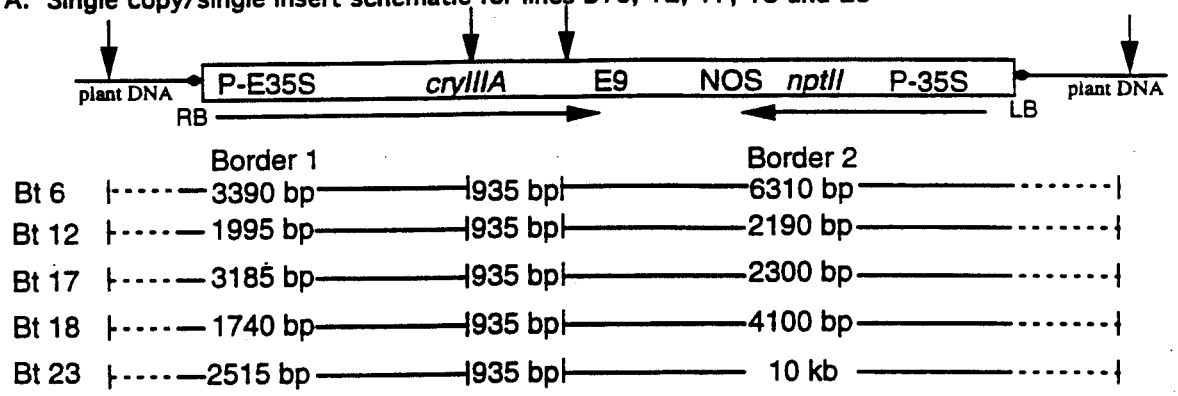


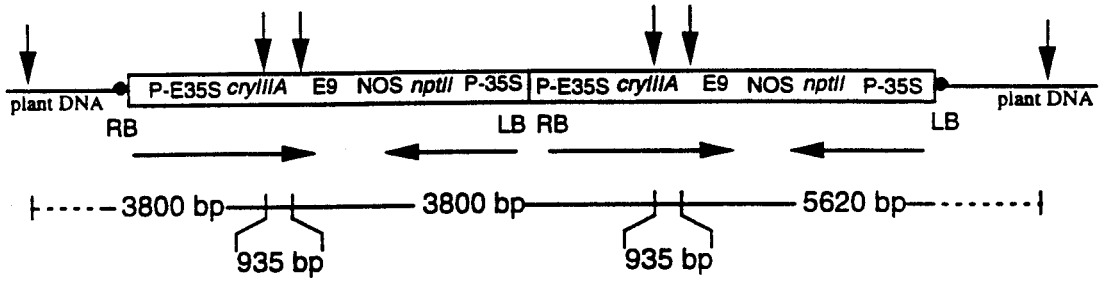
Figure V:1 Southern blot analysis using *Eco*RI.

Panel A: Autoradiogram of Southern blot analysis of approximately 10 µg of DNA isolated from young leaves of CPB resistant and Russet Burbank control potato plants which were digested with the restriction enzyme *Eco*RI. The probe was the entire PV-STBT02 plasmid. Lane 1, labelled M, is a DNA molecular weight marker (lambda DNA digested with *Bst*EII) and lane 2, labelled P, is a mixture of two digestions done separately with PV-STBT02. In one reaction the plasmid was digested with *Eco*RI (to generate the 935 bp fragment) and in the second separate reaction it was digested with *Nhe*I/*Hin*dIII (to release the DNA fragment, 4619 bp, that served as the T-DNA) and then mixed. Lanes 3-10 contain the DNA from Russet Burbank control and CPB resistant plant lines BT6, 10, 12, 16, 17, 18 and 23, respectively. Horizontal arrows denote background bands.
Panel B: A separate blot in which the same samples of DNA used in Panel A were digested with *Eco*RI and probed with DNA homologous to the *nptII* gene. A comparison to Panel A identified the border fragments containing the *nptII* gene.
Panel C: A schematic representation of the T-DNA from the plasmid PV-STBT02 integrated into the plant genome. Internal *Eco*RI restriction sites are denoted.

A. Single copy/single insert schematic for lines BT6, 12, 17, 18 and 23



B. Two copies/single insert schematic for line BT10



C. Two copies/two insert schematic for line BT16

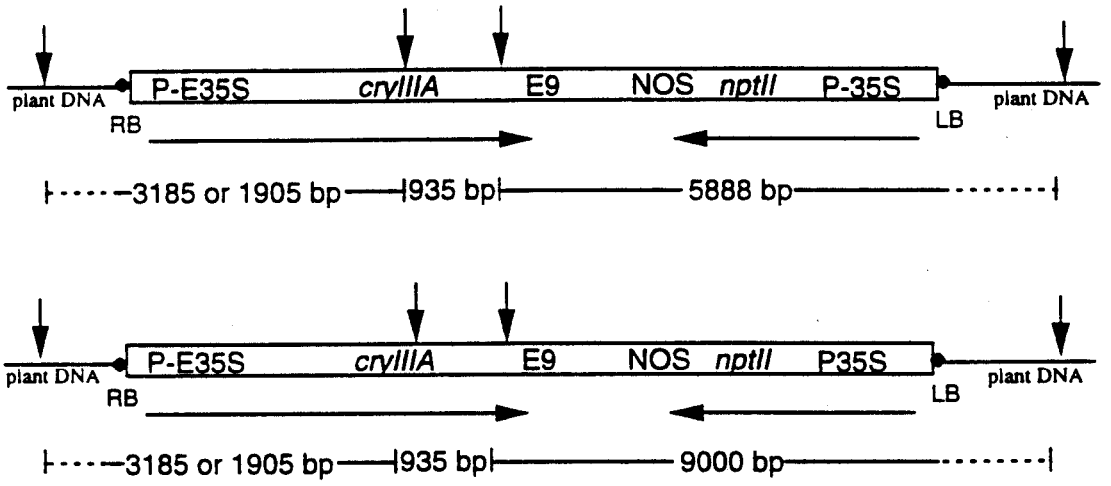


Figure V.2 Schematic illustration of the EcoRI digestion results.
The vertical arrows denote the locations of *Eco*RI sites within the T-DNA and the dashed lines indicate variable sized fragments. All border fragment sizes are estimates. Right and left borders are denoted by RB and LB, respectively, and are shown for orientation purposes (i.e., intact border sequences are not meant to be implied).

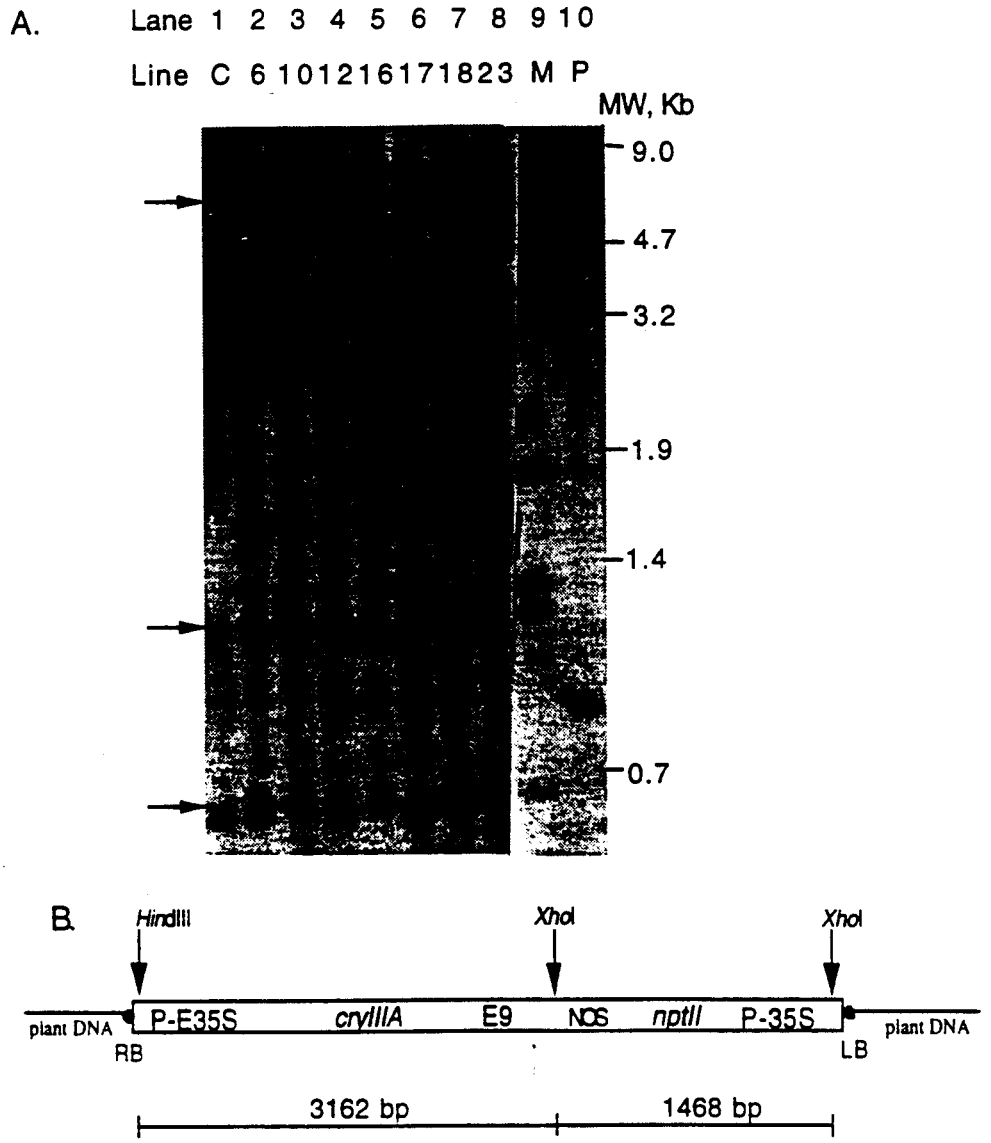
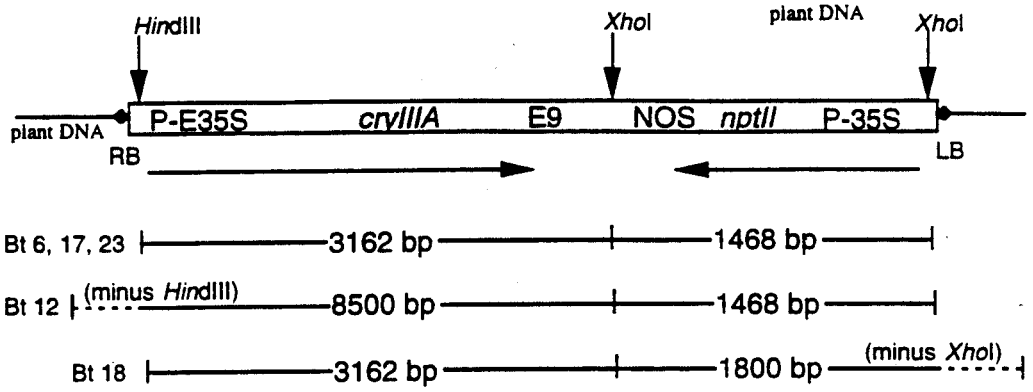
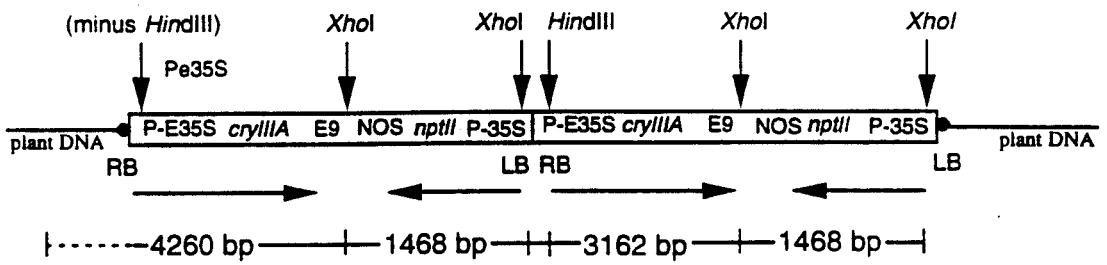


Figure V.3 Southern blot analysis using *Hin*dIII with *Xho*I.
Panel A: Autoradiogram of Southern blot analysis of approximately 10 µg of DNA isolated from young leaves of CPB resistant and Russet Burbank control potato plants which were digested simultaneously with the restriction enzymes *Hin*dIII and *Xho*I. The probe was the entire PV-STBT02 plasmid. Lane 9, labelled M, is a DNA molecular weight marker (lambda DNA digested with *Bst*EII) and lane 10 is the PV-STBT02 plasmid DNA digested separately with *Eco*RI and *Nhe*I/*Hin*dIII as described in the legend for Figure V.1. Lanes 1-8 contain the DNA from Russet Burbank control and CPB resistant plant lines BT6, 10, 12, 16, 17, 18 and 23, respectively. Horizontal arrows denote background bands.
Panel B: A schematic representation of the T-DNA from the plasmid PV-STBT02 integrated into the plant genome. Internal *Hin*dIII and *Xho*I restriction sites are denoted.

A. Single copy/single insert schematic for lines BT6, 12, 17, 18 and 23



B. Two copies/single insert schematic for line BT10



C. Two copies/two insert schematic for line BT16

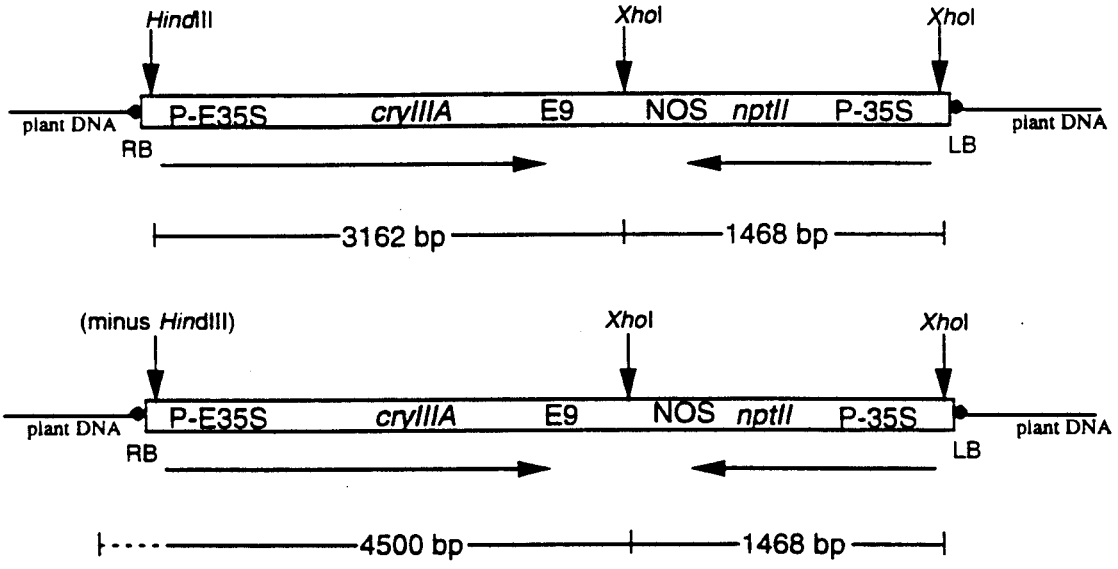


Figure V.4 Schematic illustration of the HindIII with XhoI digestion results. The dashed lines indicate variable sized fragments. All border fragment sizes are estimates. Right and left borders are denoted by RB and LB, respectively, and are shown for orientation purposes (*i.e.*, intact border sequences are not meant to be implied).

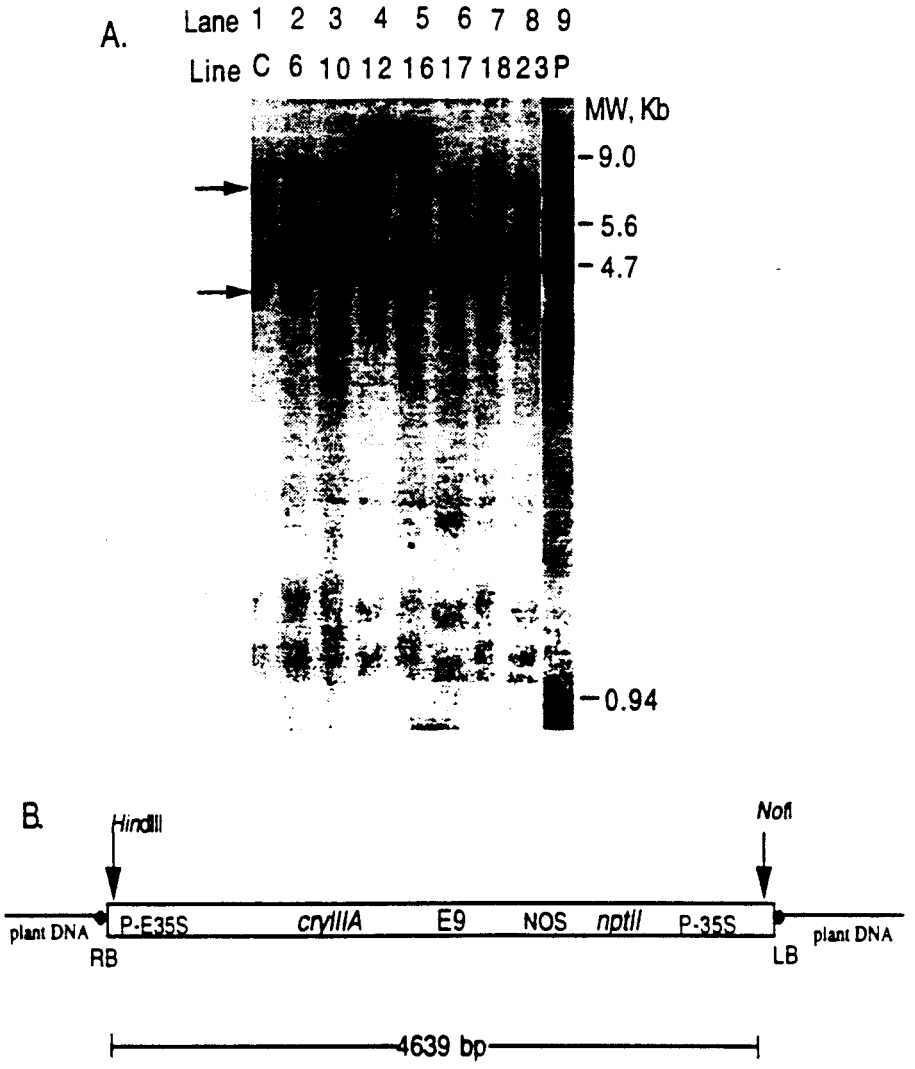
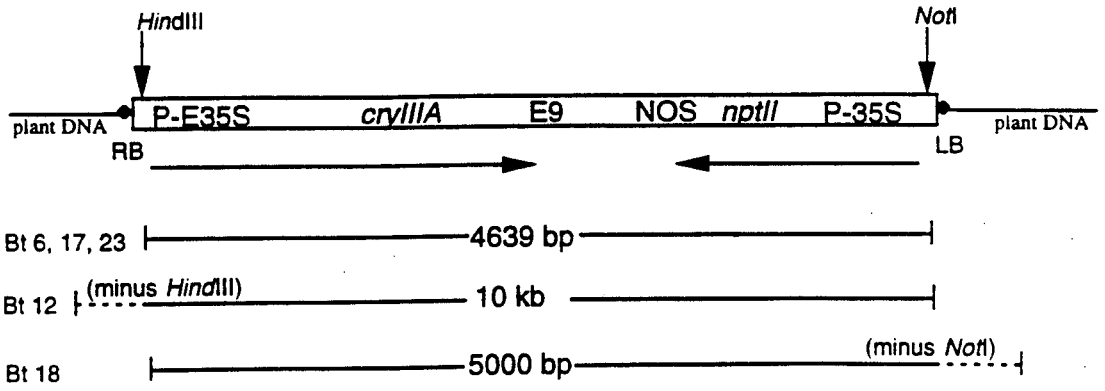
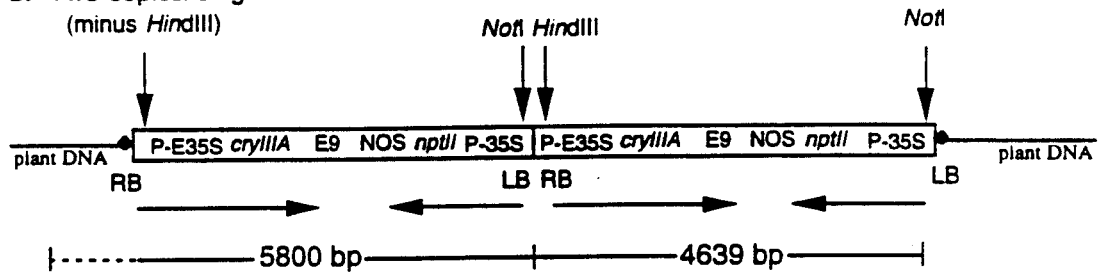


Figure V.5 Southern blot analysis using *Hin*dIII with *Not*I.
Panel A: Autoradiogram of Southern blot analysis of approximately 10 µg of DNA isolated from young leaves of CPB resistant and Russet Burbank control potato plants which were digested with the restriction enzymes *Hin*dIII and *Not*I. The probe was the entire PV-STBT02 plasmid. Lane 9 is the PV-STBT02 plasmid DNA digested separately with *Eco*RI and *Nhe*I/*Hin*dIII as described in the legend to Figure V.1. Lanes 1-8 contain the DNA from Russet Burbank control plant and CPB resistant plant lines BT6, 10, 12, 16, 17, 18 and 23, respectively. Horizontal arrows denote background bands.
Panel B: A schematic representation of the T-DNA from the plasmid PV-STBT02 integrated into the plant genome. Internal *Hin*dIII and *Not*I restriction sites are denoted.

A. Single copy/single insert schematic for lines BT6, 12, 17, 18 and 23



B. Two copies/single insert schematic for line BT10



C. Two copies/two insert schematic for line BT16

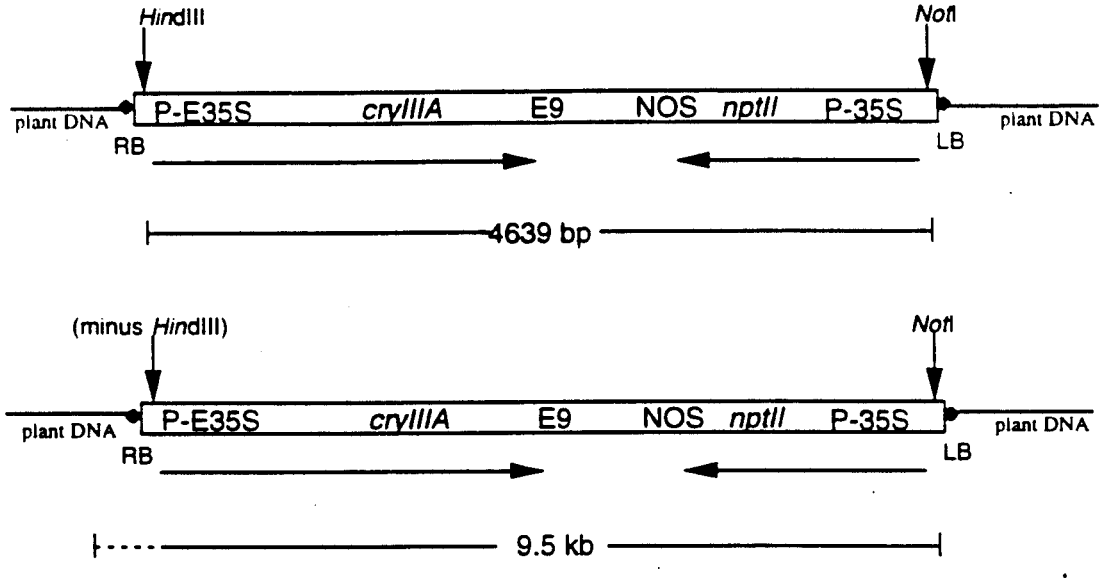


Figure V.6 Schematic illustration of the HindIII with NotI digestion results.
The dashed lines indicate variable sized fragments. All border fragment sizes are estimates. Right and left borders are denoted by RB and LB, respectively, and are shown for orientation purposes (*i.e.*, intact border sequences are not meant to be implied).

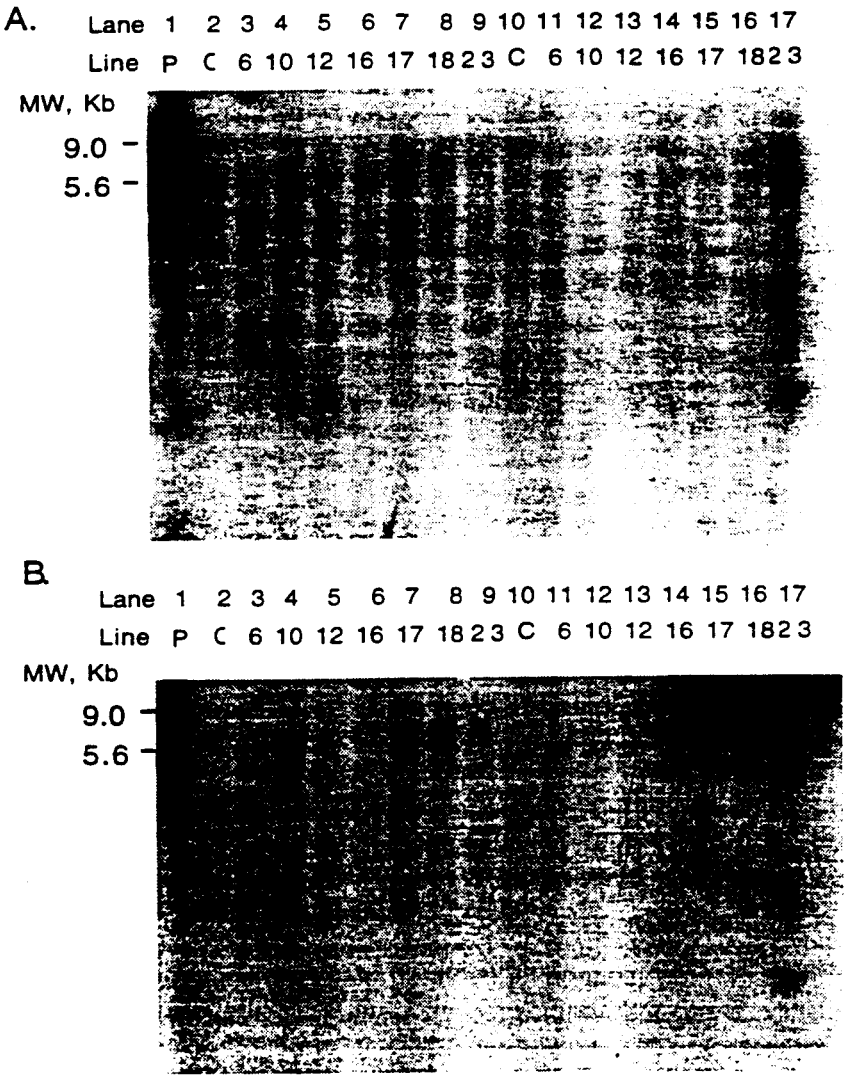


Figure V.7 Southern blot analysis confirming the absence of the *aad* and *oriV* genetic regions.

Autoradiograms of the Southern blot membrane from Figures V.1 (digestion with *Eco*RI) and V.3 (digestion with *Hin*dIII and *Xho*I), which were presented separately but analyzed on the same blot was stripped of the plasmid probe and individually analyzed for regions of homology with probes prepared against the *aad* gene and *oriV* genetic element (refer to Figure III.1 for probe locations). Refer to Figures III.1 and V.1 for plasmid sizes observed in lane 1.

Panel A: Lanes 1-9 above correspond to lanes 2-10 in Figure V.1 and lanes 10-17 above correspond to lanes 1-8 in Figure V.3. The probe was prepared against the *aad* gene.

Panel B: Same lane numbering as in Panel A. The probe was prepared against the *oriV* genetic element.

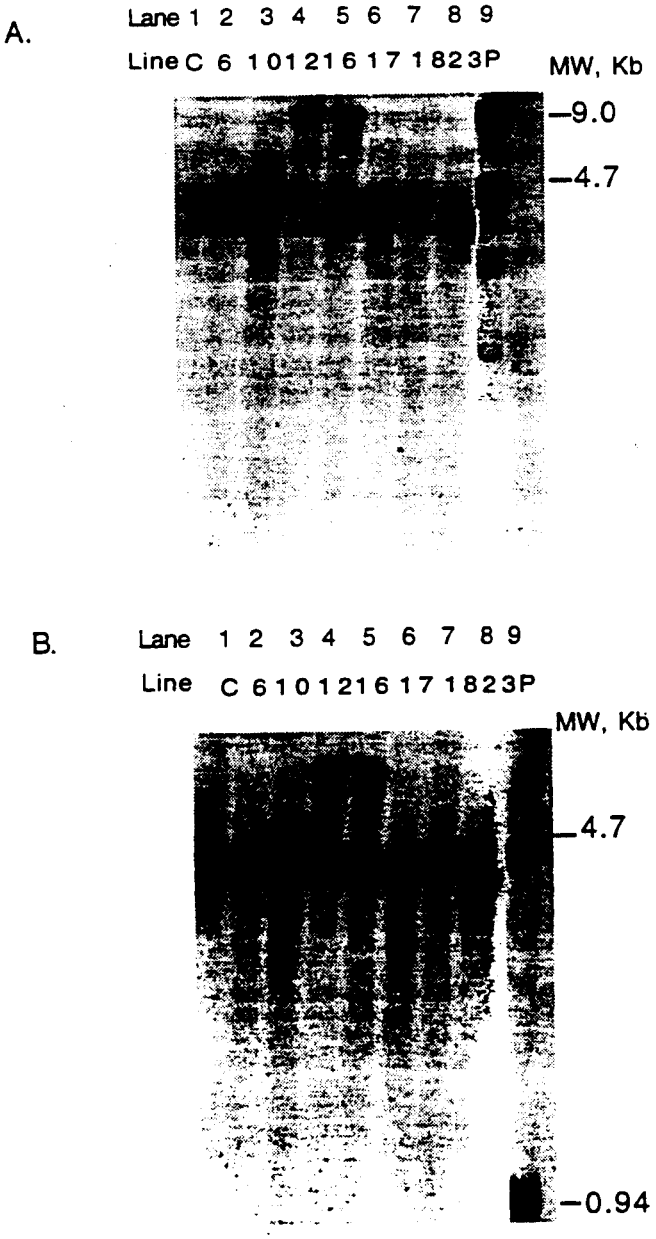


Figure V.8 Southern blot analysis indicating linkage of the *nptII* and *cryIIIA* genes.
Autoradiograms of the blot from Figure V.5 (digestion with *Hin*dIII and *Not*I) which was stripped of the plasmid probe and analyzed for homology to probes prepared against the *nptII* (Panel A) and *cryIIIA* (Panel B) genes. Lane 9 is the PV-STBT02 plasmid DNA digested separately with *Eco*RI and *Nhe*I/*Hin*dIII as described in the legend to Figure V.1. Lanes 1-8 contain the DNA from the Russet Burbank control and CPB resistant plant lines BT6, 10, 12, 16, 17, 18 and 23, respectively.

F. References

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VI. ENVIRONMENTAL CONSEQUENCES OF INTRODUCING CPB RESISTANT POTATO LINES PRODUCED UTILIZING THE PLASMID VECTOR PV-STBT02

The potential environmental consequences of introducing these CPB resistant potato lines have been evaluated. The use of these potatoes will have a positive impact on the environment by promoting integrated pest management practices and reducing reliance on traditional chemical insecticides. Extensive field test results, safety studies and independent scientific research establishes that the commercial use of these potatoes will not result in any adverse effects to the environment. These studies include assessing the toxicity to non-target organisms of the *B.t.t.* and NPTII proteins, environmental fate of the *B.t.t.* protein, transfer of the introduced genes to other plant species, the potential for these potato plants to become weeds, and the impact of these plants on potato pest management. These potential effects are discussed below.

A. *B.t.t.* Protein

The EPA and other regulatory agencies worldwide have determined that use of registered *B.t.t.* products offer no significant risks to human health or non-target organisms (EPA, 1988; EPA, 1991). Based on full product registration packages and other scientific information, the Agency found no evidence of any human or environmental safety concerns related to current uses of *B.t.t.* (EPA, 1988). In published reviews and the EPA documents, studies are referenced where the maximum hazard dose (5000 mg/kg) of *B.t.t.* microbial preparations was administered as single or multiple doses to different laboratory animals, with no adverse effects (EPA, 1991). Avian and aquatic organisms have also been fed *B.t.t.* microbial preparations, with no adverse effects. The preparations which were administered contained varying amounts of crystalline proteins from *B.t.t.*, either as a mixture with spores or encapsulated in killed *Pseudomonas fluorescens* cells (EPA, 1991). While target insects are susceptible to oral doses of *B.t.t.* proteins, there was no evidence of any toxic effects observed in non-target laboratory mammals, fish or birds given the equivalent of up to 10^6 µg of protein per gram of body weight. Since the *B.t.t.* protein expressed by CPB resistant potato plants is identical to one of the proteins present in commercial *B.t.t.* microbial preparations, the data support the safety of the *B.t.t.* protein produced in these CPB resistant potato lines.

Data was submitted to the EPA on September 10, 1993 (EPA Number 524-UTU) to support the registration and exemption from the requirement of a tolerance for the *B.t.t.* protein as a plant pesticide. Studies included within the EPA submission demonstrate the non-target and environmental safety of this protein (Appendix 16, pages 381-384). These studies confirmed the Coleopteran selectivity of the *B.t.t.* protein as expressed in CPB resistant plant lines BT6, BT10, BT12, BT16, BT17, BT18 and BT23 (Table VI.1). In addition, MacIntosh *et al.* (1990), observed no deleterious effects on non-target insects at concentrations of over 300-700 fold higher than those needed to control CPB.

An *in vitro* digestion study was conducted, which demonstrated the rapid degradation of the *B.t.t.* protein in simulated mammalian digestive fluid (EPA MRID NO. 42932218).

Even if insecticidal proteins could survive in the mammalian digestive tract, no *B.t.t.* receptors are expected on the surface of gastrointestinal tissues to permit binding of the protein to the cell surface. Data submitted to the EPA from a mouse acute gavage study with a large dose of *B.t.t.* protein (5000 mg/kg body weight) support this conclusion (EPA MRID NO. 42932217). These results are fully consistent with the history of safe use of *B. thuringiensis* preparations.

B. Neomycin Phosphotransferase II

The NPTII protein, which has no insecticidal effect, is ubiquitous in the environment and is found in microbes present on food and within the human digestive system (Flavell *et al.* 1992; Calgene, Inc., 1993). This protein has also been used as a selectable marker for animal and human cell transformation and for human gene therapy experiments (Culver *et al.*, 1991; Brenner *et. al.*, 1993). The safety of NPTII and other selectable markers were addressed in recent reviews by Fuchs *et al.* (1993a and 1993b), Flavell *et al.* (1992) and Nap *et al.* (1992). Data were submitted to the EPA on November 25, 1993, to support the exemption from the requirement of a tolerance for this protein as a pesticidal inert ingredient (EPA Pesticide Petition #4E4301; Appendix 16, pages 381-384). In addition, the U.S. Food and Drug Administration (FDA) recently approved the use of this protein as a processing aid food additive in several crops, as requested by Calgene, Inc. (FDA, 1994). All data support the safety of NPTII protein for use as a selectable marker in crops grown for human and animal consumption. This conclusion was also supported by a document published by the World Health Organization (WHO, 1993).

C. Effects of CPB Resistant Potatoes on Non-target Organisms

The Colorado potato beetle resistant plant lines BT6, BT10, BT12, BT16, BT17, BT18, and BT23 have been field tested at numerous sites across the U.S. since 1991. Plants of these lines show no deleterious effects towards non-target insects, birds, or other species that frequent potato fields (Appendix 1, page 98). Results of Monsanto sponsored tests on non-target organisms have confirmed the safety of *B.t.t.* protein expressed in plants of these lines. Non-target organisms were fed either *B.t.t.* protein or potato tubers that expressed this protein. Since it was not practical to isolate sufficient quantities of the *B.t.t.* protein from CPB resistant potatoes, the protein was obtained from *Escherichia coli* expressing the same *B.t.t.* protein present in the CPB resistant potatoes. Data demonstrating the equivalence of the *B.t.t.* protein expressed in CPB resistant potatoes and in *E. coli* and the safety of this protein to non-target organisms has been submitted to the EPA to support our request for the registration of the *B.t.t.* protein as a plant pesticide and its exemption from the requirement of a tolerance (EPA MRID Numbers 42932203 and 42932207 through 42932218). These studies are summarized below.

Non-target beneficial insects (larval and adult honeybee, ladybird beetle, green lacewing and parasitic wasp) were fed *B.t.t.* protein in the diet for up to 10 days at concentrations at least 100 times the LC_{50} (1.0 ppm) for *B.t.t.* protein to the target insect species, CPB. Consistent with published literature, *B.t.t.* protein was not toxic to

these non-target insects (Table VI.2; EPA MRID Numbers 42932207 through 42932213).

Mice were administered approximately 5000 mg/kg of the *B.t.t.* protein by gavage in one day. There was no mortality, no adverse effects, no changes in body weight gained or food consumption in dosed mice. No abnormal changes were observed in the tissues of mice necropsied seven days after dosing. The highest dose administered to mice, approximately 5000 mg/kg, was considered a "no adverse effect level." These data support the safety of the *B.t.t.* protein to mammals (EPA MRID Numbers 42932216 and 42932217).

Ten day-old bobwhite quail were fed potatoes expressing the *B.t.t.* protein to assess the wholesomeness of the potatoes if eaten by birds in a field setting. The consumption of lyophilized potatoes during this period was equivalent to 90 grams of potato/day for a 1 kg bird. No adverse effects were observed in birds fed this diet for 5 days and weight gain was comparable to birds fed control potatoes for the same period. It was concluded that the wholesomeness of the CPB resistant potatoes to birds was equivalent to control potatoes (EPA MRID Numbers 42932214 and 42932215).

A four week rat feeding study with whole raw potatoes was performed to confirm the nutritional equivalence of the seven CPB resistant potato lines to control Russet Burbank potatoes. The consumption rate was approximately 84 grams potato/kg rat body weight/day which is equivalent to the consumption of approximately 40 potatoes per day for an adult human (assumes average potato weight is 150 grams). There was no mortality in the study and all animals appeared healthy. No statistically significant differences in terminal body weight or food consumption (rat chow or potatoes) were detected between the control line and any of the CPB resistant lines. Based on the results of feeding whole CPB resistant potatoes to rats, it was concluded that the seven lines of CPB resistant potatoes were nutritionally equivalent to the Russet Burbank control line (Naylor, 1993).

The results of the above studies with various non-target organisms are consistent with published literature showing that the biological activity of *B.t.t.* protein is highly specific to target insects only (EPA, 1988; EPA, 1991). In addition, as the protein expressed in these plants is identical to that found in nature and in commercial *B.t.t.* formualtions (EPA MRID NO. 42932206), these results confirm that there are no differences in the selective toxicity of the protein expressed in the plant compared to the naturally occurring *B.t.t.* proteins. Therefore, no adverse effects are expected to non-target species from the use of the *B.t.t.* protein as expressed in CPB resistant plant lines BT6, BT10, BT12, BT16, BT17, BT18, and BT23.

Appendix 1 (page 98) contains the results of a multi-state field study conducted in 1992 at three North American locations to evaluate the impact of several insect management regimes on non-target arthropods. The CPB resistant plant lines evaluated comprised, in combination, lines BT6, BT10, BT12, BT16, BT17, BT18, and BT23. These potatoes provided complete control of all Colorado potato beetle life stages at all locations. Beneficial predaceous and parasitic arthropods such as big-eyed bugs, damsel bugs, minute pirate bugs, hymenoptera spp,. and spiders were significantly more abundant in the CPB resistant potato plots than in those treated with conventional

chemical insecticides. This increase in beneficial arthropods resulted in commercially acceptable aphid control in the CPB resistant potato plots solely through predation by those natural enemies.

In this same study data was also collected on the impact of CPB resistant potatoe plants on Collembola spp., a common detritivore of potatoes. These insects feed on decaying plant material, fungi and bacteria, and are important in the decomposition of potato foliage. The results of this study demonstrated Collembola populations to be unaffected by the *B.t.t.* protein expressed by CPB resistant potatoes (Appendix 10, page 316). Additional data demonstrating the susceptibility of earthworms to the *B.t.t.* protein has been requested by the EPA to support our registration of the protein as a plant pesticide. These studies are in progress and will be submitted to the EPA upon their completion.

D. Uncontrolled Movement of CPB Resistant Potatoes into the Environment

1. Pollen Transfer

The potato, *Solanum tuberosum*, is the only *Solanum* species cultivated within the United States. Only two other tuberizing species of *Solanum* have been confirmed to exist; however, both of these species are found in high elevation arid climates, geographically distinct from cultivated potato production areas. Neither of these species can hybridize with *S. tuberosum*. Many other species of *Solanum* exist that are considered weeds in cultivated fields. However, none of these species are closely related and can hybridize with *S. tuberosum*. In addition, Russet Burbank potatoes are male sterile and therefore, do not produce pollen; consequently, outcrossing is not possible (Gill *et. al.*, 1987; McClean and Stevensen, 1952; Pavek, pers. comm.; USDA BBEP, unpubl. report). Appendix 11 (page 323) contains additional supporting documentation on the male sterilty of Russet Burbank variety potatoes from Dr. Joseph Pavek, USDA ARS Potato Research Geneticist and an unpublished USDA BBEP report. The article by Dr. Steven Love, Associate Professor of Potato Variety Development, College of Agriculture, University of Idaho, (Section II, pages 15 through 21 of this document) addresses in more detail the potential for gene escape from CPB resistant potatoes. In this article and in Love and Pavek (1994, Appendix 12, page 327) the conclusion is reached that outcrossing of these Russet Burbank potatoes with other *Solanum* species is not possible.

2. Hybridization With Other Cultivated Varieties

Other than the male sterility of Russet Burbank potatoes, there is no genetic mechanism to prevent the hybridization of Russet Burbank CPB resistant potatoes with other cultivated varieties within the U.S. Pollination of female fertile CPB resistant potato plants would be expected to segregate in a normal Mendelian fashion, as the *B.t.t.* gene is stably integrated into the chromosome of the plant. However, due to production methods, it is unlikely that gene transfer will occur. Potato is bee pollinated, not wind pollinated, and flowers of cultivated potatoes are not attractive to bees because they lack nectar (Appendix 11, page 323). In addition, pollen transfer occurs infrequently and over short distances. Tynan and his coworkers (1990) demonstrated no pollen dispersal in a field interplanted with genetically engineered and control potatoes beyond 4 - 5 meters

and Dale *et al.* (1992) in a similar study, reported no pollen transfer beyond 10 meters. Hybrid seed that does occur is not used for further propagation and will remain in the field. If this seed germinates, long term propagation and survival of the resulting seedlings is not expected due to standard cultivation practices, and in fact has not been documented.

3. Weediness Potential

There is no indication that CPB resistant potatoes are more likely to become a weed than the non-modified parental variety or that they will increase the weediness potential of any other cultivated potato plant or wild species. At the 34 field locations at which the seven CPB resistant potato lines have been evaluated, no difference in the number of volunteers of CPB resistant or control Russet Burbank potatoes has been observed. No difference has also been noted with respect to the germination, disease and insect susceptibility, other than to CPB, of the transformed lines (see Appendices 5 and 6; beginning on pages 152 and 277, repsectively). In addition, the long term survival of volunteers would not be expected due to standard cultivation practices or herbicide application.

To further address potential differences in the ability of CPB resistant and control Russet Burbank potatoes to overwinter and become established as weeds, identical field experiments were established at three sites representing geographically diverse potato production areas (Appendix 13, page 349). Research locations were the Oregon State University-Hermiston Agricultural Research and Extension Center (HAREC), Hermiston, OR; University of Wisconsin-Hancock Research Station, Hancock, WI; and the Cornell University H.C. Thompson Vegetable Research Farm, Freeville, NY. The CPB resistant potatoes used in the study consisted of a mixture of lines BT6, BT10, BT12, BT16, BT17, BT18 and BT23 in Oregon and Wisconsin and line BT23 only, in New York. The field site at all locations was not previously planted to potatoes. Plots of CPB resistant and Russet Burbank potatoes in all locations consisted of single 20' rows, replicated three times and arranged in a randomized complete block design. Whole potato tubers were buried at a depth of 3-6 inches, 20 per plot, to simulate the random placement of tubers following harvest and fall tillage. Potato plots were planted in 1993 on November 19, in Wisconsin, November 29, in Oregon and December 8, in New York. All plots were evaluated over a 6-10 week period in the spring of 1994. Emerging volunteer potatoes were counted and removed from the study area. No volunteer potatoes were found in either Wisconsin or New York, where sub-zero temperatures were reached and snow cover was minimal (Appendix 13, Tables 1 and 2, page 352). Approximately 65% of the planted tubers emerged in Oregon, where temperatures were milder throughout the winter (Appendix 13, Table 3, page 352). Both plant types emerged at the same time, and no significant differences in overwintering survival were detected between Russet Burbank and CPB resistant potatoes. These results confirm that genetically modified CPB resistant potatoes do not have an increased ability to overwinter and become weeds compared to non-modified potatoes.

E. Impact of CPB Resistant Potatoes on Potato Pest Management

The following articles written by Drs. Richard Roush and Jeffrey Wyman of Cornell University and the University of Wisconsin, respectively, describe the impact of CPB resistant potato plants on potato pest management. It is apparent from the data developed by Monsanto and the descriptions by these experts that the superior CPB control offered by CPB resistant potatoes will enable growers to significantly reduce the amount of chemical insecticides now applied to their crop. As a result, growers will be able to utilize a host of IPM practices that cannot be implemented now because of the current dependence on broad-spectrum chemical insecticides to control this pest. An increase in the biological and cultural control of non-target potato pests and a more judicious use of chemical insecticides will result in a positive impact on the environment, which will ultimately be advantageous to the grower and the public.

TRANSGENIC HOST PLANT RESISTANCE AND INSECT MANAGEMENT IN POTATOES

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SUMMARY

Transgenic potato cultivars expressing the *B.t.t.* endotoxin gene would eliminate the need for insecticide applications for potato beetle control, greatly reducing the pesticide load in this crop, resulting in significant financial and environmental benefits, even compared to other non-pesticidal alternatives. The alternative to transgenic potatoes will most likely be continued dependence on insecticides.

Introduction

Potato production and distribution is an important portion of the agricultural economy in the northeastern US, and one of the few sectors that reliably turns a profit. In New York, for example, potato farming on some 30,000 acres generates annual cash receipts of about $60-70 million (New York Agricultural Statistics, Albany New York). Potatoes provide significant employment and cash flow in many rural communities, not just at the level of primary production, but also in processing (e.g., potato chips). The total area planted to potatoes on any given farm is typically less than 150 acres, but requires a considerable investment, usually at least $1500 per acre (Roush and Tingey 1992), stimulating sales for the local agricultural suppliers.

In the eastern US, the most important insect pest of potatoes is the Colorado potato beetle (CPB). If not checked, the CPB appears to be easily capable of population growth rates in excess of 40 fold per generation (in most areas, there are two generations per year), with overwintering survival exceeding 60% (Groden and Casagrande 1986; Harcourt 1971). As a consequence of rapid population growth, complete defoliation of the crop is possible; uncontrolled populations of the CPB can reduce potato yields as much as 85%, especially when sufficient numbers of adult beetles reach the field just as the potato sprouts are emerging from the ground (W. M. Tingey, Cornell University, pers. com.). Because of its severity, the CPB has been subjected to intensive insecticide use, both to control economically damaging densities and as insurance to prevent CPB populations from getting out of control.

This in turn has contributed to some of the worst insecticide resistance problems of any pest in the US; in many areas, there are no currently registered insecticides that will control the adult beetles effectively. Estimated yield losses of 30 percent have been documented from control failures due to insecticide resistance (Tingey, pers. com.). Only two kinds of insecticide mixtures, (1) a pyrethroid synergized with piperonyl butoxide and (2) a mixture of oxamyl and endosulfan, are generally effective in those

areas of New York where classical insecticides still work at all (Goh et al. 1988, Roush et al. 1990). One of these, oxamyl (trade name "Vydate"), has not been labeled for use on Long Island for several years (Tette and Heinmiller 1992) because of its tendency for contamination of groundwater. The failure of new insecticides seems to occur at a rate of about once every 3 years (Forgash 1984, 1985; Georghiou 1986). Thus, it is common for growers to spend more than $200 per acre for insecticides to control the potato beetle. Given the large investment in a potato crop (e.g., $1500 per acre) and the potential gross return (perhaps $3000 per acre), these costs have not significantly inhibited insecticide use (Roush, pers. obs. and conversations with growers).

In addition to the CPB, the potato leafhopper, *Empoasca fabae*, and aphids, including the green peach aphid, *Myzus persicae*, are serious insect pests of potatoes in the northeastern United States. Chemical insecticide applications made for the potato beetle appear to increase problems with aphids, apparently by the destruction of their natural enemies, parasitic Hymenoptera and lady beetles (Roush and Tingey 1993). In New York, the most important beneficial species in potatoes is the lady beetle *Coleomegilla maculata*, a predator of CPB egg masses and aphids (Groden et al. 1990, Hazard et al. 1991). Of the insecticides that can still be occasionally used for control of potato beetle adults, all but endosulfan are very toxic to the lady beetles, especially the pyrethroids (Roush and Tingey 1993). Although potato leafhoppers do not always reach damaging densities, chemical insecticides are currently the only viable option for their control when they do exceed action thresholds. Methamidophos (trade name "Monitor") is the only product consistently effective for most aphid problems. Leafhoppers are susceptible to most insecticides, but methamidophos is again the most effective foliar insecticide (Roush and Tingey 1993).

Integrated Insect Pest Management Program for Potatoes

In New York as in neighboring states, an extensive and detailed pest management program has been developed and is recommended to growers at annual meetings and in extension publications, such as the 1993 Cornell University Pest Management Recommendations for Commercial Vegetable and Potato Production. Each farm tends to adopt those portions of the program that are most applicable to local conditions. Of the 30,000 acres planted in New York during 1992, over 3500 acres were flame-treated, trap crops were used to manage adult potato beetles on ca. 500 acres and 225 acres were protected with trench traps (Tingey, pers. com. from grower survey). In addition, many growers are now utilizing *Bacillus thuringiensis* subsp. *tenebrionis* insecticides (more than 4,713 gallons were used in New York in 1992, Tingey, pers. com.), applied only when action thresholds are reached, all in an integrated effort to manage this pest in an economical, environmentally compatible manner. However, it is unusual when these tactics are sufficient to replace all insecticide use against potato beetles on any given farm. Recent estimates showed that about 43% of the total pesticide load for potatoes in New York state consisted of insecticides for the Colorado potato beetle (Tette and Heinmiller 1992).

Crop rotation. Rotation of potatoes with other non-solanaceous crops, usually oats and field corn, delays colonization by CPB adults, which allows the crop to grow to a less sensitive stage, reduces the total density of overwintered adults that find the crop, and the number of sprays needed (Wright 1984, Lashomb and Ng 1984, Roush et al. 1990).

Unfortunately, it is usually only the larger farms, often with as much as 600 acres of potatoes under production, that can afford to rotate effectively. Given the differences in yield obtained, and lack of similarly valuable alternative crops, it is often especially difficult for a grower to consider rotating to a non-solanaceous crop on muck soils. Rotation can be relied upon to completely eliminate the need for insecticide applications only when potatoes are planted no closer than 1 mile to a field planted in potatoes in the previous year (Tette and Heinmiller 1992).

Propane flaming. Flaming kills adult potato beetles by exposing them to lethal temperatures of about 160°F. Flame treatment is an effective means for controlling overwintered adults after their emergence from soil hibernation in the spring. Repeated flaming, often on a 3-4 day schedule, can provide as much as 90% kill of overwintered adults and can reduce egg hatch by 30% (Moyer et al. 1991). Unfortunately, flaming is not very effective after the plants reach six inches in height. Taller plants are less tolerant to high temperature and the canopy protects adult beetles from exposure to lethal temperatures. Control is often poor when flaming is done early in the morning, late in the evening, on cool cloudy days when adults are congregated at the base of plants or in soil crevices, or when there is more than a 15 mph wind (Moyer et al. 1991). Vacuum machines, although used in Michigan and elsewhere, are considered to be inferior to flaming in New York and are not used. Given the amount of fuel consumed and dust created, it is debatable whether either tactic is more environmentally acceptable than judicious pesticide use. Flaming, in particular, often singes the muck type soils where it is often most needed, raising a great deal of smoke. A large grass fire was started by another flamer (Roush, pers. obs.). Flaming also kills lady beetles (Roush, pers. obs.), as would also be expected for vacuuming. Vacuum machines and flamers also add to soil compaction; they treat only four rows at a time and must be used often (3 to 4 days).

Trench traps. Plastic-lined trench traps, if carefully deployed, are an effective means for controlling adults on farms where beetles hibernate in discrete areas. Adult beetles, which often disperse by walking (especially in the spring when it is still cool), encounter the trap, fall into the trench, are unable to escape across the fine coating of soil particles and dust that cover the plastic, and die of dehydration. Adults that disperse by walking comprise 50 to 75% of the overwintered population depending on air temperature and distance from the hibernation site to the nearest potatoes (Dale Moyer, Suffolk County, Long Island, Cooperative Extension Service, report on videotape). Unfortunately, the trenches can also funnel water, leading to increased problems with erosion. In contrast to relatively flat Long Island where trench traps have been applied, much of the potato production in the upstate region is on grades of more than 3%, and can be subject to severe erosion. It has been difficult to find a good site even to demonstrate trenching in the upstate (Roush, pers. obs.). In flatter areas in the upstate, especially muck soils, large numbers of adult beetles will often overwinter in fields rather than at the field margins (Roush, pers. obs.), limiting the effectiveness of trenching.

Trap plots. Following their emergence from hibernation sites, overwintered adult potato beetles will seek out and colonize the earliest emerging potatoes, especially those nearest where they overwintered. In contiguous one-year rotations, the edges of the current potato field nearest to the previous years' potato field attract high densities of emerging overwintered adults. Growers can take advantage of this phenomenon by

planting trap strips on the edges of current year fields closest to the source of overwintering adults (Roush and Tingey 1992). The effectiveness of trap strip barriers in attracting overwintered adults for control by other tactics (preferably flaming, but insecticides are often used) is limited by the ability to produce acceptable stands in the early season. Not only does this depend on the use of a cultivar with the ability to tolerate cold, wet soils, it also depends on favorable weather to provide field margins sufficiently dry for planting and equipment suitable to get onto wet soils earlier than the main field. As a practical matter, these conditions are difficult to meet (Roush, pers. obs.).

Biological Insecticides. The available biologically produced insecticides include rotenone and *Bacillus thuringiensis* subsp. *tenebrionis* products. Rotenone, which is isolated from a plant, suffers from limited effectiveness against adults, rapid degradation in sunlight, high cost, and poor supply. The most effective of the registered *Bacillus thuringiensis* subsp. *tenebrionis* insecticides, trade named "Novodor" has similar but less stringent limitations as rotenone. These limitations include a narrow window of activity against young larvae (less than 7 days old), failure to control older larvae and adults (Ferro and Gelernter 1989, Zehnder and Gelernter 1989), poor performance during periods of cool temperatures, and very short field persistence. Under ideal conditions, i.e. proper timing of the initial application (when at least 25% of the earliest deposited egg masses hatched) and with multiple applications on a 3 to 7 day schedule during the first larval generation (during June), satisfactory control can be obtained. Unfortunately, if a *Bacillus thuringiensis* subsp. *tenebrionis* insecticide cannot be applied because of rain, or if wet soils or windy conditions prevent the use of application equipment, or if residues are lost to rainfall soon after application, then the critical window of opportunity will escape. In addition, the various life stages overlap considerably in the second generation (end of July and early August). In experiments where this has been tried, the plots had to be rescued with another insecticide (cryolite, an inorganic insecticide) to prevent severe defoliation (Dale Moyer, Suffolk County Cooperative Extension, pers. com.).

Soil insecticides. Given continuing environmental concerns about ground water contamination from soil insecticides, we have investigated the use of soil insecticides in New York. Soil insecticides seem to select very efficiently for resistance in the Colorado potato beetle. We have found that none of the currently registered soil insecticides give any significant control of potato beetles, and have encouraged growers not to use them (but only foliar insecticides as needed) for aphid or leafhopper control (Roush and Tingey 1992, 1993). One of current concerns in resistance management is that some representatives of a new class of insecticides (currently in registration proceedings), the nitromethylene/ nitroguanidine heterocycles, which can be used either as a soil or foliar materials, will be marketed primarily or exclusively as a soil formulation, which would be predicted to select for resistance much more intensively than the foliar formulation.

Classically bred insect-resistant potato varieties. New varieties with classically bred resistance to all three insect pests of potatoes are under development (Tingey 1991), but cannot truly replace existing varieties due to the elaborate breeding effort required in potatoes. Thus, these efforts will not replace insecticides in the short term.

Benefits of Transgenic Potatoes

Transgenic potatoes can solve the most pressing problem in potato pest management for New York and surrounding states, the control of adult potato beetles in the early season, much more effectively than any currently available tactic. Potatoes are most vulnerable to damage when first emerging from the ground, and the adult beetles are generally insensitive to all currently registered pesticides, both because of resistance to classical insecticides and because they are not very sensitive to foliar applications of *Bacillus thuringiensis* subsp. *tenebrionis* (Zehnder and Gelernter 1989). In trials conducted on Long Island in 1992 and in greenhouse experiments, feeding damage by adult beetles on transgenic potatoes was minimal. Over time, adult beetles actually died at a much higher rate than on susceptible plants (Roush, pers. obs.). Thus, in contrast to foliar sprays, deployment of *Bacillus thuringiensis* subsp. *tenebrionis* in the potato plant can control the adult beetles or at least the damage done by them. The only other alternatives available to most growers are propane flaming, trench traps, and classical insecticides, which are of dubious environmental benefits and uneven and often limited effectiveness compared to transgenic plants, as discussed above.

Transgenic cultivars would not require insecticide applications for potato beetle control, significantly reducing the pesticide load in this crop. They would, in particular, not need applications of pyrethroids, which are especially toxic to beneficial species in this system (Roush and Tingey 1993), nor oxamyl (Vydate), an insecticide of significant mammalian toxicity and potential for ground water contamination. Elimination of insecticide-applications for potato beetle control will probably improve the biological control of aphids by predators and parasites, reducing or eliminating in turn the need for applications of methamidophos (tradename "Monitor"), the only insecticide that is generally effective against aphids (Roush and Tingey 1993). Not only is the reduced use of pesticides desirable in its own right, there is increasing concern that resistance will evolve among aphids to methamidophos, as they have to many other insecticides, leaving growers with no other control for aphids when heavy infestations occur (Roush and Tingey 1992, Roush and Tingey 1993). Because leafhoppers are very susceptible to insecticides, a reduction in problems with aphids may also encourage the use of endosulfan when insecticides must be applied for leafhopper control. Currently, because only methamidophos is effective against aphids, a grower with concerns about both aphids and leafhoppers will use it in preference to other products. Endosulfan is much less toxic to lady beetles, which are especially important for the control of aphids (Roush and Tingey 1993).

Not only will a reduction in pesticide use save money for the grower, it will also increase farm safety, minimize the potential for accidental spills, and minimize the expense of safety precautions that must now be taken. While these benefits are difficult to calculate, they are no less significant. Currently, potatoes see extensive use of organophosphate and carbamate insecticides (Tette and Heinmiller 1992), most of which have significant acute dermal and oral toxicities to mammals. With the exception of methamidophos for aphid and perhaps leafhopper control, transgenic potatoes could eliminate the need for nearly all of the organophosphate and carbamate insecticide applications now made.

Any transgenic variety introduced will probably result in greater benefits than might be suggested on a simple proportion of the acreage covered by it, since growers will likely grow the transgenics in those areas with the heaviest beetle problems, reserving non-transgenics for areas with light infestations, directly reducing the need for insecticides.

While growers will continue to use alternative controls like propane flaming and trench traps where feasible, these tactics are often not sufficient to control potato beetles by themselves. Failure to introduce transgenic plants will probably increase the incentive for growers to use any other insecticides that might be registered.

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IMPACTS OF TRANSGENIC HOST PLANT RESISTANCE TO COLORADO POTATO BEETLE ON POTATO CULTURE IN THE UNITED STATES

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In the past decade, the Colorado potato beetle (CPB), *Leptinotarsa decemlineata* (Say), has emerged as the predominant insect pest of potatoes in North America (Casagrande 1987). Both adult and larval stages can severely defoliate potatoes and reduce tuber yields to levels which have become a limiting factor in continuing potato production in some areas (Hare 1980, Ferro et al. 1983, Shields and Wyman 1984). The CPB is a particularly severe pest in eastern and northcentral production areas where control costs frequently exceed $200.00/A and is rapidly increasing in severity in the Northwest (Casagrande 1987, Ferro and Boiteau 1992). Existing management options for CPB which are practical, effective and economical are becoming increasingly limited and to achieve long term control it will be necessary to implement multifaceted management programs which integrate chemical, cultural and biological controls. Host plant resistance will be a key component of such systems.

Limitations of Current Management Alternatives for CPB

Cultural management. - Cultural controls which reduce or delay crop infestation, such as crop rotation or changing the timing of crop culture, can be effective in reducing population pressure but marketing price and land availability frequently prevent growers from using them (Wright 1984, Lashomb and Ng 1984, Voss et al. 1988). Additional off-crop manipulations of CPB ecology and behavior involving trap crops and/or habitat disruption are under investigation (Milner et al. 1992, Wyman et al. 1993), but such tactics will be most effective as components of integrated management programs and are not currently available for grower use.

Biological control. - The CPB is not effectively regulated in commercial fields by natural populations of predators and parasites (Tamaki 1981) and, in consequence, biological control plays a relatively minor role in existing integrated control programs. Enhancement and augmentation of predator populations to achieve acceptable control are possible (Hough-Goldstein and Kiel 1991, Biever and Chauvin 1992), but the need to control other insects in the potato crop with pesticides which disrupt natural control, will probably restrict biological control effectiveness to specific situations where pesticide use is limited.

Physical control. - In areas where intense CPB pressure has curtailed commercial production, physical controls have been employed to reduce otherwise uncontrollable populations. Vacuum suction (Boiteau et al. 1992), propane flaming (Moyer 1992) and polyethylene-lined trenches, are examples of successful approaches but such tactics are extremely labor-intensive, require multiple applications, and are of limited practicality in general potato production.

Conventional insecticidal control. - As a result of the limited effectiveness and/or practicality of non-chemical management approaches for CPB, current commercial control practices are almost exclusively focused on maintaining CPB populations below damaging levels with systemic and foliar insecticides. Systemic carbamate insecticides were initially extremely effective in CPB control but as usage became widespread, environmental, toxicological and resistance problems have restricted further use. Carbamates were detected as a contaminant in groundwater in 1980 (Zaki et al. 1982, Rothschild et al. 1982) and eventually withdrawn from use. Resistance to carbamate systemics (Casagrande 1987, Ioannidis et al. 1991) has also become widespread and has reduced the effectiveness of these compounds. Currently organophosphates which have limited effectiveness against CPB, are the only registered systemic insecticides available to growers for CPB control.

As availability and efficacy of systemic insecticides has declined, growers have been forced to rely increasingly on foliar insecticide use and widespread resistance to all classes of conventional insecticides has developed (Forgash 1981, Johnson and Sandevol 1986, Casagrande 1987, Kennedy and Follet 1990, Heim et al. 1990, Tisler and Zehnder 1990, Ioannidis et al. 1991). The high level of resistance and its wide distribution throughout potato production areas has greatly reduced the effectiveness of therapeutic insecticidal control and, as a result, CPB damage has increased annually. In several eastern and northcentral growing areas, the CPB has become resistant to all registered conventional insecticides and continued production is largely dependent on temporary emergency exemptions for unregistered materials and bacterial insecticides.

Resistance management strategies such as rotation of insecticidal classes across generations (Roush 1989, Tabashnik 1989) in combination with reduced selection pressure through precise targeting of highly susceptible life stages (Feldman 1990) and treatment of aggregated populations (Huang 1990), show promise in delaying the development of resistance, but these tactics are most effective in situations where resistance is at low levels, however, and cannot be applied effectively in areas where resistance is already severe. Future development and release of new insecticides with different modes of action can be expected which will provide effective short term control, but reliance on pesticide use will only provide temporary relief until new resistant strains of CPB are selected.

Biorational insecticides. - Bacterial insecticides derived from *Bacillus thuringiensis* var. *tenebrionis* (*B.t.t.*) became available for grower use in the late 1980's and no field resistance has been reported to date. These materials can be effective under certain conditions in controlling CPB populations which are resistant to conventional insecticides (Zehnder and Gelernter 1989, Ferro and Gelernter 1989, Zehnder et al. 1992) and thus, represent an important alternative to conventional insecticides. Widespread use of *B.t.t.*-based formulations has been restricted, however, by a very short residual life in the field, limited efficacy against the most damaging life stages of the CPB and the high cost of applications, which have reduced the effectiveness of existing formulations. The development of new formulations has improved both efficacy and persistence of *B.t.t.*, but control based on *B.t.t.* programs remains difficult to achieve and expensive in the field. Recent reports have indicated that CPB populations can develop resistance to *B.t.t.* sprays following repeated laboratory exposure (Whalon et al. 1993) and it is probable that extended field use of *B.t.t.* sprays will also result in

resistant field populations. Field resistance to *B. thuringiensis* var. *kurstaki* (*B.t.k.*) in the diamondback moth, *Plutella xylostella* (L) has been well documented (Tabashnik et al. 1990) following extensive use.

Host plant resistance. - Host plant resistance, through conventional plant breeding, which has great potential for the management of insect populations, has been largely unexploited in potatoes. Kennedy et al. (1985) demonstrated good CPB resistance in a wild tomato species. In potatoes there are at least two documented mechanisms for CPB resistance (Dimock and Tingey 1985). Toxic glycoalkaloids confer CPB resistance in wild solanum species, but exploitation in commercial varieties is prevented due to the high correlation between levels of foliar and tuber glycoalkaloid. Glandular trichomes also confer CPB resistance (Casagrande 1982, Dimock and Tingey 1985) and this mechanism and others have been pursued in the development of potato varieties for use in the eastern United States where CPB control is difficult to achieve (Tingey 1993, Plaisted et al. 1992). Despite recent progress toward the release of insect resistant cultivars derived through conventional breeding cultivars suitable for widespread commercial use are not available. It should be noted that all phases of the potato industry in North America including production, storage and processing are designed to utilize the horticultural characteristics of a relatively small number of existing cultivars. The time constraints involved in incorporation of new resistance traits into commercially acceptable cultivars by conventional means will continue to slow the development of such varieties. New technology involving somatic hybridization has great potential in increasing the genetic diversity available to breeders (Helgeson 1989), but commercialization of insect resistant lines with acceptable horticultural characteristics has not yet been accomplished.

Transgenic Host Plant Resistance

The transformation of existing, commercially acceptable potato cultivars using genetic engineering to incorporate specific resistance traits such as the *B.t.t.* endotoxin, will enable the potato industry for the first time to begin to realize the full potential of host plant resistance to insect pests. The *B.t.t.*-transformed potato incorporates the considerable advantages of foliar *B.t.t.* insecticides, i.e. target pest specificity, absence of toxic effects and compatibility with environmental systems, while eliminating the disadvantages associated with short residual life, limited efficacy and the cost of product manufacturing. Field trials in several production areas have demonstrated that transgenic *B.t.t.* resistance is complete (Feldman et al. 1992). All foliage feeding CPB life stages are effectively controlled season long in transformed potatoes, which are horticulturally identical to conventionally produced cultivars. The high level of control conferred by *B.t.t.* expression eliminates the need for additional insecticidal control of CPB and has the potential to benefit the potato industry and the consumer in several ways.

Benefits Associated With Transgenic Resistance

Environmental benefits.

a) Water quality. - The integrity of groundwater resources is of critical concern in potato production since potatoes are frequently grown on irrigated, course textured soils which are highly vulnerable to pesticide leaching. In the 70's and 80's systemic carbamate insecticides were widely used by potato growers because of their effectiveness in CPB control. Extensive contamination of groundwater resulted in several areas (Zaki et al. 1982, Rothschild et al. 1982) and carbamate systemics have since been withdrawn from use and replaced with intensive foliar spray programs. Transgenic CPB resistance would offer growers a selective, long lasting control alternative to systemic insecticides without risk of groundwater contamination.

b) Safety. - CPB control has been achieved for over 80 years through extensive reliance on insecticides. A wide range of chemistry has been used (Casagrande 1987) with arsenicals, chlorinated hydrocarbons, organophosphates, carbamates and synthetic pyrethroids being predominant. In the past decade many of the most extensively used materials have also been relatively toxic and, with the exception of pyrethroids, oral LD50's have been typically below 50 mg/kg. These pesticides are effective and safe when used according to the label, but inherent risks of accidental release or exposure do exist during shipping, storage, mixing and loading, application, and container disposal. A major 1992 revision to the EPA Worker Protection Standard (WPS), for agriculture pesticides (Federal Register 1992), which is designed to reduce the risk of pesticide exposure to agricultural workers, reflects these concerns. The implementation of this standard is expected to result in significant additional costs to growers which will be highest for producers of specialty crops and vegetables (O'brien-Wray 1993). Transgenic resistance would reduce pesticide use in potato production and thus reduce both the risk of accidental exposure and costs associated with WPS compliance.

c) Non-target toxicity. - Conventional insecticides commonly used in CPB management programs are generally broad spectrum in activity. Systemic insecticides are selective against herbivores feeding on the potato crop and are thus relatively target specific. Foliar insecticides on the other hand, whether applied by air or ground, have a high potential to adversely impact non-target species. Such impacts may occur not only in the crop but potentially some distance outside the crop as a result of drift from the target site. Within any cropping system, repeated applications of insecticides have a destructive impact on beneficial arthropod populations (Van den Bosch and Stern 1962), which may be important regulating factors for pest populations. Removal of such bioregulation can lead to rapid pest resurgence in the absence of natural enemies or selection of secondary pests which are normally held under effective control. A rich fauna, which includes significant numbers of predatory and parasitic insects, is associated with potatoes. The use of transgenic resistance to control CPB significantly increased populations of natural enemies in comparison to conventionally treated plots in field trials in Oregon and Wisconsin and resulted in improved biological aphid control (Reed et al. 1992).

Outside of the potato crop, broad spectrum insecticides may also adversely impact non-target invertebrate and vertebrate species such as fish and wildlife. Organophosphate, carbamate and pyrethroid insecticides which are commonly used in CPB control programs may be highly toxic to fish, birds, other wildlife and invertebrates associated with potato ecosystems. For example, granular formulations of carbamate insecticides were withdrawn from use in 1992 as a result of avian toxicity. The Karner Blue butterfly (*Lycaeides melissa* subsp. *samuelis*) is a further example, which has been designated as an endangered species. This butterfly inhabits wild areas in close proximity to potato production areas in Wisconsin and is thus potentially vulnerable to existing CPB control programs. The specific, non-toxic *B.t.t.* endotoxin would significantly reduce non-target toxicity associated with potato production.

Economic benefits

a) Reduction of input costs. - The ability to control CPB without conventional insecticides would result in direct input cost savings to growers equivalent to the cost of current insecticidal control programs. These costs reflect the level of CPB resistance to conventional insecticides and vary between production areas. Estimated 1992 costs for CPB control which were obtained from potato specialists in major production states (Personal Communications 1992) are as follows: Long Island NY - $300-350/A; Atlantic states $100 - 300/A; Maine - $50 - 100/A; Michigan - $100 - 125/A; Wisconsin - $20 - 30/A; Central Minnesota - $50 -150/A; Red River Valley (Minnesota and N. Dakota) - $30/A; Idaho - $30 - 60/A (includes cost of aphid control); Pacific Northwest - $150 - 200/A (includes cost of aphid control); Colorado - $0 - 10/A.

These input reductions would represent a significant portion of overall production costs ($1000 to 1500/A) even after deduction of the added cost for acquiring CPB-resistant seed tubers. The savings realized from reduced cost of compliance with the WPS would be additive.

b) Large vs. small farm benefits. - The economic benefits associated with transgenic resistance are independent of enterprise size and would thus benefit small scale producers equally or to a larger extent than large producers. Such scale neutrality implies that the capital investment required to adopt and use a new technology is directly proportionate to the size of the farm enterprise (Kalter and Tauer 1987, Office of Technology Assessment 1986). The farmer investment in transgenic seed in this case will be directly proportional to the number of acres planted and is consequently truly scale neutral. The investment capital required will reflect only the premium necessary to purchase transformed seed; an amount which will be equal to, or less than that already invested in beetle control by the grower. Further, since control is often more difficult to achieve on small farms, where crop rotation is less feasible, per-acre control costs for small scale growers are frequently higher. The premium placed on transgenic seed will therefore, actually be lower in relation to costs of current controls for small farms than for large. Thus, although the technology of transgenic resistance is truly scale neutral, the benefits are likely to be scale negative and favor small producers.

Additional key features which are required to ensure adoption and use of this technology on small farms are accessibility and availability of the product and the human capital or

expertise required for adoption (Bennett 1982, Volti 1992). The production system for transgenic potatoes will utilize existing certified seed growers located throughout the US which will provide equal access to all farmers large or small. The expertise required to successfully use transgenic resistance is actually considerably less, in terms of human capital and managerial skills, than that currently required for conventional management and would not present any barrier to adoption for small growers.

c) Social Impacts. - Resistance to conventional insecticides in CPB populations has resulted in escalating costs of control which are a threat to continuing production in some areas of the US. Although sociological studies have yet to be conducted to assess the impact of reduced potato production on rural farm communities, these are potentially significant. Potato production is capital and labor intensive. Costs of production are high relative to other available crops and involve considerable investment in machinery and physical plant which directly benefit ancillary agricultural businesses. Labor requirements for production of the crop and processing the raw product also generate local employment opportunities. A decline in potato production could potentially be reflected in adverse impacts on rural communities on several levels. The potential impacts of reduced potato production on local economies could be similar in several ways to the devastation which occurred in the southern US cotton growing regions following invasion of the boll weevil (*Anthonomus grandis grandis*) in the early 1900's. Crop damage of one-third to one-half was sufficient to bankrupt farmers, processors, merchants and bankers in an economy which was entirely dependent on cotton (Loftin 1945). Potato production areas are more diverse and would not be susceptible to such disruption but damage has already surpassed the 50% level in several areas and significant disruption of local economic structures could be expected as farmers switch to less intensive and less profitable alternative crops.

Compatibility of Transgenic Resistance With IPM

a) IPM and the potato industry. - Integrated pest management programs play a vital role in the overall management of the potato crop and the extensive complex of insects, diseases, weeds and other pests associated with it. Commercial IPM programs such as Potato Crop Management (PCM) (Stevenson et al. 1989) have been widely adopted by growers and are an integral part of potato production systems. PCM is currently in use on over 50,000 acres of potatoes in Wisconsin and surrounding states with reported savings exceeding $1,000,000/year and significant environmental benefits (Connell et al. 1991). PCM and other IPM programs are in continual development as technological advances become available. It is essential that new technologies such as transgenic resistance be compatible with existing IPM programs to ensure that management potential, economics and environmental protection are enhanced.

b) Transgenic resistance as a component of IPM. - The use of transgenic resistance to a single pest species would normally be considered a prophylactic approach to pest management since seed must be purchased by the farmer without prior knowledge of the extent of pest infestation. Such prophylactic approaches would be incompatible with the accepted IPM tenet that the least disruptive strategies are selected and employed only when necessary to offset economic loss. In the case of transgenic resistance to CPB, however, the ecology of the pest enables farmers to predict the extent of damage in advance and this approach is thus entirely compatible with IPM theory. The CPB is

highly specialized in association with the potato crop with limited ability for migration and movement. Infestations can be traced to previous potato crops and the extent of damage can be predicted based on the proximity of current season crops to previous crops (Lashomb and Ng 1984). Thus in the case of CPB management, growers already have the ability to select and use a non-disruptive tactic such as transgenic resistance in situations where economic damage can be predicted with a high degree of reliability, avoiding unnecessary prophylactic use.

Further, the need for repeated use of transgenic resistance in succeeding years may decline as a result of the effectiveness of the resistance and growers would be unlikely to continue to expend the premium required to acquire resistant seed when less expensive combinations of chemical and cultural controls would suffice. A rotation of control tactics across years involving one or more years of transgenic resistance alternated with chemical and/or cultural controls based on economic and ecological need, would thus be established which would enhance existing IPM programs and also serve to reduce the likelihood of CPB populations developing resistance to transgenic *B.t.t.*

c) IPM enhancement through increased biological control. - Transgenic *B.t.t.* resistance uses an extremely specific toxin contained wholly within the potato plant which is an ideal tool for incorporation into IPM programs. Since the CPB can now be targeted and controlled season long without the use of broad spectrum insecticides, the potential for conserving and enhancing existing natural enemies and increasing the level of biological control of the CPB and other pests is greatly improved. Biological control, which has previously been extremely difficult to implement in ephemeral ecosystems such as potatoes, will become an achievable goal where transgenic resistance is used to manage CPB. Field trials in Oregon and Wisconsin in 1992 have demonstrated that when CPB-resistant potatoes are used as the primary strategy for CPB management, significant increases in populations of predatory and parasitic insects result (Feldman et al. 1992, Reed et al. 1992).

Such enhancement of natural control agents, which has not previously been possible in the potato system, greatly increases the potential for achieving effective biological regulation of other key pest species such as the green peach aphid, *Myzus persicae* (Sulzer) and the potato aphid, *Macrosiphum euphorbiae* (Thomas) which are important vectors of potato virus diseases. Biological control of aphids has been extremely successful in other systems with the first major success achieved in the early 60's when several parasites were introduced into California to successfully control the spotted alfalfa aphid (*Therioaphis trifolii*) (Van den Bosch et al. 1964). In annual systems,which parallel potato production, *Aphidius sonchi* was successfully introduced into Australia to control *Hyperomyzus lactucae* the primary vector of lettuce necrotic yellows and reduce virus spread (Carver and Woolcock 1986). In the potato system several aphid species serve as vectors of virus diseases with *M. persicae* being of particular importance in the transmission of potato leafroll virus (PLRV) to processing potatoes. Reduction in PLRV spread and associated tuber net necrosis would significantly increase the value of the processing potato crop. Such reduction can only be achieved currently through repeated insecticidal use. Enhanced biological regulation, which would accompany transgenic CPB control, would reduce such insecticidal dependence.

Other key insect pest species such as the potato leafhopper, *Empoasca fabae* (Harris), and a broad range of occasional pest species such as lepidopterous larvae, flea beetles and mites would also be regulated partially by the increased activity of predators and parasites which accompany the use of transgenic resistance for CPB control.

d) Impacts on resistance management in non-target pests. - Biological regulation of aphid species is also a key consideration in insecticide resistance management for aphids on potatoes. The green peach aphid can become highly resistant to insecticides following exposure to as few as 2-3 insecticide applications through increased production of carboxylesterase (Devonshire and Moores 1982). This esterase-based resistance has severely reduced the ability of growers to manage *M. persicae* with insecticides worldwide and increased the potential for virus transmission in potatoes. The elimination of CPB spray programs (which also pre-select resistance in *M. persicae*) and the increase in effectiveness of biological regulation (which can reduce and in some cases may eliminate the need for insecticidal aphid control) are predictable results of the introduction of CPB-resistant potatoes. Both factors will greatly facilitate the management of aphids and aphid resistance in potatoes.

e) Indirect impacts on weed management. - The defoliation caused by the CPB can have important indirect impacts on IPM by disruption of foliar canopy. Foliar canopy is a critical component in integrated weed management programs which rely on limited herbicide use to achieve weed control prior to full canopy and are dependent on light exclusion by the potato canopy for subsequent suppression (Connell et al. 1992). Defoliation by CPB can disrupt the integrity of the canopy and result in mid-late season weed flushes which can adversely affect yield and harvest. Transgenic resistance, which maintains full canopy season long, is perfectly suited for use in integrated weed management programs, whereas conventional insecticide programs, which often allow some defoliation in mid-season, would necessitate greater reliance on herbicide use. An increase in herbicide use would further increase grower cost and risk of groundwater contamination.

Potential for CPB Resistance to Transgenic Potatoes Expressing *B.t.t.*

Resistance to future conventional insecticides. - The CPB has clearly demonstrated the ability to develop resistance to a broad range of insecticidal products with widely different modes of action. It is highly probable that this trend will continue as new insecticidal products with novel modes of action are developed and released by industry. Such products will receive extensive use by growers, particularly in areas where existing materials are no longer effective and resistance to the new materials will develop, notwithstanding the increased emphasis which will be placed on resistance management strategies. Resistance management strategies can be effective in reducing or delaying the onset of resistance (Leeper et al 1986, Tabashnik 1989, Roush 1989), but the level of success is affected by many factors and is ultimately dependent on wide scale, coordinated adoption of practices by growers.

Resistance to foliar *B.t.t.* - Field resistance to insecticidal products based on *B.t.k.* has been reported only infrequently despite many years of use. Resistance has been associated with unusually high exposure rates such as the continual exposure of

lepidopteran pests of stored grain in storage bins (Kinsinger and McGaughey 1979) or the intensive spray programs directed against insects such as diamondback moth which are resistant to conventional insecticides (Tabashnik et al. 1990).

No field resistance to *B.t.t.* has been reported for CPB but laboratory selection has been achieved (Whalon et al. 1993) following repeated exposure. Although laboratory selection for resistance cannot be used to reliably predict field resistance (Stone et al. 1991), it is probable that such resistance will occur in areas where foliar *B.t.t.* use is greatly expanded to replace conventional insecticides which are no longer effective. The variable efficacy of foliar *B.t.t.* which can result from short field persistence, poor plant coverage and failure to control large larvae and adults, is likely to encourage the same multiple application programs which have led to field resistance to *B.t.k.* in the diamondback moth and other lepidopteran pests.

Resistance to transgenic *B.t.t.* - If it is accepted that the CPB can develop field resistance to foliar *B.t.t.*, as seems probable, then it is also possible that the CPB will ultimately develop resistance to *B.t.t.* expressed in transgenic plants which will significantly increase beetle exposure to the *B.t.t.* protein (Gould 1988). The ability of insect herbivores to overcome plant defenses incorporated into commercial cultivars by classical breeding techniques has already been demonstrated with the Hessian fly (Sosa 1981) where resistance was documented less than 10 years after release. Kennedy et al (1985) and Dimock and Tingey (1985) have predicted that the CPB might also overcome resistance in tomatoes and potatoes derived from wild *Solanum* species and Groden and Casagrande (1986) demonstrated such resistance to *S. berthaultii* in as few as 3 generations.

The speed with which CPB might develop resistance to *B.t.t.* in transgenic potatoes cannot be predicted until further field research has been completed. Genetic simulation models and experience gained from conventional insecticidal resistance in CPB suggest, however, that continual exposure to a single gene resistance mechanism such as the *B.t.t.* protein, over an extended period of time will eventually select for resistance (Gould 1988, Roush 1989, Brattsten 1991).

Transgenic *B.t.t.* potatoes are examples of a high dose strategy for delaying resistance, whereby insecticidal mortality is high enough, in principle, to prevent the evolution of resistance. For the high dose strategy to be effective it is necessary to maintain high enough levels of the selecting toxin over extended periods of time to kill all resistant heterozygotes. This would be unlikely with conventional insecticides, which ultimately degrade, but is possible for transgenic *B.t.t.* which is continually produced by the plant and where field trials have shown high levels of expression, season long, which are sufficient to provide 100% control. Even with high expression levels, however, resistance can still occur quickly if only resistant homozygotes survive. It is also necessary for a small proportion of the susceptible homozygotes in the population to either escape exposure or be recruited by immigration to mate with the resistant homozygotes to produce heterozygotes which can be killed (Tabashnik and Croft 1982). Since the effect of the high expression levels of *B.t.t.* in the transformed potato on the ability of the CPB to evolve resistance cannot be predicted on the basis of available information, it is prudent to develop additional strategies which can be implemented to delay or prevent resistance.

Resistance Management Strategies

Preservation of *B.t.t.* susceptible genotypes. - The strategy of interplanting mixtures of resistant and susceptible plant types or otherwise providing a non-selecting crop environment, has considerable potential. By preserving *B.t.t.*-susceptible CPB genotypes in non-transformed refugia which can then mate with resistant homozygotes, if these are selected, to produce heterozygotes which can be controlled, the potential for resistance is considerably reduced (Gould 1988). Genetic simulations of Hessian fly resistance in classically bred resistant wheat cultivars show that interplanting 50% susceptible cultivars with resistant cultivars would preserve unselected genotypes of the fly and greatly extend the time required for resistance development (Gould 1986). This approach would present considerable practical difficulties in the production of resistant and susceptible cultivars with identical horticultural characteristics using conventional breeding. Transgenic resistant potatoes are identical in all respects, except *B.t.t.* expression, to susceptible cultivars, however, and would be ideally suited to this approach. The potential for economic loss through defoliation of the susceptible refugia is of concern, but several strategies for deployment exist, including trap crops and split fields using both conventional and transgenic strategies which would minimize loss to growers. The potential also exists for wild *Solanum* species which are present in most commercial potato fields as weeds and can serve as alternate CPB hosts, to act as natural refugia and preserve susceptible CPB genotypes.

Mixtures or alternations of toxins. - Other practical approaches to delaying or preventing the development of CPB resistance to transgenic *B.t.t.* are the utilization of resistance management strategies such as mixtures or alternations of toxicants with different modes of action which have been effective for conventional insecticides (Roush 1989).

The introduction of multiple CPB active proteins into the *B.t.t.* potato through genetic engineering is a long term solution to achieve resistance immunity which should continue to be pursued (Brattsten 1991). In the short term, however, the mixture of insecticidal proteins which would be necessary to achieve overall insect control, even in a transformed CPB resistant crop, would achieve the same end. Potatoes are attacked by a complex of pests and while transgenic *B.t.t.* will control the CPB, it will also be necessary to utilize conventional controls, often insecticides, to manage other pests in the system (e.g. aphids, leafhoppers). The application of such controls would expose non-target CPB to different proteins which have no cross resistance to *B.t.t.* and remove *B.t.t.*-resistant CPB homozygotes from the population. The effects in delaying resistance to *B.t.t.* in transgenic plants would be similar to the use of mixtures of conventional pesticides. A similar strategy has been successful in combining genetic plant resistance and chemicals (Smith 1989).

Additionally it is extremely unlikely that all growers will adopt the transgenic plant technology simultaneously and thus a mosaic of resistant and susceptible potato crops will in effect be created in commercial production areas ensuring the preservation of *B.t.t.* susceptible CPB genotypes and reducing the likelihood of resistance by exposure of populations to multiple toxins across space and time.

The rotation of transgenic resistance across growing seasons with chemical and/or cultural controls based on the intensity of CPB pressure has been discussed (pp 32). This tactic which would be promoted by economic considerations would also serve to reduce the potential for resistance to transgenic *B.t.t.* in the same manner as rotations or alternations of conventional insecticides. Any *B.t.t.*-resistant homozygotes selected during the years when transgenic potatoes were used would be eliminated by the combination of conventional insecticidal toxins with no cross resistance to *B.t.t.* and cultural controls.

Conclusions

1. Long term control of CPB cannot continue to be achieved using existing or future insecticidal products as a result of the demonstrated ability of CPB to evolve resistance to a wide range of insecticides.

2. *B.t.t.* foliar sprays are an alternative to conventional insecticides but several practical considerations limit the potential for widespread use. Concerns also exist over the potential for resistance development to foliar *B.t.t.* in CPB if wide scale use were achievable.

3. Alternative CPB management strategies, such as cultural, biological or physical controls, lack either the effectiveness or practicality to act as stand alone strategies suitable for wide scale adoption and are best utilized in concert with other approaches in integrated programs

4. Host plant resistance should be incorporated as a primary component of future integrated CPB management programs. CPB resistant cultivars developed by conventional breeding approaches which meet the needs of the U. S. potato industry are not currently available. Transgenic CPB resistance based on *B.t.t.* expression is a potent alternative which is practical, cost effective, suitable for all phases of the potato industry and compatible with existing IPM programs.

5. Benefits associated with transgenic CPB resistance would include:
   - Increased safety to field workers and consumers
   - Reduced toxicity to non-target species.
   - Reduced environmental hazards and improved water quality
   - Increased populations of beneficial insects in potato fields, which would enhance biological regulation of other pests such as aphids and enhance resistance management for those species through reduction of non-target selection.

6. Transgenic *B.t.t.* resistant potatoes would generate direct economic returns to growers by eliminating the current costs of conventional CPB controls and allowing continued production in areas where CPB control is now difficult to achieve. Economic benefits would be scale neutral and benefit small and large producers equally.

7. Concerns over the potential development of CPB resistance to transgenic *B.t.t.* can be addressed effectively using practical resistance management strategies, which will preserve susceptible genotypes and expose CPB populations to multiple control mechanisms through insecticide mixtures or alternations.

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F. Development of Pest and Resistance Management Strategies for CPB Resistant Potatoes

To achieve the numerous benefits, previously discussed, it is important that CPB resistant potatoes be implemented and managed properly. In this respect, these plants are no different than any other crop protection product that has been used over the last century. It is clear from the knowledge gained over that time, that to successfully maximize the long-term use of these potatoes, two interconnected management components are required. First, is the development of integrated pest management techniques that allow the farmer to optimize the utility of these plants for potato pest control. In essence, this is the development of a total insect management package that will be centered around CPB resistant potatoes. Second, to maximize the durability of these potatoes, is the development and implementation of strategies targeted to prevent the development of insect resistance to the *B.t.t.* protein produced by the plants.

For the last several years, extensive consultations have been held with the leading potato pest and resistance management researchers to develop a program to maximize the use and durability of CPB resistant potatoes. Laboratory and field studies designed in collaboration with these experts from academia and extension services are in progress and are providing the data needed to develop this management program. These studies are examining the impact of CPB resistant potatoes on populations of beneficial and pest insects endemic to the crop, the impact on the use of conventional insecticides for controlling non-target pests, the establishment of the baseline susceptibility of our insect targets to *B.t.t.*, and the impact of mixtures of resistant and non-resistant plants on yield loss.

Monsanto scientists have worked for several years on laboratory and field studies of insect resistance, and with outside collaborators nearly every suggestion made for resistance management in CPB resistant potatoes is being examined. These strategies, developed in consultation with an expert advisory panel, take into account existing research and an understanding of potato production and agronomic practices. They include:

1) High dose expression of the *B.t.t.* protein in potatoes to control CPB heterozygous for resistance alleles.

2) Refugia as hosts for sensitive insects provided through non-CPB resistant potatoes.

3) Monitoring of insect populations for susceptibility to the *B.t.t.* protein.

4) Agronomic practices that minimize insect exposure to the *B.t.t.* protein.

5) Development of novel CPB control proteins with a distinct mode of action from the *B.t.t.* protein.

Those pest and resistance management strategies best suited for use in potato production and with the potential for delaying or preventing the development of resistance will be recommended. In addition, a program is in development to educate potato growers to the most effective ways to integrate these potatoes within their current production practices. This cooperative effort between growers, academia, extension services and Monsanto will help ensure that the benefits of CPB resistant potatoes are fully realized and sustained. Monsanto's recommended approaches for the management of insect pests with insect resistant plants are described in Appendix 9, page 305.

Table VI.1 Responses of Ten Insect Species to Ingestion of *B.t.t.* Protein.
Assays were performed at a concentration of 50 µg/ml in test diet.[1]

Species	Reps	Insects Treated, No./Rep	% Survivor (Number of Insects) *B.t.t.* Protein	% Survivor (Number of Insects) Control	Physiological Effects on *B.t.t.* Protein Treated Insects
Anthonomus grandis	3	24	90.3 (65)	90.3 (65)	none
Diabrotica undecimpunctata	3	24	94.4 (68)	93.1 (67)	none
Leptinotarsa decemlineata	3	24	0.0 (68)	93.1 (67)	all larvae dead
Ostrinia nubilalis	3	24	87.5 (63)	97.2 (70)	none
Ostrinia nubilalis	3[2]	24	97.2 (70)	97.2 (70)	none
Manduca sexta	3	24	97.2 (70)	95.8 (69)	none
Helicoverpa zea	3	24	98.6 (71)	97.2 (70)	none
Heliothis virescens	3	24	94.4 (68)	94.4 (68)	none
Aedes aegypti	5	10	100.0 (50)	100.0 (50)	none
Blatella germanica	2	15	93.3 (28)	96.7 (29)	none
Myzus persicae	16	12	83.3 (150)	100.0 (192)	honeydew reduction[3]
Myzus persicae	16[4]	12	69.8 (134)	76.6 (147)	honeydew reduction[3]

1. Insect assay methods employed are summarized in Appendix 15, p. 368.
2. Test repeated due to a significant treatment related effect resulting in the first assay. Survivorship of treatment and control *O. nubilalis* in the second assay was identical. Analysis of the combined data set (6 reps, 2 assay dates) indicated that neither the day-related variation ($F = 4.55$, $df = 2$, $p > 0.06$) nor the treatment related variation ($F = 4.55$, $df = 2$, $p > 0.06$) was significant.
3. Slight reduction in honeydew production. A rating scale of 0 to 3 was used to estimate relative levels of *M. persicae* feeding based on honeydew reduction. 0 = no honeydew reduction (aphid feeding apparently normal), 1 = slight reduction, 2 = moderate reduction and 3 = no honeydew production (little or no aphid feeding). Using this scale, the mean ratings for bioassay 1 were: *B.t.t.* protein = 0.40, control = 0.19, and the ratings for bioassay 2 were: *B.t.t.* protein = 0.56, control = 0.06.
4. Test repeated due to a significant treatment related effect resulting in the first assay. Two-way analysis of variance was performed on the combined data set. Analysis of the combined data set from both assays (31 reps *B.t.t.* protein, 32 reps control, 2 assay dates) indicates that the day-related variation ($F = 10.57$, $p > 0.002$) explains much of the difference between the treatment and control groups.

Table VI.2 Sensitivity of Selected Beneficial Insects to the *B.t.t.* Protein.
Assays were performed using a concentration at least 100 times the estimated LC_{50} (LC_{50} = ca. 1.0ppm) of *B.t.t.* protein in the diet of Colorado potato beetle.[1]

Taxonomic Order	Species	Reps.	Insects Treated, No./Rep	% Mortality *Btt* Protein	% Mortality Control	LC_{50} (ppm)
Coleoptera	*Leptinotarsa decemlineata*	3	16	81	03	*ca.* 1.0[2]
Coleoptera	*Hippodamia convergens*	6	25	29	22	>100[3]
Hymenoptera	*Nasonia vitripennis*	2	25	28	30	>100[4]
Hymenoptera	*Apis mellifera* (larvae)	4	50	14	18	>100[5]
Hymenoptera	*Apis mellifera* (adults)	3	39-64	25	30	>100[6]
Neuroptera	*Chrysopa carnea*	1	30	10	23	>100[7]

1. Insect assay methods employed are summarized in Appendix 15, p. 368.
2. The activity of *B.t.t.* protein was assessed by incorporation of the test material in an artificial diet at five concentrations which ranged from 9.0 to 0.11 µg *B.t.t.* protein per gram diet. The assay employed first instart larvae. The reported values represent the percent mortatlity at 1.0 µg *B.t.t.* protein per gram diet
3. Insects were exposed to one maximum test concentration and observed daily for mortality and signs of toxicity. The reported percent mortality represent the average cummulative number of dead insects per number exposed during a ten day assay period.
4. Insects were exposed to one maximum test concentration and observed daily for mortality and signs of toxicity. The reported percent mortality represent the average cummulative number of dead insects per number exposed during a nine day assay period.
5. Larvae were exposed to one maximum dose of test substance by placing 5 µl of an aqueous solution of the test substatnce into each larval cell. Reported values represent the mean larval mortality from 1st - 2nd instar stage through adult emergence.
6. Tests were conducted using paper carton cages containing approximately 40 bees per replicate. Adults were exposed to test or control substances by adding the material to a 50:50 mixture of honey:water to achieve on a maximum concentration. The test material was introduced through the cage bottom using a glass vial and a cotton wick. Adults were observed daily for mortality and signs of toxicity until the control group mortality exceeded 20% . The percent mortality are the mean cumulative mortality during the three days assay period.
7. Larvae were exposed to one maximum test concentration and then observed daily for mortality and signs of toxicity. The percent mortality are the average cummulative number of dead insects per number exposed during a nine day assay period.

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VII. STATEMENT OF GROUNDS UNFAVORABLE

We know of no unfavorable grounds associated with CPB resistant potato lines BT6, BT10, BT12, BT16, BT17, BT18, and BT23, developed using the plasmid vector, PV-STBT02. Therefore, on the basis of the substantial potential benefits to the grower, the environment, and the significantly lower potential risk to public health, Monsanto Company requests that these potato lines no longer be regulated under 7 CFR part 340.6.

APPENDIX 1

GENETICALLY MODIFIED COLORADO POTATO BEETLE RESISTANT POTATO PLANTS, FOLIAR-APPLIED MICROBIAL *B.t.t.*, AND CONVENTIONAL INSECTICIDES: COMPARATIVE IMPACTS ON NON-TARGET ARTHROPODS

Genetically Modified Colorado Potato Beetle Resistant Potato Plants, Foliar-applied Microbial *B.t.t.*, and Conventional Insecticides: Comparative Impacts on Non-target Arthropods

by

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ABSTRACT

Replicated large plot studies were conducted in 1992 at three North American locations to evaluate the impact of several insect management regimes on non-target arthropods. Genetically modified Colorado potato beetle resistant potatoes containing an insecticidal protein from *Bacillus thuringiensis* subsp. *tenebrionis* provided complete control of all Colorado potato beetle life stages at all locations. Beneficial arthropods were significantly more abundant in genetically modified Colorado potato beetle resistant potato plots than in those treated with conventional chemical insecticides. Commercially acceptable aphid control was achieved in these plots solely through predation by natural enemies. Colorado potato beetle resistant potatoes represent an effective and environmentally compatible addition to the existing methods of managing potato insect pests.

INTRODUCTION

The Colorado potato beetle, *Leptinotarsa decemlineata* (Say), is the most destructive foliar pest of potatoes in North America. Insecticide resistance has made chemical control of the Colorado potato beetle increasingly difficult in recent years (Forgash 1985). In the east and midwestern U.S., where resistance is most troublesome, insecticide control of Colorado potato beetle costs upward of $200 to $300 per acre (Wyman, unpubl. data). Insecticide resistance is now recognized as a serious threat in all potato producing areas, and alternative management strategies which utilize a combination of control factors for the Colorado potato beetle are needed.

Bacillus thuringiensis is a common soil-borne bacterium (Martin and Travers 1989). In its spore forming stage, *B. thuringiensis* produces an insecticidal protein which is non-toxic to humans, other mammals, or beneficial organisms (EPA 1988). *B. thuringiensis* subsp. *tenebrionis* (*B.t.t.*) possesses specific activity against coleopteran insects (MacIntosh, et al. 1990), and has been widely adopted by organic producers for control of the Colorado potato beetle (OCIA Materials List). Despite its safety and environmental advantages, its use in conventional potato pest management programs has been limited due to high costs and the rather unpredictable results achieved with foliar-applied microbial sprays. Although microbial *B.t.t.* formulations

can be highly effective when properly applied, their success may be reduced by a number of factors including poor spray timing, inadequate coverage, and inclement weather (Ferro and Lyon 1991).

Plant expression of *B.t.t.* protein through genetic modification represents an alternative delivery system through which researchers have achieved complete control of the Colorado potato beetle (Perlak et al. 1993). This technology will facilitate a shift toward more biologically-based pest management programs and will allow growers to reduce pesticide inputs without suffering crop losses from the Colorado potato beetle. As reliance on broad spectrum insecticides diminishes, populations of beneficial non-target organisms such as predators and parasites are expected to increase in the cropping system. These natural enemies will contribute to the control of Colorado potato beetles and other potato pests including aphids, leafhoppers, and plant bugs.

The following data from studies conducted in 1992 at three North American locations represents preliminary results in a multi-year research project investigating the effect of several Colorado potato beetle control strategies on non-target pest and beneficial arthropods. The experimental insect control practices included conventional systemic or foliar insecticides, foliar-applied microbial *B.t.t.*, and genetically modified Colorado potato beetle resistant plants. The long term goal of this continuing research is to develop crop recommendations which incorporate Colorado potato beetle resistant plants and other selective insect controls for the integrated management of potato insect pests.

MATERIALS & METHODS

Research was conducted at three locations representing different potato production regions and their respective pest/beneficial complexes. The research sites included the Oregon State University-Hermiston Agricultural Research & Extension Center, (northcentral Oregon), the University of Wisconsin-Hancock Experiment Station (central Wisconsin), and the Agriculture Canada Charlottetown Experiment Station (Prince Edward Island). With the exception of insect control treatment factors, which were specific to each trial location, all crop production procedures reflected those used locally with conventional fertilizer, herbicide, and fungicide applications. The experimental treatment regimes were designed to compare conventional, broad-spectrum chemical controls with narrow-spectrum, selective alternatives.

Plot Design

In all trials, individual plots consisted of sixteen 54 ft rows, which were bordered on all sides by 6 feet of unprotected Russet Burbank buffer. Potato rows were 34 to 36 inches apart with tuber seed placed 9 to 14 inches apart in the row. Pest and beneficial arthropod populations were visually sampled once each week from an 18 ft by 6 row area in the center of each plot, designated the experimental unit. An unplanted single-row alleyway was maintained around this area to facilitate worker mobility. All sampling that required disruption of the foliage was performed in the potato rows immediately adjacent to, and outside of, the experimental unit.

Sampling Methods

The following methods were used in some or all locations to measure the densities of all foliar and soil-surface dwelling arthropods and their impact on the cropping system:

Beat cloth samples were taken by placing a 27 to 30 inch square cloth under the canopy and beating the foliage with a stick to dislodge resident insects. The primary insect pests that were sampled using this technique were aphids (family: Aphidae). Beneficial arthropods sampled in this manner included damsel bugs (family: Nabidae), big-eyed bugs (family: Geocoridae), lady beetles (family: Coccinellidae), green lacewings (family: Chrysopidae), brown lacewings (family: Hemerobiidae), minute pirate bugs (family: Anthocoridae), flower flies (family: Syrphidae), stink bugs (family: Pentatomidae), and spiders.

Sweep samples were taken by passing the 15 inch diameter sweep net through the canopy 25 times. This method was used to sample adult potato leafhoppers (*Empoasca fabae* (Harris)).

Visual plant counts were taken on the perimeter of the experimental unit. Potato flea beetle (*Epitrix cucumeris* (Harris)) adults, Colorado potato beetle adults, small larvae (instars 1 and 2), large larvae (instars 3 and 4), and egg masses were counted on whole plants.

Leaf samples were taken to evaluate populations of aphids and potato leafhopper nymphs, and damage (feeding holes) from adult flea beetles.

D-Vac samples were taken from foliage by moving the vacuum head through the canopy for 60 seconds/plot. This method was used to sample all foliage-dwelling insects.

Pitfall traps constructed from 16 oz plastic beer cups were placed in two locations/plot in the potato rows immediately adjacent to the experimental unit. Pitfall traps were used to estimate population densities of ground beetles (family: Carabidae), rove beetles (family: Staphylinidae), and other soil-dwelling fauna.

Yellow and green water pan traps were placed in each plot to monitor alate aphid populations throughout the season.

Defoliation estimates were made weekly to evaluate Colorado potato beetle damage.

Tubers were visually examined at harvest for wireworm damage.

All data for individual insects were subjected to multiple analyses of variance for each sampling date, and for seasonal population averages.

Regional Pest Problems and Conventional Practices

Oregon:

The most damaging pests of potatoes in Oregon are green peach aphids (*Myzus persicae* (Sulzer)), potato aphids (*Macrosiphum euphorbiae* (Thomas)), Colorado potato beetles, and wireworms. In northcentral Oregon, many growers utilize soil-applied systemic insecticides early in the season, followed by soil or foliar-applied insecticides when efficacy begins to decline (Pacific Northwest Insect Control Handbook 1992).

Green peach aphids, which are efficient vectors of potato leafroll virus (Klostermeyer 1953), are the most serious aphid pest of commercial (non-seed) potatoes. Green peach aphids overwinter on peach and other *Prunus* species in the Columbia basin. Winged adults typically infest potato fields in early spring as the weather warms (Tamaki and Olsen 1979). If these aphids have previously fed on virus-infected volunteer potatoes or other non-crop hosts, they may create points of infection within the potato field. During the season, potato leafroll virus may spread as wingless aphid populations build.

The majority of insecticide applications in the northwestern U.S. are targeted at aphids. While the Colorado potato beetle can be a serious pest, control is usually achieved as a result of aphid management. Most available insecticides provide good control of potato beetle larvae and adults since insecticide resistance has not yet been detected in this region.

Wireworms can be a severe problem in infested soils. In fields that are not fumigated prior to planting, non-systemic soil insecticides are applied to protect tubers from attack.

Wisconsin:

The primary pests of potatoes are Colorado potato beetles, potato leafhoppers, and green peach and potato aphids. Both Colorado potato beetles and potato leafhoppers can cause crop devastation if not controlled in a timely fashion, while aphids are primarily a concern due to their ability to vector potato leafroll virus. Potato flea beetles are sporadic pests which rarely require targeted insecticide applications.

Potato leafhoppers migrate into Wisconsin each spring on southerly winds and typically build to damaging levels over a very short period. As a result, they do not lend themselves to biological control since most beneficial insects have not become established in the cropping system so early in the season. Potato leafhoppers are susceptible to a variety of insecticides and can be controlled by timing applications to established economic thresholds.

Since green peach and potato aphids do not overwinter in Wisconsin, infestations usually does not occur until mid-summer. A range of 10 to 40 green peach aphids per 50 leaves is employed as a general treatment threshold for processing potatoes where potato leafroll virus is of concern, while a threshold of 5 green peach aphids per 50

leaves is recommended for seed potato production.

In the last ten years, following detection of pesticide residues in the ground water (Rothschild et al. 1982), growers in Wisconsin have shifted away from a reliance on soil-applied systemic insecticides. Instead, insect control is achieved with well timed applications of broad spectrum foliar insecticides (Chemical Recommendations for Commercial Potato Production, 1992). Insecticides specifically targeted at aphids are frequently required in mid to late season.

Prince Edward Island (PEI):

The primary insect pests in PEI which require control annually are Colorado potato beetles, potato flea beetles, and green peach and potato aphids. Insect control is commonly achieved with soil-applied systemic insecticides early in the season, followed by foliar-applied insecticides when systemics are no longer effective. Since most potatoes produced in PEI are grown as potential seed potatoes, aphid treatment thresholds are low in order to limit infection from all vectored viruses. Aphids do not overwinter in Canada, so infestation usually does not occur until mid-summer.

Because of the relatively short growing season experienced in eastern Canada, Colorado potato beetles are usually limited to one generation per year. Controls are targeted primarily at the larvae, although the adult progeny of these larvae can be a threat in some years. Insecticide resistance is a growing problem in Maine and northeastern Canada, but chemical control is still achieved without difficulty in PEI.

Colorado potato beetles and potato flea beetles are taxonomically related (family: Chrysomelidae), and share a similar life history. Flea beetle adults overwinter in the soil, larvae complete one discrete generation per year, and all stages of the insect feed on the same host plant species. Potato flea beetle larvae, which feed on potato roots, are effectively controlled with soil-applied systemic insecticides. The adult is the most damaging stage, however, as it makes numerous small holes in the foliage. Control of larvae early in the season does not always eliminate the need for late-season insecticides to control potato flea beetle adults.

Site Specific Treatments

<u>Oregon</u>

1. Russet Burbank potatoes with foliar Colorado potato beetle control.

   Permethrin (Pounce® 3.2 EC, .2 lbs ai/A) was applied every two weeks beginning June 23, with five applications total.

2. Russet Burbank potatoes with systemic insecticides for Colorado potato beetle and aphid control.

   Phorate (Thimet® 15G, 2.17 lbs ai/A) was applied on June 6, followed by foliar application of disulfoton (Di-Syston-8®, 3.36 lbs ai/A) on July 9 when aphid and Colorado potato beetle control began to decline.

3. Genetically modified Colorado potato beetle resistant Russet Burbank potatoes with systemic insecticides for aphid control.

   Phorate (Thimet® 15G, 2.17 lbs ai/A) was applied on June 6, followed by foliar application of disulfoton (Di-Syston-8®, 3.36 lbs ai/A) on July 9 when aphid control began to decline.

4. Genetically modified Colorado potato beetle resistant Russet Burbank potatoes with no additional insect control.

5. Russet Burbank potatoes with foliar-applied microbial *B.t.t.* treatment for Colorado potato beetle control.

   Microbial *B.t.t.* (M-Trak®, 0.75 qt/A) was applied weekly beginning June 23, with a total of nine applications.

6. Russet Burbank potatoes with no insect control.

All treatments were replicated six times and arranged in a Latin Square design.

Wisconsin

1. Russet Burbank potatoes with conventional foliar control of Colorado potato beetle and other pests.

   Esfenvalerate (Asana® 1.9 EC, 0.05 lbs ai/A) was applied on June 30 for control of first generation Colorado potato beetle larvae. Endosulfan (Thiodan® 50 WP, 1.0 lbs ai/A) was applied on July 15 for control of Colorado potato beetle summer adults, and methamidophos (Monitor 4® 0.75 lbs ai/A) was applied for potato leafhopper and aphid control on August 24.

2. Genetically modified Colorado potato beetle resistant Russet Burbank potatoes with selective potato leafhopper control.

   Malathion was applied at half the recommended rate (Malathion® 0.45 lbs ai/A) on July 2 when potato leafhoppers exceeded the treatment threshold of two per sweep. A second application of malathion was made on August 24.

3. Russet Burbank potatoes with foliar applied microbial *B.t.t.* and selective potato leafhopper control.

   Microbial *B.t.t.* (M Trak®, 0.75 qt/A) was applied on June 25 and July 15 for Colorado potato beetle larval control. Malathion was applied at half the recommended rate (Malathion® 0.45 lbs ai/A) on July 2 when potato leafhoppers exceeded the treatment threshold of two per sweep. As microbial *B.t.t.* has little effect on Colorado potato beetle adults, esfenvalerate (Asana® 1.9 EC, 0.05 lbs ai/A) was applied on August 24 to control potato leafhoppers and to rescue the plots from defoliation due to the Colorado potato beetle.

4. Russet Burbank potatoes with no Colorado potato beetle control and selective potato leafhopper control (designated "untreated control").

   Malathion (Malathion® 0.45 lbs ai/A) was applied at half the recommended rate on July 2 when potato leafhoppers exceeded the treatment threshold of two per sweep. A second application of malathion was made on August 24.

All treatments were replicated four times and arranged in a Latin Square design.

PEI

1. Russet Burbank potatoes with systemic insecticide for Colorado potato beetle, potato flea beetle, and aphid control.

   Phorate (Thimet® 15G, 2.17 lbs ai/A) was applied at planting.

2. Genetically modified Colorado potato beetle resistant Russet Burbank potatoes with systemic insecticide for potato flea beetle and aphid control.

   Phorate (Thimet® 15G, 2.17 lbs ai/A) was applied at planting.

3. Genetically modified Colorado potato beetle resistant Russet Burbank potatoes with no additional insect control.

4, 5, and 6. Russet Burbank potatoes with no insect control.

All treatments were replicated four times and arranged in a randomized complete block design.

RESULTS

Oregon:

Colorado potato beetle:

Genetically modified potatoes, microbial *B.t.t.*, permethrin, and the systemic insecticides all provided commercially acceptable control of Colorado potato beetles. Defoliation reached 100 percent in the untreated controls on July 17, while all other treatments incurred less than 10 percent defoliation during this time period. Widespread infection of late blight (*Phytophthora infestans*) made damage from Colorado potato beetles difficult to measure after mid-July, and defoliation estimates were discontinued as a result.

Genetically modified potatoes had fewer adults and larvae than did any other insecticide treatment (Fig. 1a, b), and egg laying was significantly reduced relative to all treatments (Fig. 1c). Weekly applications of microbial *B.t.t.* provided good Colorado

potato beetle control, and the seasonal average of larvae was lower than that in systemic insecticide-treated plots. However, while no larvae survived past the first instar in genetically modified potatoes, some late instar larvae were found in microbial *B.t.t.* plots.

Green peach aphid:

Alate green peach aphids were first detected in the Hermiston area on May 28 in yellow water pan traps. Alate aphids moved into the plots as the plants began to emerge on June 19 but did not increase substantially until after June 29, when a larger flight deposited alate aphids in large numbers throughout the field. Apterous progeny from these aphids began to build up in the plots after this point, whereupon treatment differences became evident.

Apterous aphid populations were lowest in systemic treated plots, but remained at commercially acceptable levels all season in every treatment except permethrin (Fig. 2a). In late-July to mid-August, apterous aphid populations in permethrin plots exhibited exponential-like growth, approaching 4000 per beat sample on August 13. Permethrin provided excellent control of Colorado potato beetles but was ineffective against aphids. Although a viable management option for Colorado potato beetle control, permethrin is toxic to a broad range of beneficial predators and parasites. The type of aphid population increase observed in permethrin-treated plots in this study resulted from the elimination of natural enemies as a regulating influence. This same "population bloom" did not occur in plots which received no supplemental insecticides for aphid control. Apterous green peach aphid populations increased to only 35 per beat sample in the genetically modified potato plots during this same period.

The late season population of alate aphids reflected apterous population trends, increasing significantly in permethrin-treated plots in late August (Fig. 2b). Alate populations remained below treatment threshold levels in all other treatments, where no significant differences were detected. Aphids typically mature into winged forms as a mechanism of dispersal in response to overcrowding, as was observed in permethrin plots. Since alates are capable of acquiring and transporting viruses to new locations, they may be responsible for initiating new disease outbreaks in neighboring fields. Persistent viruses such as potato leafroll virus are commonly spread between fields in this manner.

Wireworms:

Substantial wireworm populations were found in all plots. Infestation ranged from 67% of tubers in genetically modified potato plots with no insecticides, to 80% of tubers in systemic insecticide-treated potatoes. No significant differences in incidence or extent of tuber damage were detected between any treatment regimes.

Beneficial arthropod complex:

The primary predators found in the plots were generalist hemipterans and spiders. Big-eyed bugs, spiders, damsel bugs, and minute pirate bugs comprised over 97% of the predators observed in 1992, while lady beetles, brown lacewings, flower flies, and

stink bugs were present at a much lower frequency. Predacious arthropods were most abundant in genetically modified potato and microbial *B.t.t.* plots (Fig. 3). Broad spectrum insecticides were not applied in these treatments, and resident plant and detritus-feeding insect populations were high enough to maintain predator population growth.

Big eyed bugs:

Big eyed bugs were the most common predators found in the plots. Adult populations were highest during late June, giving rise to a nymphal population that increased after mid-July. Nymphs were significantly more abundant in genetically modified potato and microbial *B.t.t.* plots from July 20 to August 24 (Fig. 4 and 5). Adult populations, though greater in these treatments, were not significantly different.

Systemic insecticides generally have less impact on beneficials than do foliar insecticides, such as permethrin, because exposure is limited to those insects feeding on the plants. However, big eyed bug nymphs feed on sap during the first two instars which may account for the lower numbers observed in plots treated with phorate and disulfoton.

Spiders:

Spiders were prevalent in the experimental plots and appeared to be an important element of the natural enemy complex. Genetically modified potato and microbial *B.t.t.* plots had significantly more spiders than all other treatments from July 13 to August 3, and had more than permethrin-treated plots all season (Fig. 6).

Spider populations dropped significantly in the systemic and genetically modified potato/systemic plots after foliar application of disulfoton on July 9. Reentry of spiders into disulfoton-treated plots occurred as the material decreased in activity and pests reinfested the plots. Permethrin appeared to be toxic to spiders, as stable populations never developed in these plots despite the high pest (aphid) populations.

Damsel bugs:

Damsel bugs were most numerous in the genetically modified potato and microbial *B.t.t.* plots (Fig. 7). However, significant differences between these and the systemic treated plots only occurred for a three week period following application of disulfoton on July 9. Permethrin was detrimental to damsel bug populations, and treated plots had very few nymphs season-long.

Minute pirate bugs:

Adult minute pirate bugs, which are highly mobile, were most abundant in permethrin plots (Fig. 8) as they continually reinfested in response to large prey (aphid) populations. However, their numbers dropped sharply following each permethrin application, and very few nymphs were observed in these plots during the season. Minute pirate bug populations in general were variable throughout the season, and no significant differences between treatments were detected.

Summary:

A commercially acceptable level of Colorado potato beetle control was obtained in all plots with experimental insect management regimes. Egg laying in genetically modified potato plots was significantly lower than in any other treatments, with no larvae surviving past the first instar. Late instar larvae, which can potentially develop into destructive summer adults, were detected in all other plots.

Aphid control was achieved in all experimental treatments except permethrin. Permethrin, which is broad spectrum in activity, prevented the establishment of many beneficial arthropods such as big eyed bugs, damsel bugs, minute pirate bugs, and spiders, but provided no control of green peach aphids. As a result, aphid populations increased in an exponential-like fashion in these plots.

Both apterous and alate aphid populations were kept in check throughout the season in genetically modified potato and microbial *B.t.t.* plots, without application of chemical insecticides. In these plots, the selective control of Colorado potato beetles did not adversely affect beneficial predators and parasites, which were present in sufficient numbers to regulate aphid populations. The results of this study demonstrate the potential of *B.t.t.* to control Colorado potato beetles and enhance the potential for biological control of aphids.

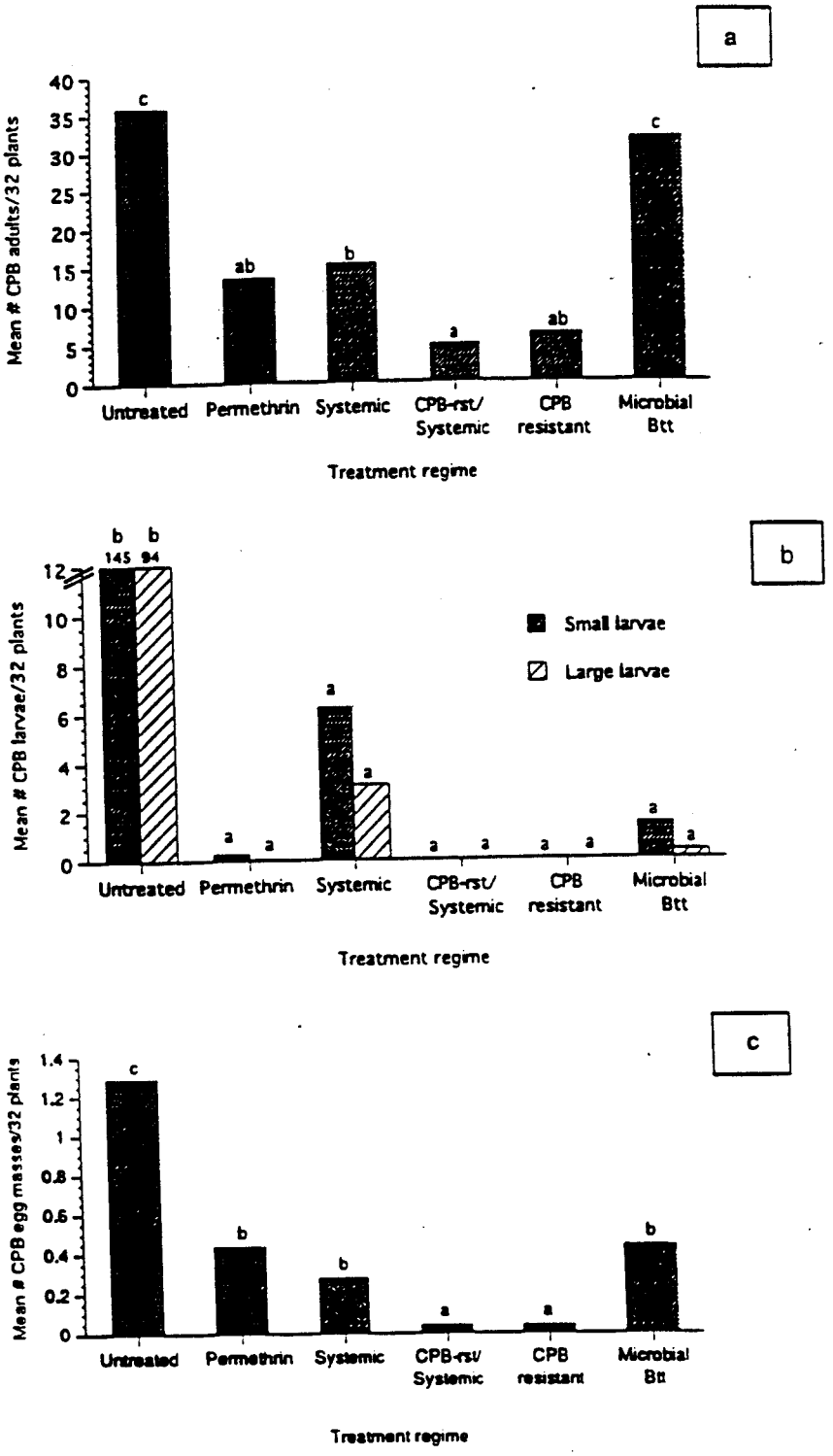

Figure 1. Colorado potato beetle (a) adults, (b) larvae, and (c) egg masses found on 32 plants in potato plots with experimental pest management regimes (average of 6 reps), Hermiston, Oregon, 1992. Means for each stage with the same letter are not significantly different at the .05 level, Fisher's (1935) protected LSD.

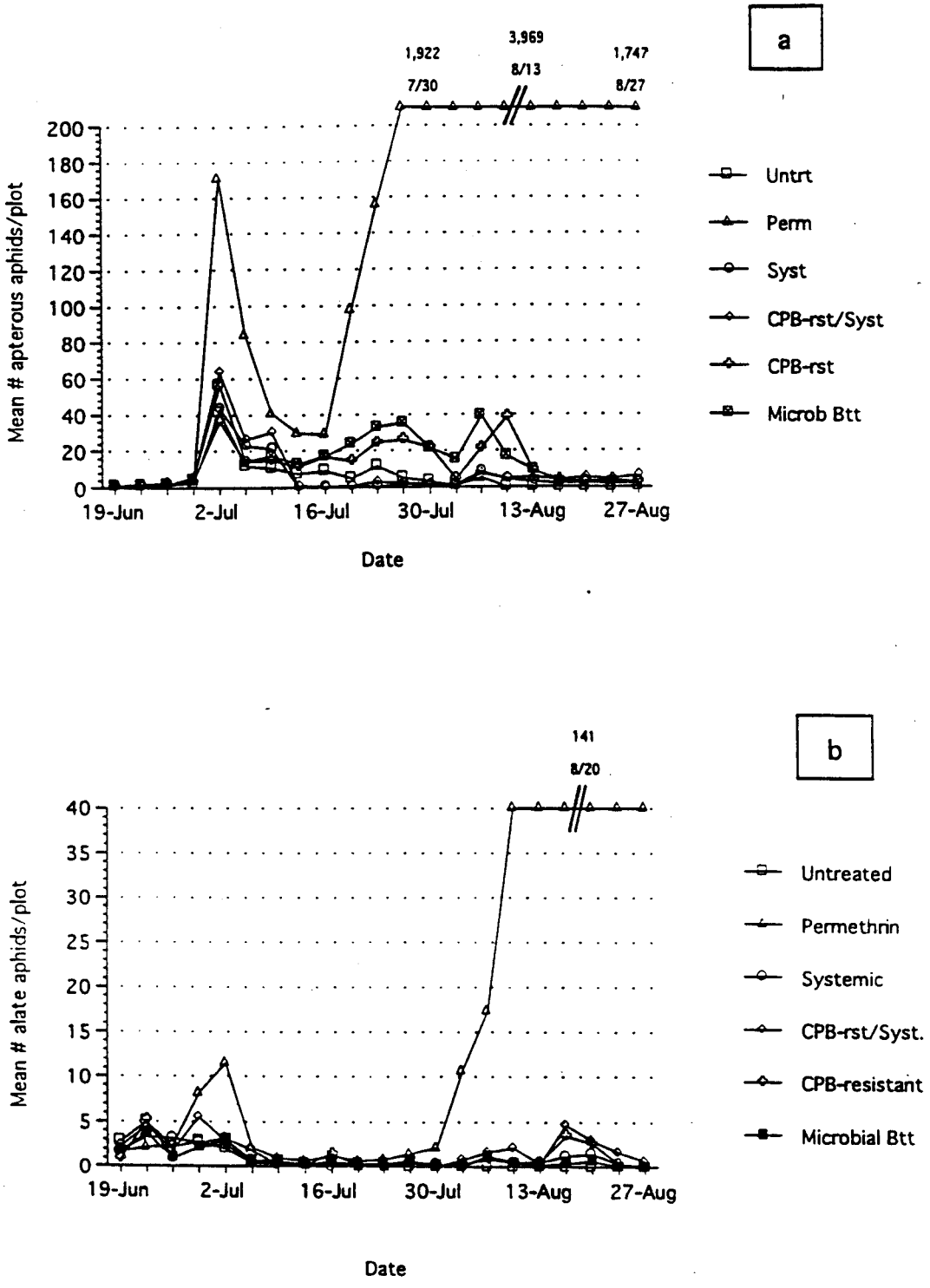

Figure 2. Seasonal distribution of (a) apterous and (b) alate green peach aphids taken from beat cloth samples in potato plots with experimental pest management regimes, Hermiston, Oregon, 1992

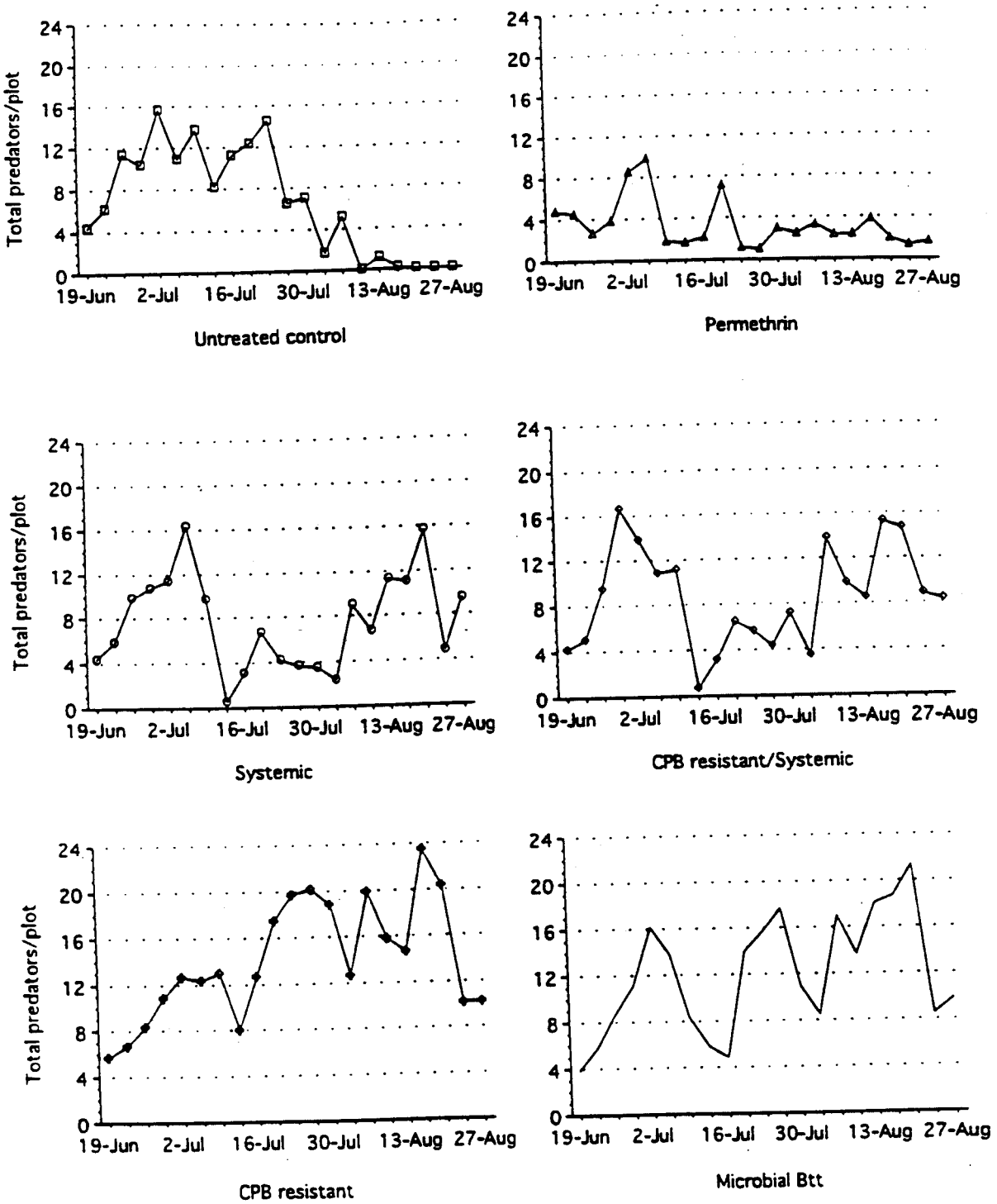

Figure 3. Seasonal distribution of generalist predators collected in beat cloth samples from potato plots with experimental pest management regimes, Hermiston, Oregon, 1992.

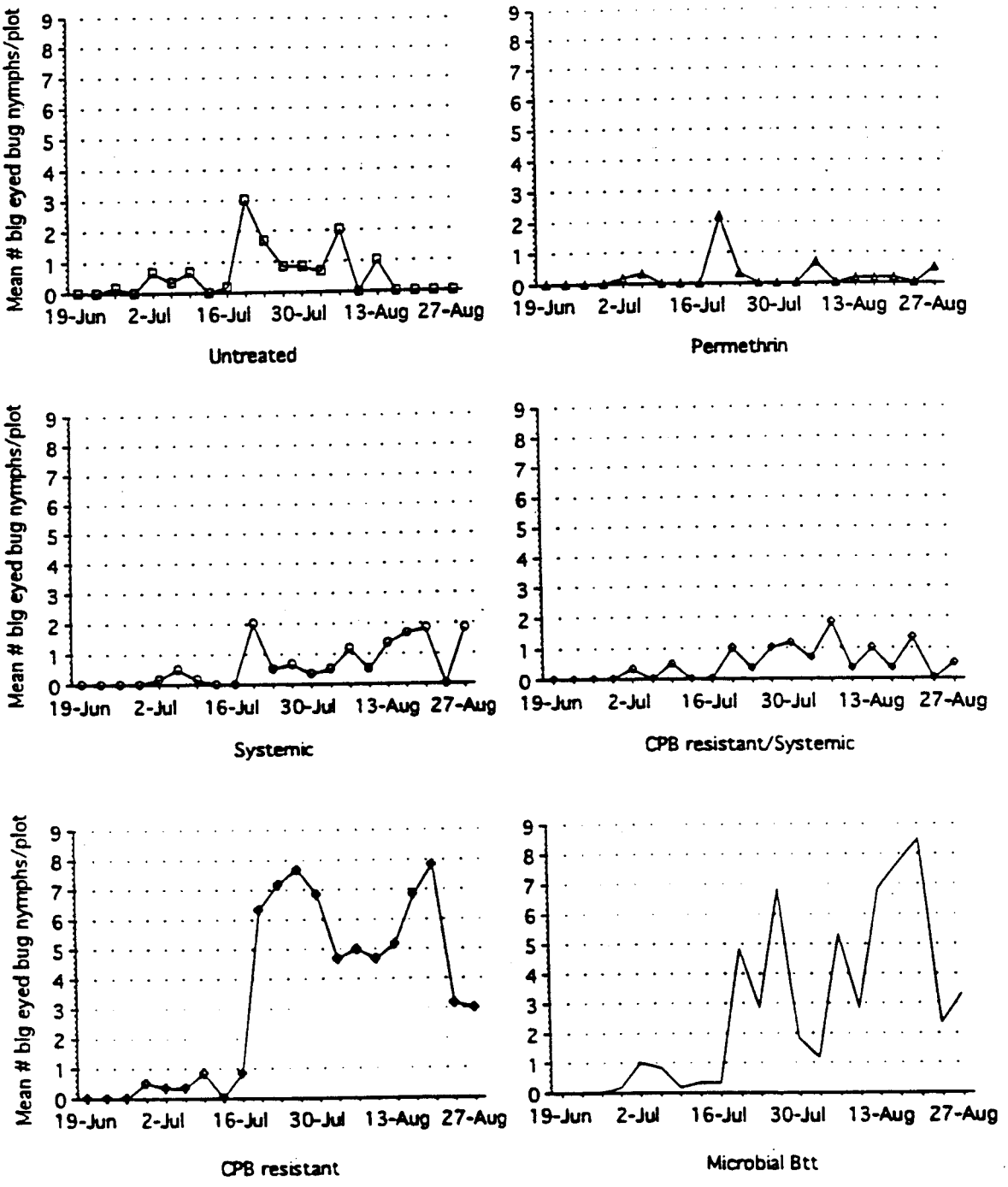

Figure 4. Seasonal distribution of big eyed bug nymphs in beat cloth samples from potato plots with experimental pest management regimes, Hermiston, Oregon, 1992.

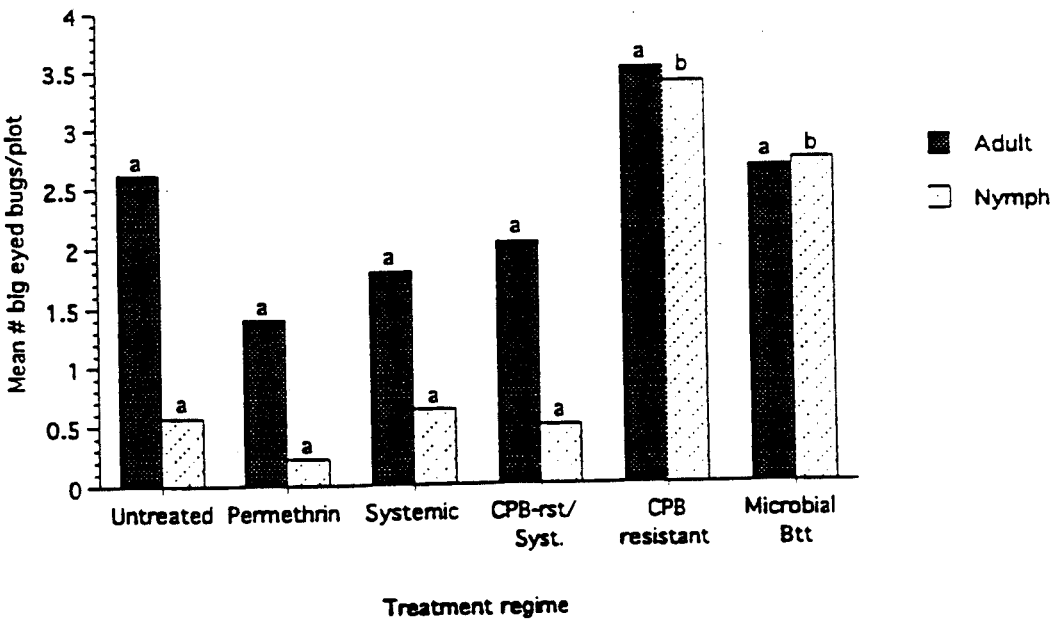

Figure 5. Seasonal average (mean of 6 reps) of big eyed bug adults and nymphs taken from beat cloth samples in potato plots with experimental pest management regimes, Hermiston, Oregon, 1992. Means for each stage with the same letter are not significantly different at the .05 level, Fisher's (1935) protected LSD.

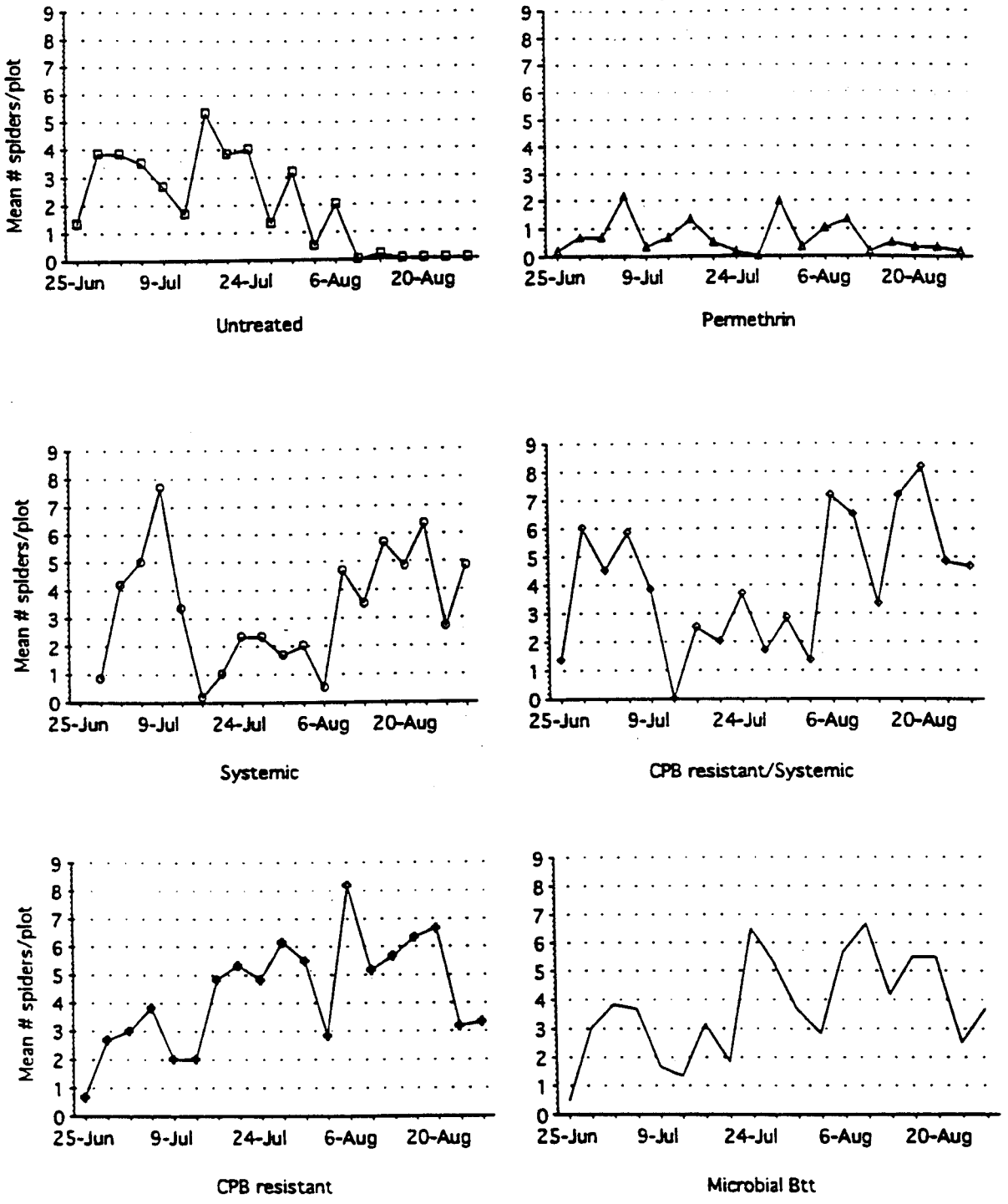

Figure 6. Seasonal distribution of spiders taken from beat cloth samples in potato plots with experimental pest management regimes, Hermiston, Oregon, 1992.

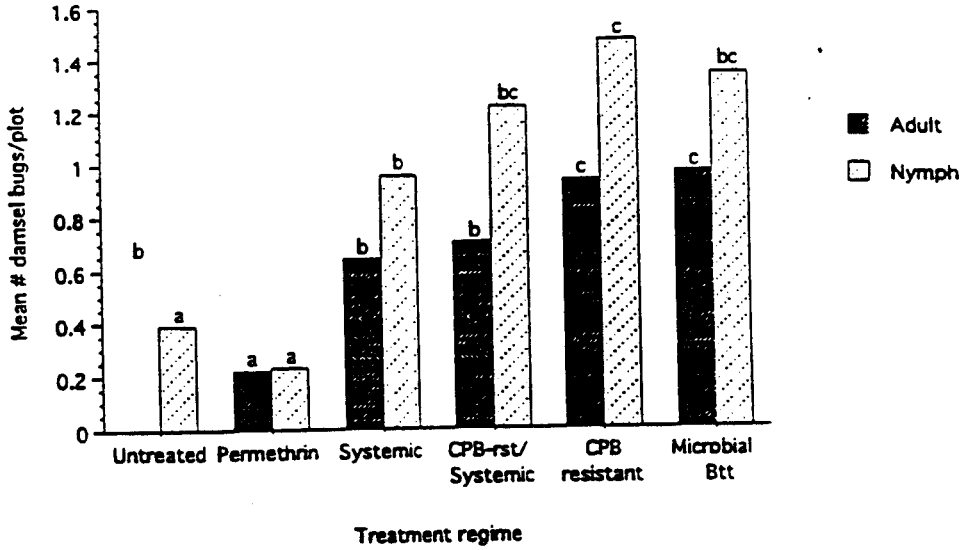

Figure 7. Seasonal average of damsel bug adults and nymphs collected in beat cloth samples from potato plots with experimental pest management regimes (average of 6 reps), Hermiston, Oregon, 1992. Means for each stage with the same letter are not significantly different at the .05 level, Fisher's (1935) protected LSD.

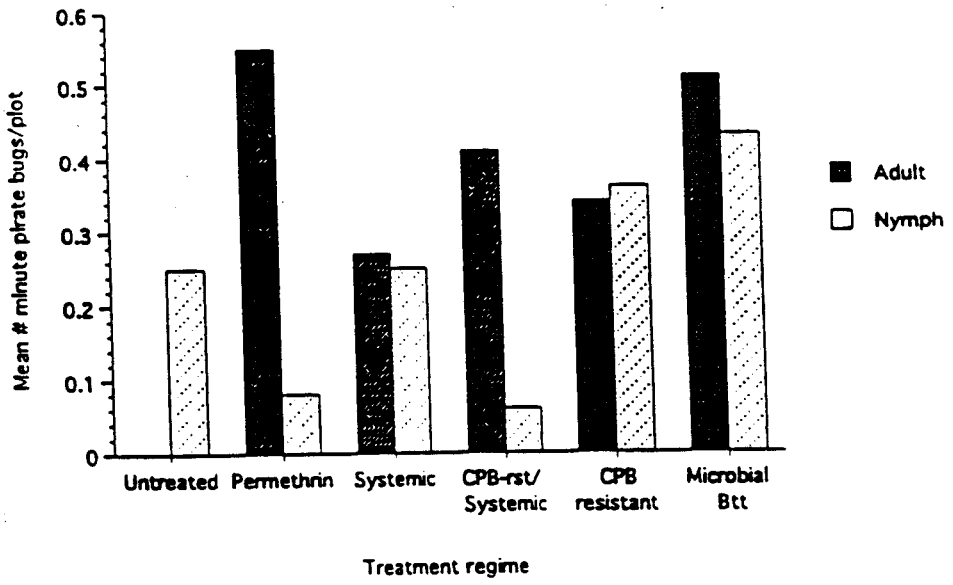

Figure 8. Seasonal average of minute pirate bug adults and nymphs taken from beat cloth samples in potato plots with experimental pest management regimes (mean of 6 reps), Hermiston, Oregon, 1992. (No significant differences)

Wisconsin:

Colorado potato beetle:

Early season adult populations were low with means of 5 adults per 20 plants in untreated plots on June 11 (Fig. 9). Overwintered adults gave rise to significantly fewer eggs in the genetically modified potato plots, while no differences in egg deposition were found between other treatment regimes (Fig. 10).

Cool weather resulted in a single larval generation which caused 50% defoliation of the untreated control by mid-July (Fig. 11). Defoliation of genetically modified potato, microbial *B.t.t.*, and conventional insecticide plots was limited to 10%. However, unlike the conventional insecticide and microbial *B.t.t.* treatments, very few small larvae (Fig. 12a) and no large larvae (Fig. 12b) were found on genetically modified potato plants throughout the season. Those small larvae that were found on these plants were probably neonates which had not yet fed on the foliage, as none developed to later instars.

Summer adults, which began to emerge on July 20, completely defoliated the untreated controls by August 13. Adult populations remained low in the genetically modified potato plots all season without additional insecticide applications. In contrast, adults began to increase in microbial *B.t.t.* plots in late August, necessitating treatment with esfenvalerate to rescue the plots from defoliation.

Potato leafhopper:

Potato leafhopper populations were unusually high, exceeding the treatment threshold of 2 per sweep on June 26 (Table 1), before natural enemy populations had a chance to establish. Malathion provided excellent control of potato leafhoppers with no apparent effect on Colorado potato beetle larvae, as seen in the untreated control (Fig. 12). After the July 2 treatment, potato leafhopper populations in genetically modified potato, microbial *B.t.t.*, and untreated plots remained below threshold until late-August, when plots were re-treated.

Aphids:

Aphid populations were extremely low throughout the season and never approached the seed treatment threshold of 5 aphids per 50 leaves. Combined counts of green peach and potato aphids in untreated controls (prior to defoliation) averaged 4.8 per 50 leaves. Although populations were low overall, differences between treatment regimes were detected on several dates using both leaf and beat cloth sampling methods (Tables 2 and 3).

The conventional insecticide treatment utilized for Colorado potato beetle control (esfenvalerate/endosulfan) effectively eliminated apterous aphids, maintaining the population at less than 1 per 50 leaves from late June through mid August. Aphid populations in genetically modified and microbial *B.t.t.* plots were significantly lower than in the untreated control from July 8 to 24 (leaf counts, $p < .05$). Similar results were obtained in beat samples, but the differences were not significant. The higher aphid populations observed in the control plots may have resulted from plant stress and

early senescence which was caused by insect feeding.

Potato flea beetles:

The potato flea beetle summer adult population peaked in late August, after untreated control plots were totally defoliated by Colorado potato beetles. Significantly fewer potato flea beetles were recovered from D-Vac samples on August 20 in genetically modified potato and conventional control plots than from microbial *B.t.t.* plots (Figure 13). Untreated control plots were not sampled at this time because of the lack of foliage.

Beneficial arthropod complex:

Predator populations were generally low and reflected the limited availability of prey. The greatest numbers of predators were collected from beat samples prior to defoliation in the controls, between July 10 and July 31 (Fig. 14). The most predators were found in the untreated controls where prey (Colorado potato beetles, potato leafhoppers, aphids, and others) were most abundant. Conversely, predator populations were lowest in the conventional control treatment, where broad spectrum insecticides were used and prey insects were few. Intermediate predator populations were found in the genetically modified and microbial *B.t.t.* treatments. Although overall numbers of individual predator species were generally low and differences were not significant, these data suggest that pest management programs which utilize selective insect controls, such as *B.t.t.*, may foster higher predator populations than do conventional insecticide programs.

Predaceous species recovered in pitfall traps were comprised primarily of ground beetles and rove beetles. Several other species normally associated with foliage were also found in the traps (e.g. spiders, minute pirate bugs, lady beetles, and lacewings). Predator numbers did not differ significantly between treatments but as was observed in beat samples, the highest numbers were detected in untreated controls and the lowest in conventionally treated plots.

The D-Vac was the most effective technique for sampling a wide variety of natural enemy species. No samples were taken in control plots due to a lack of foliage, but significantly more minute pirate bugs, lady beetles, and spiders were present in the genetically modified potato and microbial *B.t.t.* plots than were found in the conventionally treated plots (Fig. 15). Although, the number of predatory species was significantly greater in the microbial *B.t.t.* plots than the genetically modified potatoes, the profile of these species was similar, indicating that the method of *B.t.t.* delivery has no effect on non-target organisms.

Hymenopteran species, including those which are important aphid parasites, were most effectively sampled with the D-Vac. Although species determination has not been conducted, significantly more hymenopterans were recovered from genetically modified potatoes than from conventional control plots (Fig. 16). Hymenopterous species were also abundant in microbial *B.t.t.* plots, but not significantly more so than in the control.

An assessment of the total insect fauna in the potato plots was made using the insects recovered from the D-Vac sample (Fig. 17). Over 1000 insects/60 seconds were

recovered in both the microbial *B.t.t.* and genetically modified potato plots. In comparison, the conventional insecticide treatment reduced the insect fauna by over 50%.

Summary:

Genetically modified potato plants and conventional insecticides provided season long control of all Colorado potato beetle life stages. Foliar-applied microbial *B.t.t.* prevented defoliation from larvae but did not protect the plants from summer adult feeding, which necessitated application with a conventional insecticide to rescue the plots from crop loss. Aphid populations were unusually low, and commercially acceptable control was achieved in plots with all treatment regimes.

Malathion provided excellent control of potato leafhoppers when applied at half the recommended rate, and did not appear to negatively impact other insect species. Since potato leafhopper control is typically required in the early season before beneficials are established, this tactic may provide a safe control option for an integrated management system.

Natural enemy populations were generally low, and no differences were detected in beat samples or pitfall traps. However, significantly more predators (minute pirate bugs, lady beetles, and spiders) and hymenopteran parasitoid species were recovered in D-Vac samples from genetically modified potato and microbial *B.t.t.* treated plots than from conventional insecticide plots.

The total number of insects recovered from D-Vac samples was significantly reduced in potato plots treated with broad spectrum insecticides. No difference in the insect fauna was detected between foliar-applied *B.t.t.* and genetically modified potato plots. Since untreated control plots were completely defoliated by the August 20 sample date, a comparison with this treatment could not be performed.

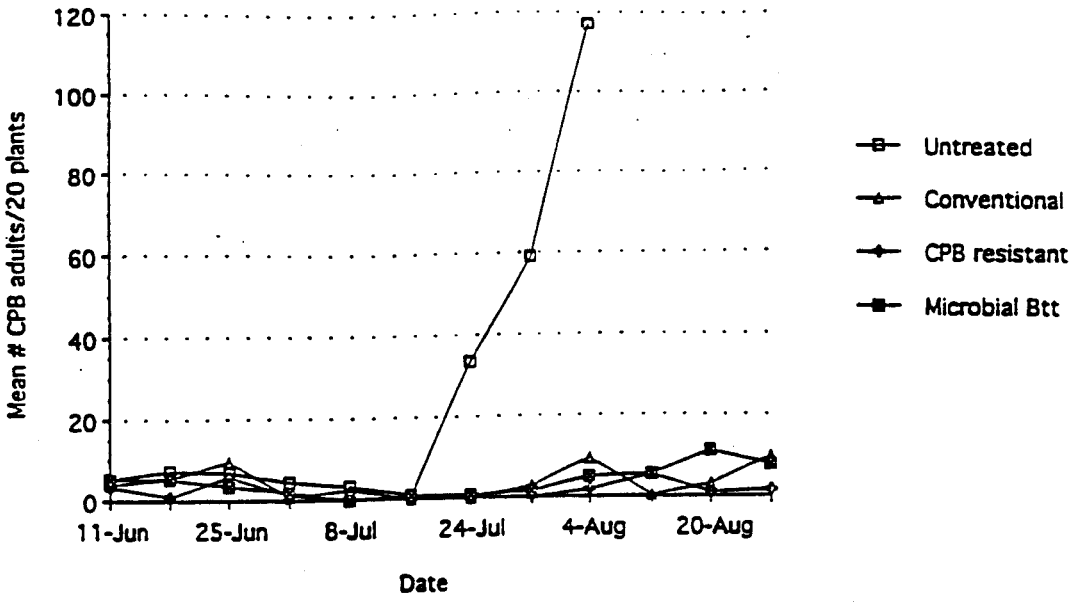

Figure 9. Seasonal distribution of adult Colorado potato beetles in potato plots with experimental pest management regimes, Hancock, Wisconsin, 1992. (Insect counts in untreated plots discontinued after August 4 due to lack of foliage.)

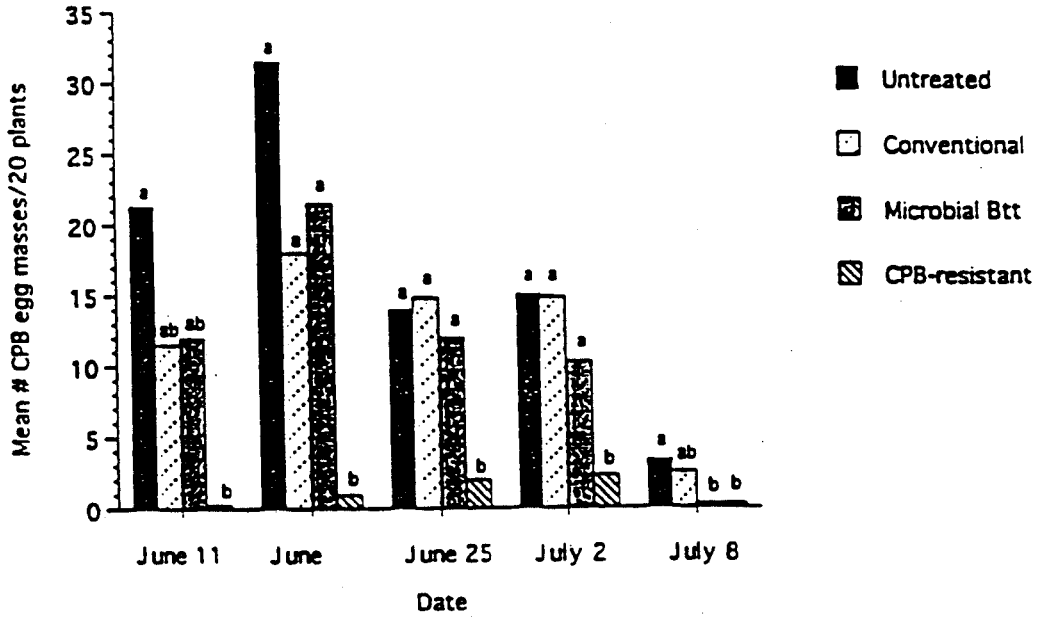

Figure 10. Colorado potato beetle egg masses (mean of 4 reps) on 20 plants in potato plots utilizing experimental pest management regimes, Hancock, Wisconsin, 1992. Means for each date with the same letter are not significantly different at the .05 level, Fisher's (1935) protected LSD.

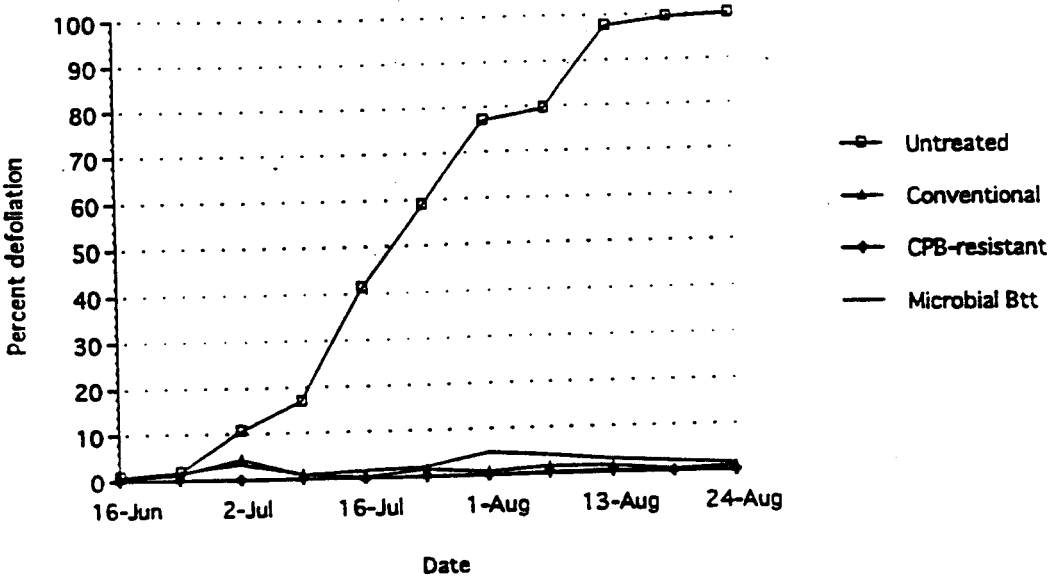

Figure 11. Defoliation caused by Colorado potato beetle feeding in potato plots with experimental pest management regimes, Hancock, Wisconsin, 1992.

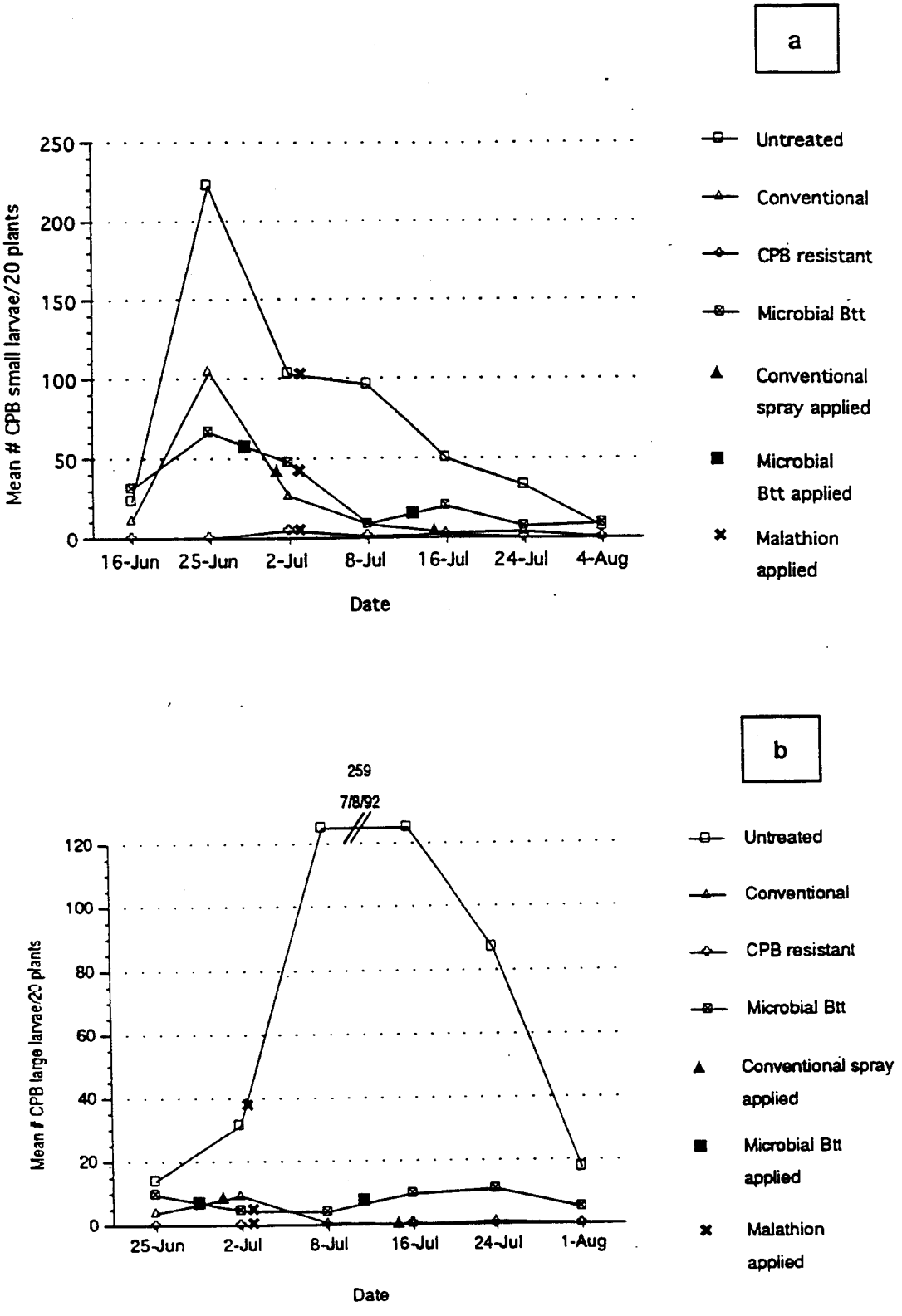

Figure 12. Seasonal distribution of CPB (a) first and second instar larvae and (b) third and fourth instar larvae in potato plots with experimental pest management regimes, Hancock, Wisconsin, 1992

Table 1. Potato leafhopper adult populations in potato plots with experimental treatment regimes, Hancock, WI 1992

Treatment	Potato leafhoppers/50 sweeps								
	6/26	6/30	7/8	7/17	7/24	8/1	8/5	8/13	8/20
Untreated	113 a [1]	184 a *	19 a	19 a	28 a	59 a	33 b	26 bc	NA
Conventional	116 a *	17 b	1 b *	7 b	7 c	19 b	32 b *	5 c	1 b
CPB resistant	82 a	164 a *	2 b	18 a	17 b	49 a	68 a	94 a	110 a
Microbial Btt	75 a	217 a *	2 b	11 ab	18 ab	47 ab	62 a	63 ab	41 b

[1] Means followed by the same letter are not significantly different at the .05 level, Fisher's (1935) protected LSD.

* = spray applied

Table 2. Potato and green peach aphids counted in leaf samples in potato plots utilizing experimental pest management regimes, Hancock, Wisconsin 1992

Treatment	Aphids/50 leaves						
	6/30	7/8	7/17	7/24	8/1	8/5	8/13
Untreated	2 a [1]	3 a	10 a	4 a	5 a	2 ab	3 a
Conventional	3 a	1 b	0 b	0 b	1 b	1 b	1 a
CPB resistant	2 a	1 b	1 b	1 b	4 ab	7 a	1 a
Microbial Btt	1 a	0 b	0 b	1 ab	5 a	2 ab	3 a

[1] Means followed by the same letter are not significantly different at the .05 level, Fisher's (1935) protected LSD.

Table 3. Potato and green peach aphids counted in beat cloth samples in potato plots utilizing experimental pest management regimes, Hancock, Wisconsin 1992

Treatment	Aphids/beat sample						
	7/10	7/17	7/24	7/31	8/6	8/13	8/21
Untreated	43 a [1]	27 a	15 a	5 a	NA	NA	NA
Conventional	1 b	2 b	3 a	0 b	6 a	2 b	1 a
CPB resistant	18 ab	11 ab	9 a	1 b	16 a	8 a	4 a
Microbial Btt	27 ab	20 ab	2 a	2 b	14 a	4 ab	4 a

[1] Means followed by the same letter are not significantly different at the .05 level, Fisher's (1935) protected LSD.

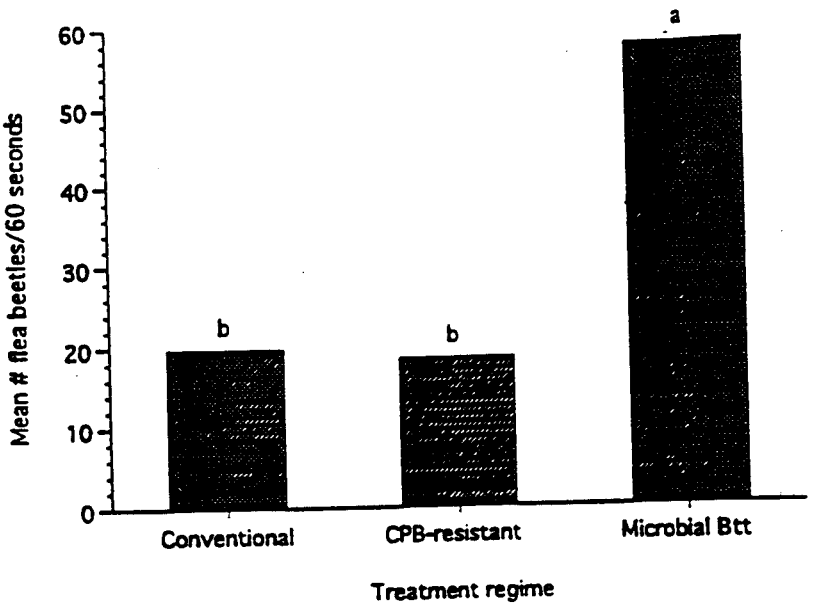

Figure 13. Potato flea beetles (mean of 4 reps) collected in 60 second D-Vac samples from potato plots utilizing experimental pest management regimes, Hancock, Wisconsin, 1992. Means with the same letter are not significantly different at the .05 level, Fisher's (1935) protected LSD.

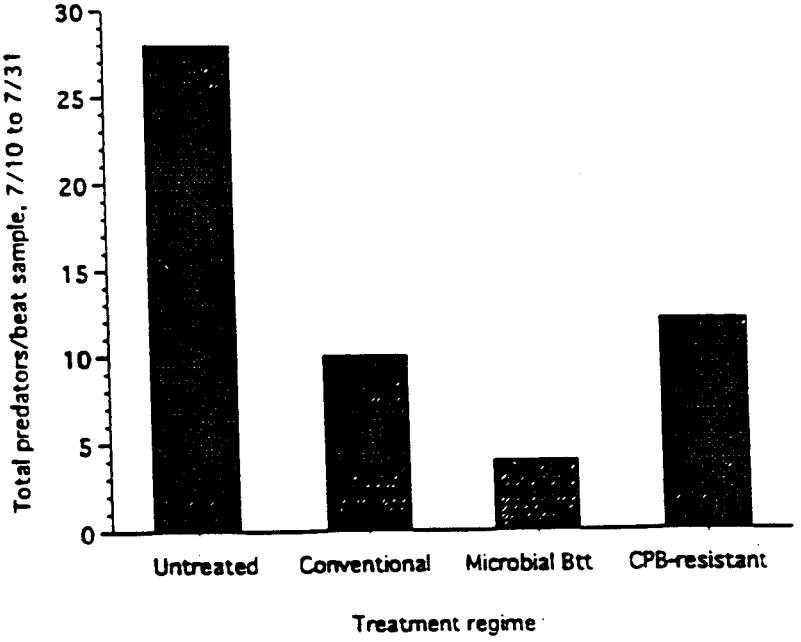

Figure 14. Total predators (mean of 4 reps) collected in beat samples between July 10 and July 31 in potato plots with experimental pest management regimes, Hancock, Wisconsin, 1992. (No significant differences)

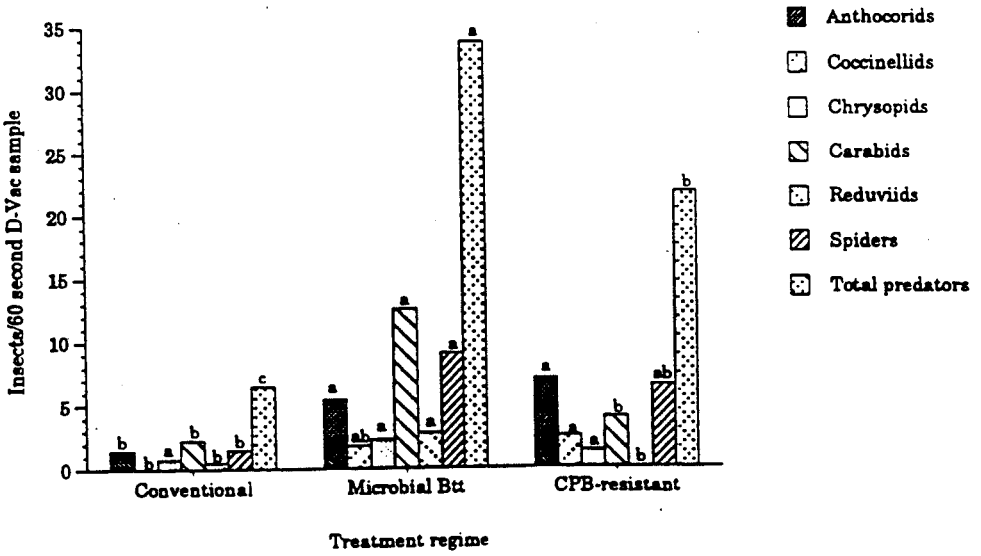

Figure 15. Individual and total predators (mean of 4 reps) collected in 60 second D-Vac samples in potato plots utilizing experimental pest management regimes, Hancock, Wisconsin, on August 20, 1992. Means for each predator with the same letter are not significantly different at the .05 level, Fisher's (1935) protected LSD.

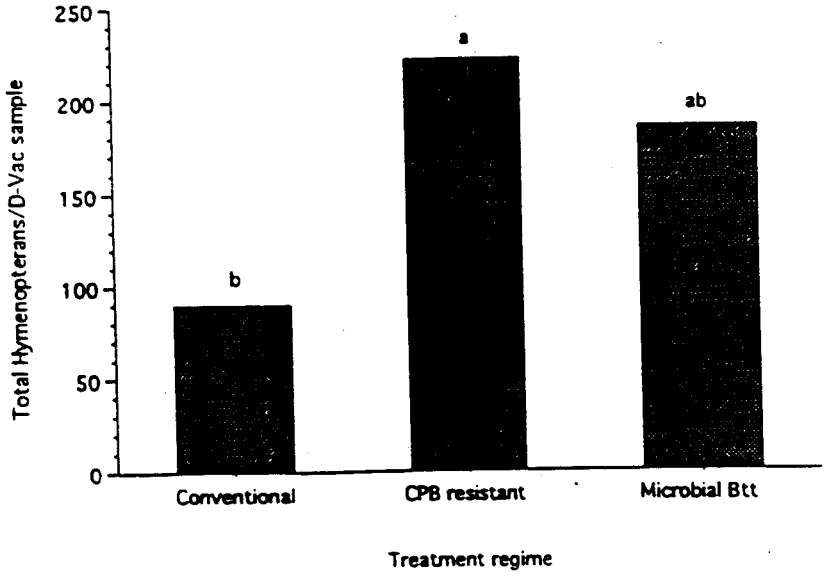

Figure 16. Total Hymenopterous insects (mean of 4 reps) collected in 60 second D-Vac samples in potato plots utilizing experimental pest management regimes, Hancock, Wisconsin, on August 20, 1992. Means with the same letter are not significantly different at the .05 level, Fisher's (1935) protected LSD.

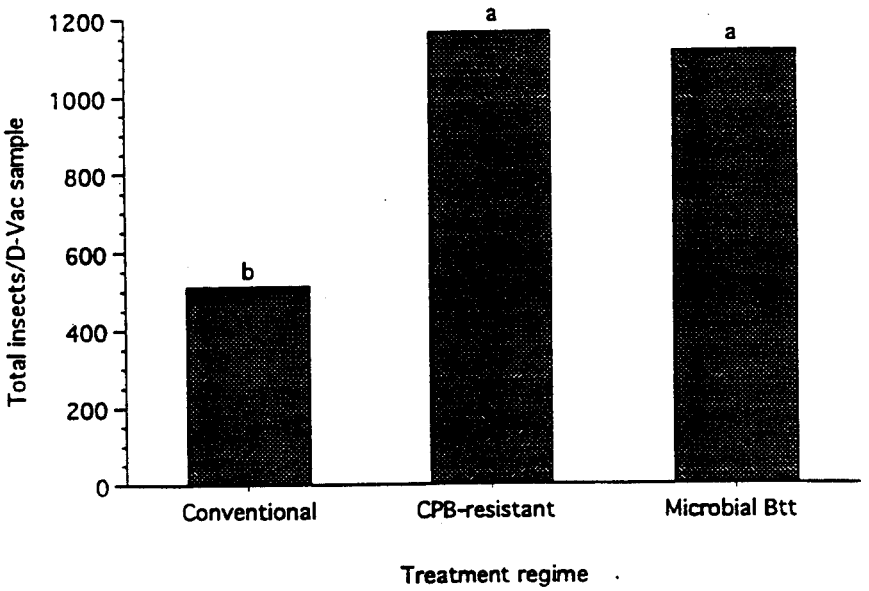

Figure 17. Total insects (mean of 4 reps) collected in 60 second D-Vac samples from potato plots utilizing experimental, pest management regimes, Hancock Wisconsin, on August 20, 1992. Means with the same letters are not significantly different at the .05 level, Fisher's (1935) protected LSD.

PEI

It was intended that the treatments in this study mirror those in the Hermiston, Oregon experiment. However, due to extreme winter weather conditions in 1991-92, insect populations in all plots were suppressed and treatment thresholds for foliar insecticide applications were never reached. With the exception of at-planting systemics, no insecticides were applied to the plots.

Colorado potato beetle:

Although Colorado potato beetle populations were atypically low in all plots, significantly fewer adults and larvae were found in genetically modified potato plots than in all others, including those treated with phorate (Fig. 18a and b). Early season adult Colorado potato beetles gave rise to one larval generation that peaked in the untreated controls and the non-resistant phorate treatment between August 4 and 25. Virtually no larvae were found in any genetically modified potato plot throughout the season.

Aphids:

Potato aphid populations were significantly lower in systemic insecticide treated plots than in untreated plots between July 27 and August 10 (Fig. 19). By the time the population peaked from August 20 to August 25, residues had begun to decline such that no differences between treatments were detected. Green peach aphids were extremely scarce all season, reaching a maximum of 3.25 per beat sample and 12.5 per 50 leaves in the untreated control. Aphid populations did not differ between genetically modified and unmodified potato plots within each treatment regime.

Potato flea beetles:

Adult potato flea beetle populations and the corresponding leaf damage were lower in phorate treated than in untreated plots. Flea beetle feeding damage was also reduced in genetically modified potato plots with no insecticide. While differences in feeding damage were not evident early in the season, these plants had significantly fewer leaf feeding holes than all three untreated controls on July 27, August 20, September 10, 15, and 21 (Fig. 20). Adult flea beetles were also less abundant in the genetically modified plots, but these differences were not statistically significant.

Beneficial arthropods:

Predator and parasite populations reflected the low pest populations. No significant differences in lady beetles, damsel bugs, flower flies, soldier beetles, or ground beetles were detected between plots.

Summary:

Insect populations were generally low, and treatment thresholds for Colorado potato beetles and aphids were not reached. Potato flea beetle summer adult feeding was significantly reduced in genetically modified potato plots.

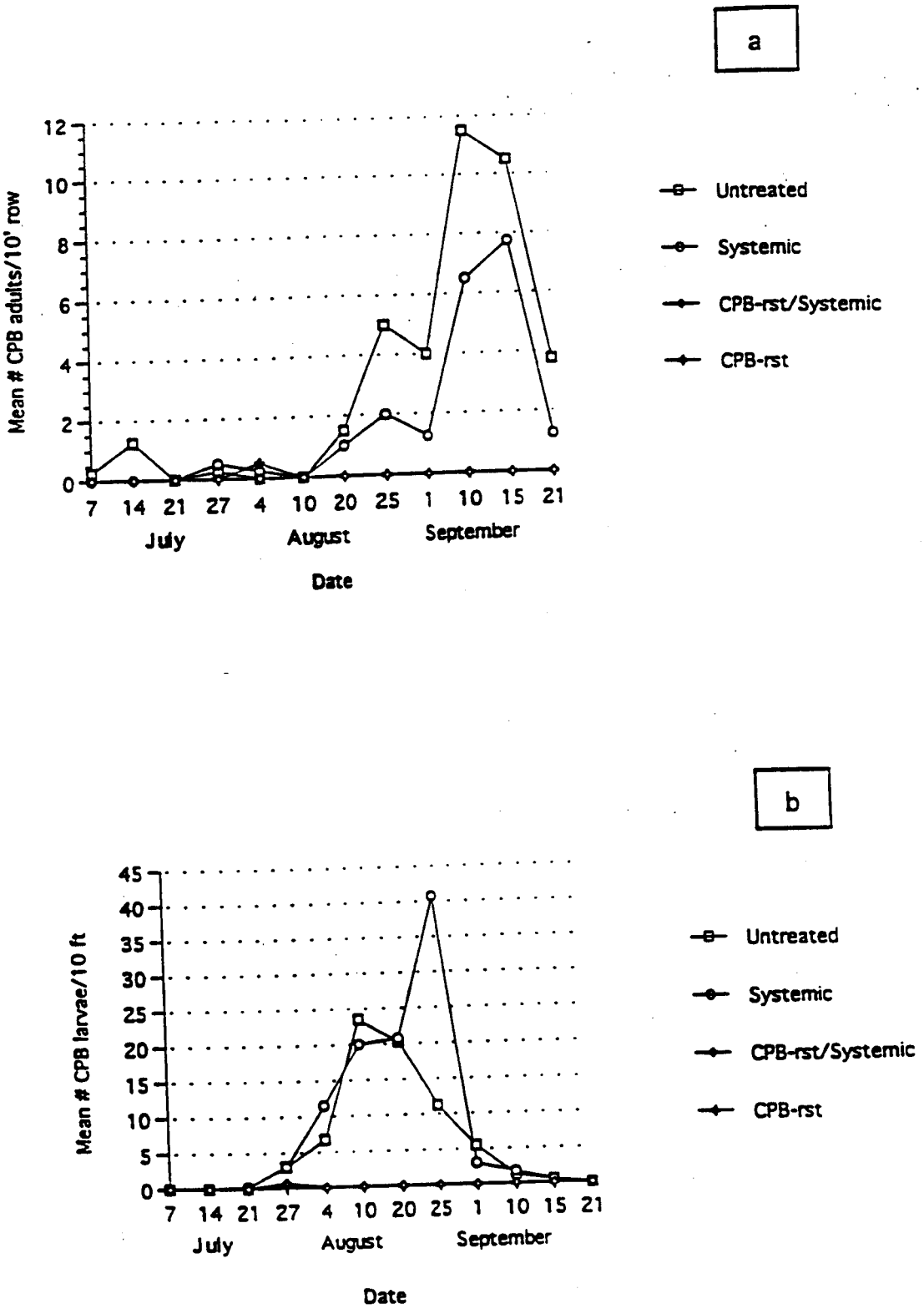

Figure 18. Seasonal distribution of Colorado potato beetle (a) adults and (b) larvae in potato plots utilizing experimental pest management regimes, Charlottetown, PEI, 1992.

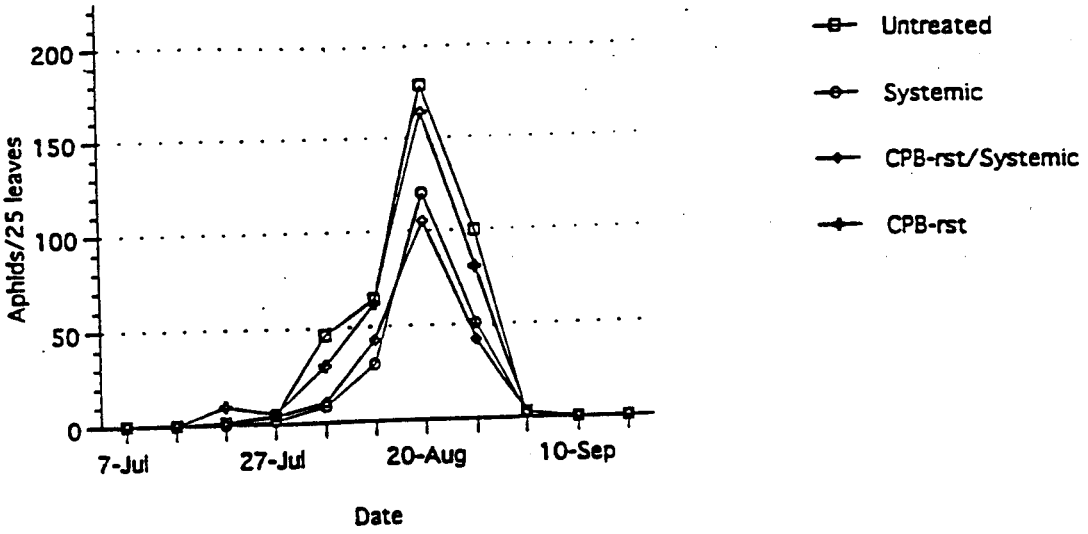

Figure 19. Seasonal distribution of potato aphids per 25 leaves (mean of 4 reps) in potato plots utilizing experimental pest management regimes, Charlottetown, PEI, 1992.

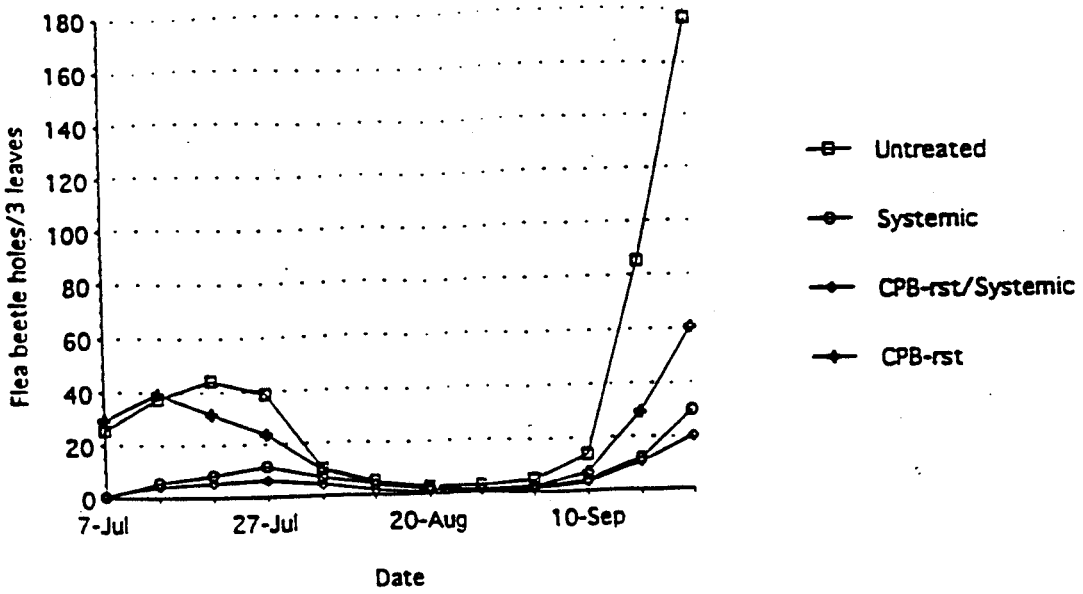

Figure 20. Seasonal distribution of potato flea beetle leaf feeding holes (mean of 4 reps) in potato plots utilizing experimental pest management regimes, Charlottetown, PEI, 1992.

CONCLUSIONS

Genetically modified Colorado potato beetle resistant potato plants provided season long control of Colorado potato beetles at all locations. No larvae were found to survive past the first instar, and the numbers of adults and egg masses on these plants were lower than in all other treatments. Microbial *B.t.t.* effectively protected the plots from Colorado potato beetle feeding damage, but allowed some larvae to survive to later instars. It is expected that such larval "escapes" will successfully pupate and emerge as summer adults. These insects are difficult to control with either microbial *B.t.t.* or chemical insecticides, and can cause substantial defoliation in a short period. Summer adult Colorado potato beetles will also overwinter to establish the succeeding year's population in potatoes.

Significantly more predators and parasites were found in the genetically modified potato and foliar-applied microbial *B.t.t.*-treated plots than in conventional insecticide treatments in both Wisconsin and Oregon, demonstrating the safety of the *B.t.t.* protein to non-target insects. As a result of elevated predator populations, aphids were maintained at commercially acceptable levels in these plots without supplemental insecticides. In contrast, aphid populations in Oregon rose exponentially in esfenvalerate-treated plots, where beneficial arthropods were eliminated and no chemical aphid control was achieved. This population response demonstrates the tremendous reproductive potential of aphids and the importance of natural enemies in their population regulation.

Data from PEI and Wisconsin suggest that plant expression of *B.t.t.* has some activity against potato flea beetles. Although summer generation adult populations were not significantly lower in genetically modified plots than in untreated controls in PEI, feeding damage was reduced. Significantly fewer potato flea beetles were recovered from Colorado potato beetle resistant plots in Wisconsin than from microbial *B.t.t.* plots. Since potato flea beetles and Colorado potato beetles are both in the family Chrysomelidae, it is possible that potato flea beetles are susceptible to *B.t.t.* Further studies specifically investigating the effect of plant expressed *B.t.t.* on potato flea beetle adult and larval feeding and development will be conducted in 1993 and 1994.

Results from this multi-year research program will be used to develop crop recommendations which incorporate genetically modified Colorado potato beetle resistant potatoes and other selective controls for the integrated management of potato insect pests. Data from 1992, which will be confirmed in subsequent studies, clearly demonstrates that genetically modified potatoes provide superior season-long control of all life stages of the Colorado potato beetle. The safety of the *B.t.t.* protein to non-target arthropods enables natural enemy populations to develop without disruption by chemical insecticides. Beneficial arthropods can significantly reduce the populations of non-target potato pests such as aphids. Upon their commercialization, these potatoes will represent an effective and environmentally compatible addition to the existing methods of potato pest management.

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APPENDIX 2

ECONOMIC BENEFITS OF TRANSGENIC HOST PLANT RESISTANCE TO COLORADO POTATO BEETLE IN THE UNITED STATES

ECONOMIC BENEFITS OF TRANSGENIC HOST PLANT RESISTANCE TO COLORADO POTATO BEETLE IN THE UNITED STATES

by

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ABSTRACT

Control of Colorado potato beetle (Leptinotarsa decemlineata) using conventional methods is becoming quite difficult. Potato cultivars transformed to contain the endotoxin from Btt (Bacillus thuringiensis var tenebrionis) have provided complete resistance in field trials. CPB-resistant potatoes would likely have the following impacts on the potato market: reduced yield loss, lower costs of production, increased supply, lower prices to growers, higher grower profits, increased exports, decreased imports and lower prices to consumers. Off-farm benefits would extend to agribusinesses and rural communities as well.

INTRODUCTION

Control of agricultural pests is an important economic issue that extends well beyond the farm. The invasion of the boll weevil (Anthonomus grandis grandis) in the southern United States caused drastic sociological and economic changes. Financial ruin came to farmers, processors, merchants, bankers and the entire regional economy because of crop damage of one-third to one-half (Loftin 1945). Although today's potato industry is different from the former cotton industry in the South, yield losses due to Colorado potato beetle (Leptinotarsa decemlineata) can exceed the 85 percent level (Hare 1980, Ferro et al. 1983, Shields and Wyman 1984).

Another potato pest, the late blight fungus (Phytophthora infestans), caused severe hardship in Ireland. Beginning in 1845, blight destroyed potato crops that were the main source of food for the Irish people. With no resistance to the fungus, plants died within days of infection (Salaman 1985). The Great Irish Potato Famine caused deaths and immigration from which Ireland never fully recovered. Before the famine the population of Ireland was 10 million; now it is only 3.5 million.

Today's farmers have some effective weapons against agricultural pests, including potato blight. In spite of modern integrated pest management, however, control of the Colorado potato beetle is becoming more difficult. It has emerged as the most serious potato insect problem in North America (Casagrande 1987). Control options are becoming increasingly limited due to declining availability of effective systemic insecticides and widespread resistance to foliar sprays. Alternative cultural, biological and physical methods offer limited hope for long term control (Wyman, et al. 1993).

What does offer hope is transgenic potatoes. The potato has become a primary target for applied plant molecular genetics. According to Vayda and Belknap (1992):

"...difficulties associated with the breeding, the ease by which it is genetically transformed and its clonal mode of propagation make potato an ideal candidate for molecular manipulation."

The attention of molecular geneticists is welcome in the potato industry where the traditional focus has been on controlling pests with chemicals rather than breeding pest resistant varieties (Guenthner, 1992a,b).

Existing potato cultivars can now be transformed to contain the endotoxin from Btt (Bacillus thuringiensis var tenebrionis) to control Colorado potato beetle. This provides a control option -- host plant resistance -- that has not previously been available to the potato industry. Field trials have shown that the transgenic potatoes provide complete resistance (Feldman, et al. 1992).

The purpose of this paper is to analyze the potential economic impacts of a genetically modified potato to control Colorado potato beetle.

ECONOMICS OF BIOTECHNOLOGY

Economists expect agricultural biotechnology to cause dramatic changes. Kalter and Tauer (1987) say that "over the next twenty years, the technological revolution could cause changes... more rapid and pervasive than in any previous time period." Hueth and Just (1987) say that "biotechnology promises to have a greater impact on society than any other presently foreseeable development."

Agricultural productivity can be increased by: (1) raising the yield potential of the plant or (2) allowing current yield potential to be obtained. CPB-resistant potatoes is an example of the latter; hybrid seed corn is an example of the former. During the 1935-39 period less than 10 percent of U.S. corn growers were using hybrid seed. By 1949-53 more than 80 percent were and average yields increased by 52.4 percent. Projections for biotechnology-driven productivity gains are expected to exceed those caused by the adoption of hybrid seed corn. (Hueth and Just 1987).

Increases in agricultural productivity have kept costs down, but perhaps profits as well. Heady (1949) claimed that continuous technological change results in chronic excess resources. He argued that the over supply of resources and the slow movement of resources out of agriculture cause it to be a low-return industry. The low returns, however, would only be on the average. Early adopters of new technology could gain higher profits.

Economic theory suggests that advances in technology are good for consumers as well as producers. Empirical studies have supported economic theory but most have been limited to technologies that have been on the market for some time. Analyzing the impacts of technology that is not yet on the market is much more difficult. Fishel (1987) argues that the difficulties can be overcome by the establishment of close

working relationships between economists and scientists and overcoming the reluctance of economists to use "soft" data. Analysis of future impacts, however, involves forecasting which is less precise than after-the-fact analysis.

Several economists have responded to the challenge of evaluating new rather than old technology. The earliest efforts were in animal agriculture but there have been a few studies of plant agriculture. Florkowski and Hill (1990) analyzed the following plant biotechnologies: symbiotic changes, new rhizobia strains, altered protein content, new resistant varieties, frost tolerance, herbicide tolerance, heat tolerance, plant growth regulators and ice-retarding bacteria. The focus was on nine different crops, none of which was potatoes. Their model predicted that the commercialization of new biotechnology would result in increased supply, lower prices, increased exports and reduced environmental problems. Individual farm income may increase or decrease depending on location and adoption of the technology.

A study by Gotsch, et al. (1993) was also conducted to evaluate different plant biotechnologies. They built a linear programming model for a farm in Switzerland growing faba beans, corn, oats, potatoes, rapeseed, barley, sugar beets, rye and wheat. Data was obtained from a Delphi survey of international experts regarding promising new technologies. The survey participants identified the following as likely developments:

1. improved nitrogen efficiency in wheat
   a. 10% yield increase, no quality loss
   b. 20% yield increase, quality-related price loss 8.4%

2. improved resistance of wheat against leaf diseases
   a. no yield change, no quality loss
   b. no yield change, quality-related price loss 3.7%

3. virus resistance in potatoes
   a. 15% yield increase, no quality loss
   b. 15% yield increase, quality-related price loss 10%

4. herbicide resistant sugar beet varieties
   a. no yield or quality impacts.

These biotechnological developments in the Gotsch model were evaluated under three scenarios:

1. the current situation

2. higher fertilizer and pesticide costs (300% of current)

3. liberal market (booming economy with 50% higher labor costs).

Under all three scenarios the potato biotechnology developments improved grower profits the most.

ON-FARM IMPACTS OF CPB-RESISTANT POTATOES

Although the impacts of CPB-resistance on potato supply, demand and prices have not been empirically estimated, the following direction of impacts are quite likely: reduced yield loss, reduced production costs, reduced acres, increased production, lower prices and increased grower profits. These are summarized below.

Impacts on Yields

Obtaining the true yield potential in potato production is becoming increasingly difficult as pest control options become fewer. Several recent articles have evaluated the impact of the loss of insecticides on the potato industry. Lee and Guenthner (1990) claimed that removing pesticides from the potato industry would devastate growers and consumers. Osteen and Guenthner (1990) estimated a $8.6 million loss to growers if phorate was taken off the market. The assumption was that other insecticides would continue to be available, but they would be more expensive and less effective.

More recently Knutson (1993) conducted a study on the loss of pesticides in the fruit and vegetable industry. The focus of the potato portion of the study was on three states: Idaho, Maine and North Dakota. In the latter two states, "multiple applications are required to control Colorado potato beetle." Under the zero-insecticide-use scenario, expected yields decrease 50 percent in North Dakota and Maine and 25 percent in Idaho.

The Knutson study analyzed the impacts of all insecticides being withdrawn from the market, which may be viewed by some as unlikely. However, the availability of effective chemical control of Colorado potato beetle is already a problem. In some production areas the number of currently registered insecticides which are effective against CPB has been reduced to 1-2 from 15-20 in 1980. (Casagrande 1987).

Although a quantified estimate is not made, CPB resistant potatoes would reduce potato yield losses.

Impacts on Costs

Growing potatoes in the U.S. is a high-cost, risky enterprise. Production costs exceed $1000 per acre in most areas and are double that in others (USDA 1988). A significant portion of production cost goes for pest control. Estimated 1992 costs for controlling Colorado potato beetle are as follows (personal communication with Dr. J. Wyman, University of Wisconsin): Long Island NY - $300-350/A; Atlantic states - $100-300/A; Maine $50-150/A; Michigan - $100-125/A; Wisconsin - $20-30/A; Central Minnesota - $50-150/A; Red River Valley (Minnesota, North Dakota) - $30/A; Idaho - $30-60/A (includes cost of aphid control); Washington/Oregon - $150-200/A (includes cost of aphid control); and Colorado - $0-10/A.

In the most simplistic analysis, a CPB-resistant potato would mean that grower costs would be reduced by the amount of their current control costs. On the other hand, costs

would increase by the amount of premium paid for the transgenic seed potatoes. If the cost of the seed is less than the current control costs the growers are better off.

The simplistic analysis, however, ignores differences in yield. Since current control methods are becoming inadequate, many growers are suffering yield losses due to defoliation by Colorado potato beetles. Although current yield losses have not been quantified, the fact that there is a yield loss suggests a much larger benefit to growers. One of the conclusions in the study by Gotsch (1993) was:

"technologies which cause an increase in yield (e.g. virus resistant potato) are more competitive than those which only cause a reduction in pesticide or mineral fertilizer input."

In the case of CPB-resistant potatoes, there would be a two-fold impact: increased yields as well as lower pesticide use.

Knutson (1993) estimates that under a zero-insecticide-use scenario production costs per hundredweight would increase 36 percent in Idaho, 113 percent in Maine and 88 percent in North Dakota. The cost-saving impact of CPB-resistance under current conditions would be less than those numbers, but would undoubtedly be significant.

Impacts on Acreage, Supply, Prices and Profits

In addition to impacts on yields and costs, CPB-resistant potatoes would also impact acreage, supply prices and profits. Assuming no changes in potato demand or potato acreage, higher potato yields would mean lower potato prices. Demand and acreage, however, would not remain the same. Guenthner et al. (1991) found that demand for U.S. potatoes is increasing at the rate of 2 to 3 percent per year. The growth in demand would tend to moderate the price impact of increases in supply.

Guenthner (1992c) has also found that growers respond to changes in potato prices by adjusting their acreage planted (response to changes in costs was insignificant). The response to lower prices is to reduce acreage, which in turn has a positive influence on prices for the next crop. Since potato growers are free to increase or decrease acreage without government controls, CPB-resistant potatoes would cause the potato market to head toward a new equilibrium.

It is likely that the new equilibrium would be at lower acreage, larger total supply, and reduced prices. It is also expected that profits would increase for those growers who adopt the new technology.

Impacts on Small Farms

Both small and large growers would benefit from CPB resistance. A new technology is scale-neutral if the investment required to adopt it is directly proportionate to the size of the farm (Kalter and Tauer 1987, Office of Technology and Assessment 1986). Buckwell and Moxey (1990) concluded that biotechnology is scale neutral in principle.

Since grower investment in transgenic potatoes depends only on the amount of seed purchased and not on new expensive equipment, the product fits into this category. Moreover, Tweeten and Welsh (1987) say:

"Biotechnologies on the horizon do not promise to revolutionize the structure of agriculture (farm size, numbers, legal organization, tenure, etc.) as much as the tractor and its complements already have. A major reason is that biotechnology is much less scale-biased toward large units than is mechanical technology."

In order for small farmers to adopt new technology two additional things are required:

1. accessibility and availability of the product and

2. the human capital or expertise needed for adoption (Bennett 1982, Volti 1992).

The two requirements are easily met with the CPB-resistant product. Since the existing network of North American seed potato growers will be used to distribute the product, small growers will be able to buy the product through their traditional seed potato sources. No additional expertise is needed to plant the CPB-resistant product. Growers would simply plant it like any other seed potato. Expertise to manage the crop would be less than that needed to control CPB under present conditions.

OFF-FARM IMPACTS OF CPB-RESISTANT POTATOES

Impacts on Consumers and Trade

The impact of agricultural biotechnology is not limited to farms. Increased supplies and lower prices would increase consumer net benefits (Stanley 1991), increase exports and decrease imports. Since the U.S. potato industry is increasingly dependant on overseas markets (Lin, et al. 1992) the positive trade impacts would be welcome.

Impacts on Agricultural Supply Businesses

CPB-resistant potatoes could cause reduced sales for some agribusinesses. An obvious group would be those who currently provide CPB control materials and services. Since long run control depends on an integrated approach in order for host plant resistance to continue to be effective, the demand for alternate methods would not disappear.

Other agribusinesses whose sales are directly related to potato acreage may be hurt. Since more potatoes could be grown on fewer acres with CPB-resistant seed, demand for services such as custom spraying might be reduced. On the other hand, businesses whose sales are related to total potato volume, such as those that sell handling or storage materials, might gain sales.

Impacts on Potato Processors

Another important group of agribusinesses is potato processors. Due to transportation economics, most potato processing facilities are very close to the rural communities where potatoes are grown. The only exception is the potato chip industry, where plants are located near population centers. The facilities for fresh packing, dehydrating and freezing are almost always located in potato growing regions (Greig and Blakeslee 1988).

The Colorado potato beetle threatens the continued production of potatoes in some regions (Hare 1980, Ferro et al. 1983). Since facilities are located near production and it is not feasible to move existing facilities, CPB presents a very real threat to potato processors and fresh shippers. An effective control method would provide the firms and their employees more stability than they now have.

Impacts on Rural Communities

Some sociologists concerned about the decline of rural communities have blamed agricultural technology. Economists disagree. Non-agricultural technology likely has more to do with the decline in rural communities than does agricultural technology. Modern transportation and communications technology in particular have strengthened links between rural residents and larger communities, making links with smaller communities less vital (Tweeten and Welsh 1987).

Potato input supply firms and potato processing firms provide employment, pay local taxes, buy local products and provide the economic foundation of many rural communities. Money paid by processors to growers, the local labor force, local businesses and local governments is spent and re-spent on other goods and services, partly within the local community. Robison, et al. (1993) found that one-third of the economic activity in Eastern Idaho was linked to the potato industry. That is not just for one small community but an entire region with urban centers, manufacturing, nuclear research, mining, forestry, tourism and other important industries.

Other growing regions in the Midwest and East that are more severely threatened by CPB may be just as dependant on the potato industry. Businesses and families have made significant investments in local potato-driven communities. Many of those communities are currently threatened by a pest, the Colorado potato beetle, that is becoming increasingly difficult to control. CPB-resistant potatoes can offer these communities economic stability.

CONCLUSIONS

The Colorado potato beetle is the most serious potato insect problem in North America. Declining availability of effective systemic insecticides and increasing resistance to foliar sprays are making the problem worse.

The exact economic benefits of new biotechnologies are difficult to forecast before the technology is introduced, but benefits to both growers and consumers have been

estimated in several recent studies. Likely results of CPB-resistant potatoes include: reduced yield loss lower costs of production, increased supply, lower grower prices, higher grower profits, increased exports, decreased imports, and lower prices to consumers. In addition to growers and consumers, agribusinesses and rural communities would benefit.

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APPENDIX 3

THE T-DNA SEQUENCE, DELINEATED BY THE RIGHT AND LEFT BORDERS, OF THE PLASMID PV-BTST02.

APPENDIX 3. THE T-DNA SEQUENCE, DELINEATED BY THE RIGHT AND LEFT BORDERS, OF THE PLASMID PV-BTST02.

Genetic endpoints are marked with arrows and bp numbering is shown on the right side. Locations of the restriction sites used in the Southern analysis, described in Section V, are indicated with vertical arrows.

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APPENDIX 4

AMINO ACID SEQUENCE FOR THE *B.t.t.* AND NPTII PROTEINS PRESENT IN THE POTATO PLANTS MODIFIED WITH THE VECTOR PV-STBT02

APPENDIX 4. AMINO ACID SEQUENCE FOR THE *B.t.t.* AND NPTII PROTEINS PRESENT IN THE POTATO PLANTS MODIFIED WITH THE VECTOR PV-STBT02.

A. *B.t.t.* Protein Sequence:

```
  1  MTADNNTEAL DSSTTKDVIQ KGISVVGDLL GVVGFPFGGA LVSFYTNFLN
 51  TIWPSEDPWK AFMEQVEALM DQKIADYAKN KALAELQGLQ NNVEDYVSAL
101  SSWQKNPVSS RNPHSQGRIR ELFSQAESHF RNSMPSFAIS GYEVLFLTTY
151  AQAANTHLFL LKDAQIYGEE WGYEKEDIAE FYKRQLKLTQ EYTDHCVKWY
201  NVGLDKLRGS SYESWVNFNR YRREMTLTVL DLIALFPLYD VRLYPKEVKT
251  ELTRDVLTDP IVGVNNLRGY GTTFSNIENY IRKPHLFDYL HRIQFHTRFQ
301  PGYYGNDSFN YWSGNYVSTR PSIGSNDIIT SPFYGNKSSE PVQNLEFNGE
351  KVYRAVANTN LAVWPSAVYS GVTKVEFSQY NDQTDEASTQ TYDSKRNVGA
401  VSWDSIDQLP PETTDEPLEK GYSHQLNYVM CFLMQGSRGT IPVLTWTHKS
451  VDFFNMIDSK KITQLPLVKA YKLQSGASVV AGPRFTGGDI IQCTENGSAA
501  TIYVTPDVSY SQKYRARIHY ASTSQITFTL SLDGAPFNQY YFDKTINKGD
551  TLTYNSFNLA SFSTPFELSG NNLQIGVTGL SAGDKVYIDK IEFIPVN
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B. NPTII Protein Sequence:

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  1  MIEQDGLHAG SPAAWVERLF GYDWAQQTIG CSDAAVFRLS AQGRPVLFVK
 51  TDLSGALNEL QDEAARLSWL ATTGVPCAAV LDVVTEAGRD WLLLGEVPGQ
101  DLLSSHLAPA EKVSIMADAM RRLHTLDPAT CPFDHQAKHR IERARTRMEA
151  GLVDQDDLDE EHQGLAPAEL FARLKARMPD GEDLVVTHGD ACLPNIMVEN
201  GRFSGFIDCG RLGVADRYQD IALATRDIAE ELGGEWADRF LVLYGIAAPD
251  SQRIAFYRLL DEFF
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APPENDIX 5

USDA PERMIT FINAL REPORTS

1991 TRIAL OF POTATOES TOLERANT TO COLORADO POTATO BEETLE

(USDA PERMIT #91-011-04)

FINAL REPORT

Gregory B. Parker
Monsanto Co.

This trial of genetically modified Russet Burbank potatoes was a field evaluation of transgenic potatoes expressing a gene conferring tolerance to feeding by the Colorado Potato Beetle. The objective of the trial was to evaluate the efficacy of the gene in controlling feeding damage by Colorado Potato Beetle (CPB) under field conditions. It was conducted by Monsanto in collaboration with Oregon State University, Hermiston, Oregon; University of Wisconsin, Hancock, Wisconsin; and USDA/ARS, Moxee, Washington during May - October, 1991.

Experimental layout

Planting material consisted of transplants of Russet Burbank transformed with a gene from *Bacillus thuringiensis* ssp *tenebrionis* coding for an insecticidal protein toxic to CPB, and non-transgenic Russet Burbank transplants and seed pieces. Transplants were produced at the Ashland, Maine facilities of the Maine Seed Potato Board and transported to the release sites in accordance with USDA permit number 91-011-05. Fifty lines of transgenic Russet Burbank (RB-Btt) plants and additional non-transgenic Russet Burbank lines were arranged in four replications of a randomized complete block design. Up to 40 plants per plot were transplanted. In some plots at some locations, fewer than this number were planted. At each site, transgenic plants occupied an area of approximately 0.75 acre. The whole trial occupied approximately 1.0 acre. Agronomic practices of pest control (except for CPB) and irrigation typical of irrigated potatoes in the area were followed. Major operations conducted during the field trial at each site are listed in Tables 1 - 3.

Plant growth and general observations

Transplant survival was excellent. Plants developed normally during the season. Most lines were indistinguishable from non-transgenic Russet Burbank. The few lines which showed vine abnormalities were characterized by less vigorous vine growth and atypical vine and leaf morphology. These abnormal lines were not selected for further evaluation. Most lines had tubers of a size and shape consistent with those of non-transgenic Russet Burbank grown from transplants. Atypical tubers were generally smaller

and/or rougher in appearance than typical tubers. Yield, specific gravity, and processing characteristics of selected RB-Btt lines were within the range of variability of non-transgenic Russet Burbank lines in the trial.

The plots were regularly monitored for *Agrobacterium* infection symptoms. None could be found.

Responses to specific issues:

1) Horizontal movement:

No evidence of movement of the CPB tolerance trait was observed.

2) Changes in survival characteristics:

There was no evidence of changes in the survival characteristics of the transgenic potato plants. Volunteer potato plants were observed at all locations during the next growing season. The number observed was typical for non-transgenic potatoes given the mild winter.

3) Expression level of the genes:

The expression level of the Btt gene was assessed by comparing defoliation caused by larval and adult CPB in transgenic lines to that in non-transgenic lines, as well as by Western blot. Expression of the gene resulted in virtually complete protection against feeding by all stages of the CPB despite heavy insect pressure during the growing season.

4) Stability and inheritance of the new genes:

Because potatoes are vegetatively propagated, no Mendelian analysis of inheritance was performed. Our experience to date indicates trait stability over several generations of vegetative propagation.

5) Published data:

At this point, we are not aware of any published data by Monsanto for this specific test.

Table 1. Schedule of major operations - Hermiston, Oregon

5/3/91	Received transplants from Maine; USDA permit number 91-011-05. Plants placed in greenhouse in accordance with protocol, awaiting field preparation.
5/30/91	Transplanting to field complete
9/23/91	Harvest 4,913 pounds of tubers. Soil disked and planted to barley.
10/9/91	Tubers not saved for sampling disposed of in trench as per protocol.
12/31/91	11,369 pounds of tubers picked up from Moxee and transported to Hermiston for grading in accordance with USDA permit number 91-263-01M.
4/7/92	Last of remaining tubers at Hermiston buried in trench

Samples were shipped to Monsanto in St. Louis and to Aberdeen, Idaho for further analysis in contained facilities under USDA permit #91-196-04 and #91-260-01M, respectively. All tubers in samples not destroyed as part of analytical procedures have been destroyed in accordance with conditions of this latter movement permit.

Table 2. Schedule of major operations - Moxee, Washington

5/3/91	Received transplants from Maine; USDA permit number 91-011-05. Plants placed in greenhouse in accordance with protocol, awaiting field preparation.
5/22/91	Transplanting to field complete
10/15-16/91	Harvest 11,369 pounds of tubers.
11/15/91	Remaining tubers lifted and allowed to remain on soil surface for freezing per conversation with Terry Ely, local APHIS inspector.
12/31/91	11,369 pounds of tubers picked up from Moxee and transported to Hermiston for grading in accordance with USDA permit number 91-263-01M.
3/18/92	Overwintered tubers on surface disked into soil

Table 3. Schedule of major operations - Hancock, Wisconsin

5/17/91	Received transplants from Maine; USDA permit number 91-011-05. Plants placed in greenhouse in accordance with protocol, awaiting field preparation.
5/23/91	Transplanting to field complete
10/3-4/91	Harvest 9,379 pounds of tubers.
10/5/91	Field disked.
6/6/92	All remaining tubers from storage disposed of by field spreading and disking. Plant growth from tubers was killed by glyphosate application on July 27, 1992

Samples were shipped to Monsanto in St. Louis and to Aberdeen, Idaho for further analysis in contained facilities under USDA permit # 91-196-04 and 91-260-01M, respectively. Samples were shipped to Richland, WA, Caldwell, ID, and Moses Lake, WA in accordance with USDA permit # 91-276-02-M, 91-261-01-M, and 91-276-01-M, respectively. All tubers in samples not destroyed as part of analytical procedures have been destroyed in accordance with conditions of these movement permits.

1991 SEED INCREASE OF POTATOES TOLERANT TO COLORADO POTATO BEETLE

(USDA PERMIT #91-050-02) (Mons #91-036)

FINAL REPORT

Gregory B. Parker
Monsanto Co.

This release of genetically modified Russet Burbank potatoes was a seed increase of transgenic potatoes expressing a gene conferring tolerance to feeding by the Colorado Potato Beetle (CPB). It was conducted by Monsanto in collaboration with the Maine Seed Potato Board, Ashland, Maine, from June - October, 1991.

Experimental layout

Planting material consisted of transplants of Russet Burbank transformed with a gene from *Bacillus thuringiensis* ssp *tenebrionis* coding for an insecticidal protein toxic to CPB (RB-Btt), and non-transgenic Russet Burbank (RB) transplants and seed pieces. Transplants were produced at the Ashland, Maine facilities of the Maine Seed Potato Board. Fifty lines of RB-Btt plants and additional RB lines were planted in single blocks. Two blank rows separated the trial from any adjacent potatoes. Up to 400 plants per block were transplanted. Transgenic plants occupied an area of approximately 1.25 acre. The whole trial occupied approximately 1.75 acre. Agronomic practices of pest control and irrigation typical of irrigated potatoes in the area were followed. No commercial production of seed potatoes occurred within 20 feet of the trial. During the 1992 growing season, the plots were planted with short straw oats. In 1993, the plots will be planted with either oats, buckwheat, or ryegrass. Major operations conducted during the field trial are provided in Table 1.

Plant growth and general observations

Transplant survival was excellent. Plants developed normally during the season. Most RB-Btt lines were indistinguishable from RB. The few lines which showed vine abnormalities were characterized by less vigorous vine growth and atypical vine and leaf morphology. These abnormal lines were not selected for further evaluation. Most RB-Btt lines had tubers of a size and shape consistent with those of RB grown from transplants. Atypical tubers were generally smaller and/or rougher in appearance than typical tubers.

The plots were regularly monitored for *Agrobacterium* infection symptoms. None could be found.

Responses to specific issues:

1) Horizontal movement:

No evidence of movement of the CPB tolerance trait was observed.

2) Changes in survival characteristics:

There was no evidence of changes in the survival characteristics of the transgenic potato plants. Volunteer potato plants were not observed in 1992. The site was inspected weekly from June 15, 1992. The lack of volunteers was likely the result of the ground freezing to a depth of six feet during the winter.

3) Expression level of the genes:

The expression level of the Btt gene was not measured in this trial. These same lines were tested in other trials by comparing defoliation caused by larval and adult CPB in transgenic lines to that in non-transgenic lines, as well as by Western blot. Expression of the gene resulted in virtually complete protection against feeding by all stages of the CPB despite heavy insect pressure during the growing season.

4) Stability and inheritance of the new genes:

Because potatoes are vegetatively propagated, no Mendelian analysis of inheritance was performed. Our experience to date indicates trait stability over several generations of vegetative propagation.

5) Published data:

At this point, we are not aware of any published data by Monsanto for this specific test.

Table 1. Schedule of major operations - Ashalnd, Maine (all dates 1991)

June 10 Transplanting to field complete

September 22 Harvest 11,683 pounds of tubers.

Field disked within four weeks of harvest.

Seed tubers were shipped to Hermiston, OR and Riverhead, NY under USDA permit #92-007-04M and to Othello, WA, Hancock, WI, and Aberdeen, ID under #92-007-03M. The 7,500 pounds of tubers not shipped or replanted were destroyed in February, 1992 by spreading them out on the plot area to freeze.

1992 TRIAL OF POTATOES RESISTANT TO INSECTS, DISEASES AND THAT CONTAIN HIGH LEVELS OF SOLIDS

(USDA PERMIT # 91-360-01)
(MONSANTO # 91-098)

FINAL REPORT

John Cudnohufsky
HybriTech Seed International

This trial was a field evaluation of genetically modified potatoes expressing either a gene providing tolerance to feeding by the Colorado Potato Beetle (CPB), resistance to Potato Leaf Roll Virus (PLRV), resistance to Potato Virus Y (PVY) or increasing the solids levels in tubers. It consisted of eleven separate experiments. The experiments were conducted by Monsanto in collaboration with the Maine Seed Potato Board, Aroostook County, Ashland, Maine; a private seed grower in Otsego County, Michigan; Oregon State University, Umatilla County, Hermiston, Oregon; the University of Idaho, Canyon County, Parma, Idaho and Bingham County, Aberdeen, Idaho; USDA-ARS, Benton Co., Prosser, Washington, and the Washington State University, Grant County, Moses Lake, Washington during April - October, 1992.

EXPERIMENTAL LAYOUT

Trial 91-001: Horticulture and processing evaluation of second year Russet Burbank *Bacillus thuringiensis* subsp. *tenebrionis* (B.t.t.) lines at Aberdeen, ID and Othello, WA.

Purpose: Evaluate the horticulture and processing characteristics of Russet Burbank potatoes genetically modified to express the insecticidal protein from B.t.t. for control of the CPB.

Summary: Since the objective was to observe normal potato growth characteristics with no pest interference, the plots were kept insect and disease free. The plot design was a six replicate, randomized complete block. Plot size at Aberdeen, ID was three feet wide by 20 feet long. See

Table 1 for schedule of major operations. Plot size at Othello, WA was nine feet wide by eight feet long. See Table 2 for schedule of major operations. A 10 meter buffer separated each trial from adjacent potatoes. The total trial area at either location occupied an area less than 0.3 Acres. Eighteen lines of transgenics and two control lines were evaluated. See Appendix 1 for list of lines and agronomic data evaluated at Aberdeen,ID and Appendix 2 for Othello, WA data.

Overall, the transgenic potatoes grew as expected and were similar to the non-transgenics in horticultural characteristics. At both locations there were no differences in insect susceptibility between the transgenic lines. Despite pesticides spray applications, the controls had numerous CPB larvae and adults. Some non-transformed control and transformed potatoes demonstrated physiological abnormalities. At Othello, observations soon after the Russet Burbank potatoes emerged from the ground, showed both controls (non-transformed) and transformed plants having a "curling, distorted leaf shape and yellow coloration". These symptoms were variously attributed to a disease or herbicide carryover or heat stress. The symptoms lessened after one week, but a few plants still showed symptoms two weeks after they initially appeared. The plants generally grew out of the symptoms and there were no yield differences between transgenic and non-transgenic plants. Leaf roll symptoms, including inter-veinal chlorosis, were recorded later in the season, for both transgenics and controls. At Aberdeen, one transformed line had "more of the physiological seed problem" (which was attributed to heat stress, early in the season) than other lines, otherwise there was normal growth. See Appendices 1 and 2 for details at Aberdeen and Othello, respectively. All seed was tested before planting for the presence of virus, and had negative ELISA readings. The symptoms were inconsistent among plants within a plot. These symptoms were variously attributed to herbicide carryover, seed dormancy problems or heat stress.

There were no differences between transformed or non-transformed plants with respect to early blight, late blight, leaf roll, or mildew. See Appendix 5 for representative evaluation sheets. Occasionally, observations noted that transformed plants were shorter or larger than controls or had more or less yellow mottling.

There were no unusual or unexpected agronomic results which would pose

a threat to the environment.

All tubers in this trial were lifted and left on the soil surface. Only those lines that were selected for post-harvest tests were collected and stored. This trial area was not planted to potatoes in 1993. The plots were observed in spring 1993 and all volunteers destroyed.

Trial 91-002: Second year efficacy evaluations of B.t.t. lines at Hermiston, OR.

Purpose: Re-evaluate the efficacy of B.t.t. in plants against CPB populations.

Summary: Eight transformed potato lines and one control were evaluated. The line numbers are listed in Appendix 3. This trial had a randomized, non-replicated design. Plot size was 3 feet wide by 25 long. A ten meter buffer separated this trial from adjacent potatoes. The total trial area was less than 0.02 Acres. Natural infestations of CPB were allowed to occur. All transgenic lines provided complete control of CPB throughout the season. See Table 3 for schedule of major operations.

All lines met or exceeded the selection criteria when compared to the non-transformed controls. They will be tested again in future studies. There were no unusual or unexpected agronomic results which would pose a threat to the environment. All tubers in this trial were lifted and left on the soil surface. Only those lines that were selected for post-harvest tests were collected and stored. This trial area was not planted to potatoes in 1993. The plots were observed in spring 1993 and all volunteers destroyed.

Trial 91-003: First year efficacy, horticultural and processing evaluation of B.t.t. lines at Aberdeen, ID.

Purpose: Evaluate the efficacy of B.t.t. in plants against CPB populations and assess horticultural and processing characteristics of resistant lines compared to non-transgenic checks.

Summary: Plantlets of different lines of transgenic Russet Burbank, Shepody, Norchip, Atlantic and Superior potatoes were compared to non-transgenic controls. The plots were 3 feet wide by 20 feet long, and replicated four times. One border row was planted between each treatment row to provide an equal source of CPB. A ten meter buffer separated this trial from adjacent potatoes. The total plot area was less than 0.6 acres. See Table 1 for schedule of major operations. At planting, there were 35 lines of transgenic Russet Burbank, 22 lines of Shepody, 5 lines of Norchip, 26 lines of Atlantic, and 11 lines of Superior. Some lines were off-type and were discarded soon after planting. The line numbers that were evaluated full season are listed in Appendix 4.

Selection criteria used to choose lines for further study included plant vigor, foliage type, control of CPB, tuber type and yield. Post-harvest analyses of the best lines, included measuring bruising potential and fry color. The best lines from each variety will be tested in future studies. There were no unusual or unexpected agronomic results which would pose a threat to the environment. All tubers in this trial were lifted and left on the soil surface. Only those lines that were selected for post-harvest tests were collected and stored. This trial area was not planted to potatoes in 1993. The plots were observed in spring 1993 and all volunteers destroyed.

Trial 92-004: First year efficacy and horticulture evaluation of solids lines at Parma, ID.

Purpose: Evaluate the efficacy of inserted genes in mediating increased or modified solids production in potato tubers, and assess the horticulture and processing characteristics of transformed lines. A secondary objective for those lines also containing the B.t.t. gene is to assess the efficacy of the B.t.t. gene in controlling CPB.

Summary: Plants were observed for vigor, foliage type, yield, tuber type, bruising potential, and frying characteristics. Plot size was 3 feet wide by 10 feet long. See Table 4 for schedule of major operations. There were 326 lines of Russet burbank, 27 lines of Atlantic, and 28 lines of Norchip tested. The constructs that survived the selection criteria and were evaluated at the end of the season are listed in

Appendix 4. Several lines from each variety were observed to offer advantages over non-transformed controls. These lines will be observed in future tests. There were no unusual or unexpected agronomic results which would pose a threat to the environment. All tubers in this trial were lifted and left on the soil surface. Only those lines that were selected for post-harvest tests were collected and stored. This trial area was not planted to potatoes in 1993. The plots were observed in spring 1993 and all volunteers destroyed.

Trial 92-005: First year efficacy, horticulture and processing evaluation of PLRV lines at Prosser, WA and Parma, ID.

Purpose: Inoculate new transgenic lines of Russet Burbank potato for levels of resistance to PLRV and screen for efficacy under field conditions.

Summary: Potatoes were inoculated by placing 10-15 aphids infected with PLRV on each plant. Plot size was 3 feet wide by 20 feet long at Prosser and 3 feet wide by 25 feet long at Parma. See Table 5 and Table 4 for schedule of major operations at Prosser and Parma, respectively. There were 261 transgenic and 24 control lines planted at both locations. See Appendix 7 for list of 40 lines that were carried through to harvest and evaluated for net necrosis.

All tubers in this trial were lifted and left on the soil surface. Only those lines that were selected for post-harvest tests were collected and stored. This trial area was not planted to potatoes in 1993. The plots were observed in spring 1993 and all volunteers destroyed.

Trial 92-006: Second year efficacy, horticulture and processing evaluation of PLRV lines at Prosser, WA; Parma, ID; and Hermiston, OR.

Purpose: Determine if any lines selected from 1991 field trials possess commercial levels of resistance to potato leaf roll virus (PLRV).

Summary: The plot design was a two replicate, randomized design. These treatments were part of the first year screening trial and received the same treatments.

All tubers in this trial were lifted and left on the soil surface. Only those lines that were selected for post-harvest tests were collected and stored. This trial area was not planted to potatoes in 1993. The plots were observed in spring 1993 and all volunteers destroyed.

Trial 92-007: PLRV tolerance in second year lines at Hermiston, OR.

Purpose: Verification of PLRV transgenic resistance under natural pressure.

Summary: There were 16 transgenic lines tested. The plants were arranged in a two replicate design and inoculated with PLRV infected aphids. See table 3 for schedule of major operations. Plot size was 3 feet wide by 20 feet long.

This trial was infected early in the season with late blight disease and the plants were in poor health during the season. Some transgenic lines demonstrated a low level resistance to PLRV compared to the control. The list of lines tested are indicated with an asterisk in Appendix 7.

All tubers in this trial were lifted and left on the soil surface. Only those lines that were selected for post-harvest tests were collected and stored. This trial area was not planted to potatoes in 1993. The plots were observed in spring 1993 and all volunteers destroyed.

Trial 92-008: CPB population dynamics

Purpose: To determine the effect on populations of predators and other beneficial and pest arthropods of Russet Burbank potato genetically modified to express B.t.t. protein toxic to CPB.

Summary: Transgenic Russet Burbank potatoes were planted in 54 foot x 54 foot blocks replicated six times in a latin square design. There were two transgenic treatments and four non-transgenic treatments. A 10 meter buffer separated this trial from adjacent potatoes. The plot size was less than 1.07 acres. See Table 3 for schedule of major operations. The trial compared the influence of different pest

management practices on the control of Colorado potato beetle (CPB) and the impact on other insects in potato agroecosystems. The results indicate that the second year transgenic seed grew true to type, controlled all stages of the CPB, and had yields equal to the non-transformed controls. Populations of certain beneficial and predator insects were higher in plots that contained transformed potatoes than in plots which used foliar insecticides. These early results indicate that transgenic potato plants which control CPB will be useful in Integrated Pest Management programs for potatoes. The line numbers evaluated are listed in Appendix 3.

There were no unusual or unexpected agronomic results which would pose a threat to the environment. All tubers in this trial were lifted and left on the soil surface. Only those lines selected for post-harvest tests were collected and stored. This trial area was not planted to potatoes in 1993. The plots were observed in spring 1993 and all volunteers were destroyed.

Trial 92-010: Second year efficacy and horticulture evaluation of solids lines at Aberdeen, ID.

Purpose: Evaluate the efficacy of inserted genes in mediating increased or modified solids production in potato tubers, and assess the horticulture and processing characteristics of transformed lines.

Summary: Twelve second year lines were compared to one control in a replicated trial.

All tubers in this trial were lifted and left on the soil surface. Only those lines selected for post-harvest tests were collected and stored. This trial area was not planted to potatoes in 1993. The plots were observed in spring 1993 and all volunteers were destroyed.

Trial 92-011: Shrinkage and sprouting influenced PVY resistance trial in Hermiston, OR.

Purpose: Compare transgenic plants with and without insecticide treatments to evaluate virus resistance under field conditions. Tubers

will be stored and evaluated for the presence of PVY.

Summary: Russet burbank plots were six rows wide and fifteen feet long. Each plot was bordered with nontransgenic Russet burbank potatoes and surrounded with unplanted space for access to observation areas. See Table 3 for schedule of major operations.

Trial 92-014: Seed production trial

Purpose: Evaluate seed production practices with second year Bt lines

Summary: Russet Burbank potatoes were planted in a single row, non-replicated design. There was a 10 meter buffer separating the transgenic potatoes from adjacent potatoes. The total plot size was less than 0.2 acre. See table 6 and Table 7 for schedule of major operations at Maine and Michigan, respectively.

The line numbers evaluated are listed in Appendix 3. The transgenic plants grew identical to non-transformed controls. There were no unusual or unexpected agronomic results which would pose a threat to the environment.

All tubers in this trial were lifted, then harvested and stored for use in 1993 trials. This trial area was not planted to potatoes in 1993. The plots were observed in spring 1993 and all volunteers destroyed.

Plant growth and general observations

Transplant survival was fair to good. Plants from both plantlets or tubers generally developed normally during the season. All plants were examined closely for such common growth parameters as emergence, vigor, growth habit, leaf type, color, disease susceptibility, insect susceptibility, flowering, maturity, tuber type, yield, storage and processing characteristics. Even though there were minor individual differences between some plants, at some locations, the field results indicate that the potatoes transformed to control Colorado potato beetle or have resistance to PLRV or resistance to PVY or have higher solids, only have this single trait difference from the non-transformed controls. The transformed potatoes are usually indistinguishable from non-

transformed plants. Cooperators at all locations were required to observe the transgenics for *any differences* from the controls and record any possible adverse environmental effects. Overall, the results of the field trials showed that the transformed potatoes have similar horticulture traits to the non-transformed control potatoes and do not demonstrate any other competitive advantages in the environment. Lines which showed vine abnormalities were characterized by less vigorous vine growth and atypical vine and leaf morphology. These abnormal lines were not selected for further evaluation. Most lines had tubers of a size and shape consistent with those of non-transgenic Russet Burbank grown from transplants. Atypical tubers were generally smaller and/or rougher in appearance than typical tubers. Yield, specific gravity, and processing characteristics of selected RB-Btt lines were within the range of variability of non-transgenic Russet Burbank lines in the trial.

Responses to specific issues

1) Horizontal movement:

No evidence of movement of the CPB tolerance trait was observed.

2) Changes in survival characteristics:

There was no evidence of changes in the survival characteristics of the transgenic potato plants. Volunteer potato plants were observed at all locations during the next growing season. The number observed was typical for non-transgenic potatoes given the mild winter.

3) Expression level of the genes:

The expression level of the Btt gene was assessed by comparing defoliation caused by larval and adult CPB in transgenic lines to that in non-transgenic lines, as well as by Western blot. Expression of the gene resulted in virtually complete protection against feeding by all stages of the CPB despite heavy insect pressure during the growing season.

4) Stability and inheritance of the new genes:

Because potatoes are vegetatively propagated, no Mendelian analysis of inheritance was performed. Our experience to date indicates trait stability over several generations of vegetative propagation.

5) Published data:

At this point, we are not aware of any published data by Monsanto for this specific test.

SCHEDULE OF MAJOR OPERATIONS
USDA release permit# 91-360-01

Table 1. Schedule of major operations - Idaho-Aberdeen

DATE	
4-16 and 5-1-92	Received tubers from Maine Seed potato board; USDA permit number 92-007-03M.
4-30-92	Received plantlets from St Louis, MO; USDA permit number 92-007-01M. Plants placed in greenhouse in accordance with protocol, awaiting field preparation.
5-7-92	Transplanting to field complete.
10-6-92	Harvest 11,402 pounds of tubers.
10-19-92	Field disked.
11-13-92, 1-6, 1-12-93	All remaining tubers from storage disposed of by field spreading and disking.

Samples were shipped to Monsanto in St. Louis , for further analysis in contained facilities under USDA permit # 92-223-01M. All tubers in samples not destroyed as part of analytical procedures have been destroyed in accordance with conditions of these movement permits.

Table 2. Schedule of major operations - Washington-Othello

DATE	
4-22-92	Received tubers from Maine; USDA permit number 92-007-03M.
	Seed was cut and stored in accordance with protocol, awaiting field preparation.
5-5-92	Transplanting to field complete.
10-5-92	Harvest 12,319 pounds of tubers.
10-28 and 12-16-92	Tubers not saved for sampling disposed of by putting back on field at test site as per protocol.
2-1-93	All remaining tubers from storage disposed of by field spreading and disking.

Samples were shipped to Monsanto in ST. Louis and to Aberdeen ID, for further analysis in contained facilities under USDA permit # 92-223-02M and 92-223-04M, respectively. All tubers in samples not destroyed as part of analytical procedures have been destroyed in accordance with conditions of these movement permits.

Table 3 Schedule of major operations - Oregon - Hancock

DATE	
4-14-92	Received tubers from McCains, Canada; USDA permit number 92-007-06M.
4-17-92	Received tubers from Wisconsin; USDA permit number 92-007-04M.
4-23-92	Received tubers from Maine; USDA permit number 92-007-03M.
5-21-92	Planting to field completed.
10-8-92	Harvest of 1750 pounds of tubers completed. 400 pounds are in storage for further tests and for planting in 1993 trials. Tubers not saved for sampling disposed of in trench as per protocol.

Samples were shipped to Monsanto in St. Louis and to Aberdeen ID, for further analysis in contained facilities under USDA permit # 92-223-01M and 92-223-04M, respectively.

All tubers in samples not destroyed as part of analytical procedures have been destroyed in accordance with conditions of these permits.

Table 4. Schedule of major operations - Idaho-Parma

DATE	Virus and solids
4-9, 4-29, 5-5-92	Received plantlets from St Louis; USDA permit number 92-007-01M. Plants placed in greenhouse in accordance with protocol, awaiting field preparation.
6-8-92	Transplanting to field complete.
9-22-92	Harvest 10,700 pounds of tubers.

Tubers not saved for sampling disposed of per protocol. All remaining tubers from storage disposed of by field spreading to allow freezing and disking.

Samples were shipped to Monsanto in St. Louis and to Aberdeen ID, for further analysis in contained facilities under USDA permit # 92-223-03M.

All tubers in samples not destroyed as part of analytical procedures have been destroyed in accordance with conditions of these movement permits.

Table 5. Schedule of major operations - Washington-Prosser

DATE	
4-14-92	Received plantlets from St Louis, MO; USDA permit number 92-007-01M. Received tubers from McCains; USDA permit number 92-007-06M. Plants placed in greenhouse in accordance with protocol, awaiting field preparation.
5-13-92	Transplanting to field complete.
9-23-92	Harvest of 4410 pounds of tubers completed. Field has been left fallow. 320 pounds are in storage for planting in 1993 trials. All other tubers have been put on the field site to be killed by freezing.

Table 6. Schedule of major operations - Maine -Ashland

DATE	
1-13-92	Seed potatoes were stored on site from the previous years field trial. USDA permit number #91-007-04.
	Received plantlets from St Louis, MO; USDA permit number 92-007-07M.
	Plants placed in greenhouse in accordance with protocol, awaiting field preparation.
6-8-92	Transplanting to field complete.
9-18-92	Harvest of 38,276 pounds of tubers completed.
10-15-92	Field cultivated with barber shank implement.
	Samples were shipped to Monsanto in ST. Louis and to Aberdeen ID, for further analysis in contained facilities under USDA permit # 92-223-01M and 92-223-04M, respectively. Samples were also shipped to Florida for winter tests in accordance with USDA field release permit # 92-262-02 and USDA movement permit # 92-174-06M.
	All tubers in samples not destroyed as part of analytical procedures have been destroyed in accordance with conditions of these movement permits.

Table 7. Schedule of major operations - Michigan-

DATE	
4-15-92	Received plantlets from New York; USDA permit number 92-007-02M. Plants placed in greenhouse in accordance with protocol, awaiting field preparation.
6-9-92	Transplanting to field complete.
9-4-92	Hand harvested 50 pounds of tubers.
10-92	All tubers disposed of by field spreading and disking. Field was disked in spring 1993.

Appendix 1

1992 Second year Bt agronomic data, Aberdeen, ID*, trial # 91-001

Treatment	-%- Stand	Emergence Abnormality† % Incidence	Emergence Abnormality† Severity	Total Yield (cwt/A)
RBBT02- 2	90	11	3.0	462
RBBT02- 4	93	23	4.7	414
RBBT02- 6	92	14	4.7	428
RBBT02- 9	91	10	2.7	416
RBBT02- 10	96	18	5.0	404
RBBT02- 11	91	22	5.2	402
RBBT02- 12	88	27	6.5	441
RBBT02- 13	91	18	5.5	443
RBBT02- 16	89	22	6.2	429
RBBT02- 17	88	12	4.3	439
RBBT02- 18	91	9	3.0	434
RBBT02- 19	97	27	5.7	399
RBBT02- 20	84	30	6.2	397
RBBT02- 21	91	21	5.2	405
RBBT02- 23	88	12	5.2	476
RBBT02- 25	86	22	6.3	436
RBBT02- 26	91	12	3.2	454
RBBT02- 28	93	26	6.3	428
RBBT 01 (control)	93	9	3.3	477
RBBT 02 (control)	96	23	5.2	452
LSD (.05)	NS	11	2.2	36

* Trial consisted of six replications, in a randomized complete block design. Treatments consist of the Russet Burbank variety transformed with construct PV-STBT02.

† Emergence abnormality quantifies the physiological phenomenon found in the seed of these clones in the spring of 1992. Incidence is the percent of abnormal plant, severity is a rating of the degree of expression of affected plants on a 0-10 rating with 10 =severe.

Appendix 2 - 1992 - Second year Bt agronomic data, Othello, WA, trial # 91-001

Treatment	% Stand 60 Day PP	% Abnormal Leaf Growth*	Total Yield (cwt/A)
RBBT02- 2	94	33	317
RBBT02- 4	92	55	402
RBBT02- 6	95	53	508
RBBT02- 9	98	20	419
RBBT02- 10	98	60	405
RBBT02- 11	96	78	475
RBBT02- 12	98	61	441
RBBT02- 13	94	65	489
RBBT02- 16	96	28	352
RBBT02- 17	84	95	423
RBBT02- 18	95	55	436
RBBT02- 19	94	25	337
RBBT02- 20	85	50	354
RBBT02- 21	74	55	372
RBBT02- 23	98	50	396
RBBT02- 25	93	57	400
RBBT02- 26	97	61	422
RBBT02- 28	92	78	450
RBBT 01 (control)	93	18	423
RBBT 02 (control)	97	5	434

* Trial consisted of six replications, in a randomized complete block design. Treatments consist of the Russet Burbank variety transformed with construct PV-STBT02.

Appendix 3.

List of genetically modified lines used in field trials, #91-002, #91-008, #91-014.

RBBT02 - 06
RBBT02 - 10
RBBT02 - 12
RBBT02 - 16
RBBT02 - 17
RBBT02 - 18
RBBT02 - 23
RBBT02 - 28
RB Control

APPENDIX 4 - List of Bt genetically modified and control lines used in field trial #91-003.

RUSSET BURBANK	SHEPODY	NORCHIP	ATLANTIC
RBBT 04-1	SHBT 04-1	NCBT 04-2	ATBT 04-1
RBBT 04-2	SHBT 04-2	NCBT 04-3	
RBBT 04-3	SHBT 04-7	NC 01	ATBT 04-6
RBBT 04-4	SHBT 04-11	NC 02	
RBBT 04-5	SHBT 04-12		ATBT 04-12
RBBT 04-6	SHBT 04-18		ATBT 04-13
RBBT 04-7	SHBT 04-34		ATBT 04-14
RBBT 04-8	SHBT 04-41		ATBT 04-17
RBBT 04-10	SHBT 04-42		ATBT 04-24
RBBT 04-11	SHBT 04-44		ATBT 04-26
RBBT 04-13	SHBT 04-47		ATBT 04-27
RBBT 04-14	SH 01		ATBT 04-28
RBBT 05-1	SH 02		ATBT 04-30
RBBT 05-2			ATBT 04-31
RBBT 05-4			ATBT 04-32
RBBT 05-6			ATBT 04-33
RBBT 05-7			ATBT 04-36
RBBT 05-8			
RBBT 05-10			ATBT 04-40
RBBT 05-11			
RBBT 05-12			ATBT 04-43
RBBT 05-13			ATBT 04-44
RBBT 05-14			
RBBT 05-15			ATBT 04-47
RBBT 05-16			AT 01
RBBT 05-17			AT 02
RBBT 05-18			
RB 01			
RB 02			
RB 03			
RB 04			
RB 05			

Appendix 5 - Representative field evaluation forms.

Appendix 5

41-001

201

Monitoring for Disease/Insect/Weediness Characteristics

- Make observations at least once every 4 weeks during the growing season.
- Compare control versus transgenic lines for obvious differences using the following criteria:
  - DISEASE (resistance/susceptibility to diseases not specifically engineered to resist).
  - INSECTS (resistance/susceptibility to attack by insects not specifically engineered to resist).
  - WEEDINESS (less susceptibility to herbicides not specifically engineered to resist, unusual proliferation, etc.).
- Record observations below.

Observations about:

Disease No obvious differences.

Insects No obvious differences

Weediness No obvious differences

[CBI DELETED]

26 May 92
Date

IAREC, Othello, WA.
Site, city

Site, State

208

[redacted]

Field Monitoring for Disease/Insect/Weediness Characteristics

- Make observations at least once every 4 weeks during the growing season.
- Compare control versus transgenic lines for obvious differences using the following criteria:
  - DISEASE (resistance/susceptibility to diseases not specifically engineered to resist).
  - INSECTS (resistance/susceptibility to attack by insects not specifically engineered to resist).
  - WEEDINESS (less susceptibility to herbicides not specifically engineered to resist, unusual proliferation, etc.).
- Record observations below.

Observations about:

Disease No obvious differences which could be directly related to disease but see "unexpected occurrence report from 2 June 92.

Insects No obvious differences

Weediness No obvious differences by stated criteria but see "unexpected occurence ..." report from 2 June 92

observation 2 June 92

[CBI DELETED] 3 June 92
Date

IAREC O'Dell
Signature

Signature Title

000184

Appendix 5 91-001 209

Test number:

Unexpected occurrence or deviation from protocol

Note all unexpected occurrences that could impact the test results. Also note all deviations from the test protocol. When completed, send a copy of this form to the Study Director.

Unexpected occurrence/protocol deviation: Many of the plants in this study were observed to exhibit characteristics ~~to~~ not typical of a healthy Russet Burbank potato plant. The atypical plant showed curling distorted leaf shape and yellow coloration. Photos of symptoms were taken.

Why occurred: Suspected to be seed borne herbicide carryover.

What, if anything, was done to address this event: John Cushnuffsky was notified as well as Glen Regan. Terry Eli[SP?] observed the plots (APHIS)

Date(s) of ~~occurrence~~ observation: 6/2/92

Initial(s) of observer: JNR + DT

[CBI DELETED] Date: 6/3/92

41-001

AGRONOMIC TRAITS CHECKOFF

X= normal cultivar characteristics.
O= off-type-,resistant, or otherwise different from cultivar in any way.

	Physical character.						Disease Susceptibility								Insect Suscept.							
Line #	Plant vigor	Stunted growth	Chlorotic color	Leaflet shape	Flowering	Others?	Early Blight	Late Blight	Leaf spot	Rusts	Verticillium	Mildew	Others?	Aphids	Colorado potato beetle	Cutworms	Leafhoppers	Spider mites	Others?	Aberdeen Second year Bt Comments/description
Bt 2	X	X	X	X	X															
Bt 4	X	X	X	X	X															
Bt 6	X	X	X	X	X															
Bt 9	X	X	X	X	X															
Bt 10	X	X	X	X	X															
Bt 11	X	X	X	X	X															
Bt 12	X	X	X	X	X															
Bt 13	X	X	X	X	X															
Bt 16	X	X	X	X	X															
Bt 17	X	X	X	X	X															
Bt 18	X	X	X	X	X															
Bt 14	X	X	X	X	X															
Bt 20	X	X	X	X	X															
Bt 21	X	X	X	X	X															
Bt 23	X	X	X	X	X															
Bt 25	X	X	X	X	X															
Bt 26	X	X	X	X	X															
Bt 28	X	X	X	X	X															

date: 29 July 93

[CBI DELETED]

Appendix 6 - List of constructs that have passed the first year criteria.

RBHS 01
RBHS 02
RBHS 03
RBHS 05
RBHS 06
RBHS 07
RBHS 09
RBHS 10
RBHS 11
RBHS 13
RBMT 01
ATMT 01
NOMT 01

Appendix 7 - List of transgenic lines tested for virus resistance.

Russet Burbank

LR02-01*	LR11-02
LR02-02*	LR11-04
LR02-03*	LR11-06
LR02-04*	LR11-07
LR02-05*	LR11-09
LR02-06*	LR11-11
LR02-07*	LR11-12
LR03-01*	LR11-13
LR03-02*	LR11-16
LR03-03*	LR11-18
LR03-04*	LR11-22
LR03-05*	LR11-23
LR03-06*	
LR03-07*	
LR03-08*	
LR03-09*	
LR05-02	
LR05-25	
LR06-12	
LR07-33	
LR08-47	
LR09-04	
LR09-05	
LR09-10	
LR09-11	
LR09-13	
LR09-15	
LR09-22	
RB 02	
RB 04	

* Lines with an asterisk were evaluated for a second year. These lines were in trial # 92-006 at Prosser, Wa; Parma, ID and Hermiston, OR. and in trial#92-007 in Hermiston, OR.

1992 TRIAL WITH POTATOES THAT HAVE BEEN GENETICALLY IMPROVED TO CONTROL COLORADO POTATO BEETLE, BE RESISTANT TO VIRUSES AND CONTAIN HIGHER LEVELS OF SOLIDS.

(USDA PERMIT # 92-002-01) (MON # 91-099) (WI # 92-38)

FINAL REPORT

John Cudnohufsky
HybriTech Seed International

This trial was a field evaluation of genetically modified potatoes expressing either a gene providing tolerance to feeding by the Colorado Potato Beetle or increasing the solids levels in tubers. It consisted of eight separate experiments. There was no field release of potatoes genetically modified to resist viruses as requested in the permit, but experiments were conducted in a greenhouse. The experiments were conducted by Monsanto in collaboration with the University of Wisconsin, at the Hancock Research Farm, Waushara Co., WI and a private Wisconsin grower, Oneida County, WI during April - October, 1992.

EXPERIMENTAL LAYOUT FOR COLORADO POTATO BEETLE (CPB) RESISTANT POTATO TRIALS

Planting material consisted of transplants or seed pieces of potatoes transformed with a gene from *Bacillus thuringiensis* ssp *tenebrionis* coding for an insecticidal protein toxic to CPB, and non-transgenic transplants and seed pieces. Transplants or seed pieces were either produced at Monsanto facilities St. Louis, MO; Maine Seed Potato Board facilities at Ashland, Maine; a private seedling/tuber facility; or produced on the Hancock Research farm and transported to the release sites in accordance with USDA movement permit numbers 91-365-01M, 92-007-01M, 92-007-03M, 92-007-05M.

91 lines of transgenic potato plants modified to control the CPB and additional non-transgenic lines were arranged in one to six replications of a randomized complete block design. Up to 100 plants per plot were transplanted. In some plots, fewer than this number were planted. Transgenic plants occupied an area of approximately 0.1 to 0.4 acre. Each trial occupied approximately 0.25 to 1.5 acre. Agronomic practices of pest control (except for not controlling CPB in some trials) and irrigation practices typical of potatoes grown in the area were followed. Major operations conducted during the field trial are listed in Table 1.

Trial 91-001: Horticulture and processing evaluation of second year Russet Burbank B.t.t. lines.

Purpose: Evaluate the horticultural and processing characteristics of Russet Burbank potatoes genetically modified to express the insecticidal protein from *Bacillus thuringiensis var. tenebrionis* for control of the Colorado Potato Beetle.

Summary: Since the objective was to observe normal potato growth characteristics with no pest interference, the plots were kept insect and disease free. The plot design was a six replicate, randomized complete block. Plot size was three feet wide by 20 feet long. A 10 meter buffer separated the trial from adjacent potatoes. The total trial area occupied an area less than 0.6 acres. Eighteen lines of transgenics and two control lines were evaluated. See Appendix 1 for list of lines evaluated.

The emergence, early growth, insect damage and disease susceptibility characteristics were the same for transformed and non-transformed potatoes. Overall, the transgenics were equivalent to the control plants. Some non-transformed control potatoes had: "Upward curling of leaves, possibly virus", recorded. Similar symptoms were observed on some transformed plants. All seed was tested before planting for the presence of virus, and had negative ELISA readings. The symptoms were inconsistent (not all plants in a row would show them) and recorded as, "Virus ?" or "herbicide damage ?". These symptoms were initially attributed to herbicide carryover or seed dormancy problems and since they diminished over time, were not considered significant.

Occasionally, observations noted that transformed plants were shorter or larger than controls or had more or less yellow mottling, but these minor differences were not considered significant by the cooperators.

Observations at mid-season showed no differences in growth between controls and transformed potatoes. The "curled leaves" symptoms that were noted above, were not observed. Early dying (verticillium) was recorded on both transformed and control plants, with some transformed plants being either more or less susceptible than the controls. There were no differences between transformed or non-transformed plants with respect to early blight, late blight, leaf roll, or mildew. Tuber type and yields were equal to the non-transformed controls. See Appendix 1 for yield data. There were no unusual or unexpected agronomic results which would pose a threat to the environment. Overall, the transgenic potatoes were considered identical to the non-transformed controls.

The line numbers that were evaluated are listed in Appendix 1.

Trial 91-002: Second year efficacy evaluations of B.t.t. lines.

Purpose: Re-evaluate the efficacy of B.t.t. in plants against CPB populations.

Summary: Eight transformed potato lines and one control were evaluated. The line numbers are listed in Appendix 2. This trial had a randomized, non-replicated design. Plot size was 3 feet wide by 25 long. A ten meter buffer separated this trial from adjacent potatoes. The total trial area was less than 0.02 Acres. Natural infestations of CPB were allowed to occur. All transgenic lines provided complete control of CPB throughout the season.

Some "herbicide injury" symptoms were observed early in the season on both non-transformed and transformed potatoes, but the transformed potatoes had more plants with severe ratings. Plant vigor which was recorded two weeks later showed transformed potatoes having a range of vigor (some better and some less) than the controls. Mid-season evaluations showed no visual differences

in plant growth. All lines met or exceeded the selection criteria when compared to the non-transformed controls. They will be tested again in future studies. There were no unusual or unexpected agronomic results which would pose a threat to the environment.

Trial 91-003: First year efficacy, horticultural and processing evaluation of B.t.t. lines.

Purpose: Evaluate the efficacy of B.t.t. in plants against CPB populations and assess horticultural and processing characteristics of resistant lines
compared to non-transgenic checks.

Summary: Plantlets of different lines of transgenic Russet Burbank, Shepody, Norchip, Atlantic and Superior potatoes were compared to non-transgenic controls. The plots were 3 feet wide by 10 feet long, and replicated two times. Two border rows were planted between each two treatment rows to provide an equal source of CPB. A ten meter buffer separated this trial form adjacent potatoes. The total plot area was less than 1.3 acres. There were 19 lines of transgenic Russet Burbank tested, 19 lines of Shepody, 2 lines of Norchip, 24 lines of Atlantic, and 9 lines of Superior. The line numbers that were evaluated are listed in Appendix 3.

Selection criteria used to choose lines for further study included plant vigor, foliage type, control of CPB, tuber type and yield. Post-harvest analyses of the best lines, included measuring bruising potential and fry color. The best lines from each variety will be tested in future studies. There were no unusual or unexpected agronomic results which would pose a threat to the environment.

Trial 92-008: CPB population dynamics

Purpose: To determine the effect on populations of predators and other beneficial and pest arthropods of Russet Burbank potato genetically modified to express B.t.t. protein toxic to CPB.

Summary: Russet Burbanks (RBBT 02) were planted in 54 foot x

54 foot blocks replicated four times in a latin square design. There was one transgenic treatment and three non-transgenic treatments. A 10 meter buffer separated this trial from adjacent potatoes. The plot size was less than 1.07 acres. The trial compared the influence of different pest management practices on the control of Colorado potato beetle (CPB) and the impact on other insects in potato agroecosystems. The results indicate that the second year transgenic seed grew true to type, controlled all stages of the CPB, and had yields equal to the non-transformed controls. Populations of certain beneficial and predator insects were higher in plots that contained transformed potatoes than in plots which used foliar insecticides. These early results indicate that transgenic potato plants which control CPB may be useful in Integrated Pest management programs. There were no unusual or unexpected agronomic results which would pose a threat to the environment.

The line numbers that were evaluated are listed in Appendix 2.

Trial 92-009: CPB resistance management; refugia

Purpose: To examine options for managing the development of resistance to B.t.t. in CPB.

Summary: Transgenic Russet Burbank potatoes (RBBT 02 tubers stored from 1991 trials at Hancock, WI) were planted in pure stands and in mixtures with non-transgenic potatoes, within 12 foot by 12 foot cages. There were 40 to 48 plants per treatment in a cage, and each treatment was replicated three times in a randomized complete block design. Total plot size was less than 0.32 acres. A 10 meter buffer separated this experiment from adjacent potatoes.

The trial compared the influence of different pest management practices on the control CPB and the impact on other insects in potato agro-ecosystems. The results indicate that the second year transgenic seed grew true to type, controlled all stages of the CPB, and had yields equal to the non-transformed controls. Observations of CPB larvae and adult movement, and beneficial insects will be useful in determining the best resistant management programs for potatoes grown in Wisconsin. There were no unusual or unexpected

agronomic results which would pose a threat to the environment.

All tubers in these trials were lifted and left on the soil surface. Only those lines that were selected for post-harvest tests were collected and stored. This trial area was not planted to potatoes in 1993. The plots were observed in spring 1993 and all volunteers were destroyed.

Trial 92-014: Seed production trial

Purpose: Evaluate seed production practices with second year Bt lines

Summary: Russet Burbanks were planted in a single row, non-replicated design. There was a 10 meter buffer separating the transgenic potatoes from adjacent potatoes. The total plot size was less than 0.2 acre. The line numbers that were evaluated are listed in Appendix 2. The transgenic plants grew identical to non-transformed controls. There were no unusual or unexpected agronomic results which would pose a threat to the environment. See Table 2 for schedule of major operations.

All tubers in this trial were lifted, left on the soil surface, then harvested and stored for use in trials in 1993. This trial area will not be planted to potatoes in 1993. The plots were observed in spring 1993 and all volunteers destroyed.

EXPERIMENTAL LAYOUT FOR SOLIDS INCREASE TRIAL

Planting material consisted of transplants of potatoes transformed with a gene expressing carbohydrate metabolizing enzymes or increased phytohormones, and non-transgenic transplants. Transplants were either produced at Monsanto facilities, St. Louis, MO; or at the Maine Seed Potato Board facilities at Ashland, Maine and transported to the release sites in accordance with USDA movement permit numbers 91-365-01M, 92-007-01M, 92-007-03M, 92-007-05M.

210 lines of transgenic potato plants and additional non-transgenic lines were arranged in a two replication, randomized complete block design. Up to 20 plants per plot were transplanted. In some plots fewer than this number were planted. Transgenic plants occupied an area of approximately 1.4 acres. The entire trial occupied approximately 2.0 acres. Agronomic practices of pest control and irrigation typical of irrigated potatoes in the area were followed. Major operations conducted during the field trial at each site are listed in Table 1.

Trial 92-004: First year efficacy and horticulture evaluation of solids lines.

Purpose: Evaluate the efficacy of inserted genes in mediating increased or modified solids production in potato tubers, and assess the horticulture and processing characteristics of transformed lines. A secondary objective for those lines also containing the B.t.t. gene is to assess the efficacy of the B.t.t. gene in controlling CPB.

Summary: Plants were observed for vigor, foliage type, yield, tuber type, bruising potential, and frying characteristics. Several lines from each variety were observed to offer advantages over non-transformed controls. These lines will be observed in future tests. There were no unusual or unexpected agronomic results which would pose a threat to the environment.

The line numbers that were evaluated are listed in Appendix 4. All tubers in this trial were lifted and left on the soil surface. Only those lines that were selected for post-harvest tests were collected and stored. This trial area was not planted to potatoes in 1993. The plots were observed in spring 1993 and all volunteers destroyed.

Plant growth and general observations

Transplant and seed piece survival was excellent. Plants developed normally during the season. Most lines were indistinguishable from non-transgenic controls. The few lines which showed vine abnormalities were characterized by less vigorous vine growth and atypical vine and leaf morphology. These abnormal lines were not selected for further evaluation. Most lines had tubers of a size and shape consistent with those of non-transgenic Russet Burbank grown from transplants. Atypical tubers were generally smaller and/or rougher in appearance than typical tubers. Yield, specific gravity, and processing characteristics of selected RB-Btt lines were within the range of variability of non-transgenic Russet Burbank lines in the trial.

Responses to specific issues

1) Horizontal movement:

No evidence of movement from the test site of the CPB tolerance or virus resistance or high solids traits were observed.

2) Changes in survival characteristics:

There was no evidence of changes in the survival characteristics of the transgenic potato plants. Volunteer potato plants were observed at all locations during the next growing season. The number observed was typical for non-transgenic potatoes.

3) Expression level of the genes:

The expression level of the Btt gene was assessed by comparing defoliation caused by larval and adult CPB in transgenic lines to that in non-transgenic lines, as well as by Western blot. Expression of the gene resulted in virtually complete protection against feeding by all stages of the CPB despite heavy insect pressure during the growing season. The expression of the virus and solids genes was assessed by measuring disease susceptibility or solids increases at the end of the season.

4) Stability and inheritance of the new genes:

Because potatoes are vegetatively propagated, no Mendelian analysis of inheritance was performed. Our experience to date indicates trait stability over several generations of vegetative propagation.

5) Published data:

At this point, we are not aware of any published data for this specific test.

USDA release permit# 92-002-01

Table 1. Schedule of major operations - Hancock, WI

DATE	
4/15/92	Received tubers from Maine; USDA permit number 92-007-03M.
4/27/92	Received plantlets from St Louis, MO. USDA permit number 92-007-01M.

Plants placed in greenhouse and tubers stored in accordance with protocol, awaiting field preparation.

5/8/92	Planting of tubers started.
6/4/92	Planting of seed and transplants to field complete.
9/21/92	Harvest 14,968 pounds of tubers.
9/22/92	Tubers from trials shredded and field disked.

Fields were monitored in 1993 and volunteers destroyed with glyphosate. There were no differences is emergence of volunteers, between transgenic and control potatoes.

Samples were shipped to Monsanto in ST. Louis and to Aberdeen ID for further analysis in contained facilities, under USDA permits # 92-223-01M and 92-223-02M, and 92-223-04M respectively.

Up to 556 pounds of tubers are labeled and stored at the Hancock facilities for additional laboratory work.

All tubers in samples not destroyed as part of analytical procedures have been destroyed in accordance with permits.

USDA release permit# 92-002-01

Table 2. Schedule of major operations - WI

DATE

5-8-92 Received minitubers from Valley Tissue Culture; USDA permit number 92-007-07M.

Tubers stored in accordance with protocol, awaiting field preparation.

5-21-92 Transplanting to field complete.

9-22-92 Harvest 4700 pounds of tubers. All tubers stored for trials next year.

10-27-92 Field disked.

Samples were shipped to Monsanto in ST. Louis and to Aberdeen, ID for further analysis in contained facilities under USDA permit # 92-223-01M and 92-223-04M, respectively. 75 pounds shipped to Maine and Florida for winter field tests under USDA permit #92-262-02.

All tubers in samples not destroyed as part of analytical procedures or stored for trials the following year have been destroyed in accordance with conditions of these permits.

Appendix I.

1992 Wisconsin Yield Data, trial # 91-001*

Treatment	Total Yield		
RBBt02- 12	213	a	
RBBt02- 9	214	a	
RBBt02- 4	220	a	
RBBt02- 13	221	a	
RBBt02- 2	235	a	b
RBBt02- 19	235	a	b
RBBt02- 17	241	a	b
RBBt02- 26	243	a	b
RBBt02- 20	244	a	b
RBBt02- 16	244	a	b
RBBt02- 21	244	a	b
RBBt02- 28	245	a	b
RBBt02- 25	249	a	b
RBBt02- 10	249	a	b
RB Control	255		b
RBBt02- 18	258		b
RBBt02- 11	259		b
RBBt02- 23	261		b
RBBt02- 6	264		b

* Trial consisted of six replications, in a randomized complete block design.
The data from the two, independent controls was combined.

Significance level is $p < 0.05$. Means with different letters are significantly different from each other. Treatments consist of the Russet Burbank variety transformed with construct PV-STBT02.

Appendix 2.

List of genetically modified lines used in field trials, #91-002, #91-008, #91-009, #91-014.

RBBT02 - 06
RBBT02 - 10
RBBT02 - 12
RBBT02 - 16
RBBT02 - 17
RBBT02 - 18
RBBT02 - 23
RBBT02 - 28

APPENDIX 3
List of genetically modified and control lines used in field trial #91-003.

RUSSET BURBANK	SHEPODY	NORCHIP	ATLANTIC	SUPERIOR
RBBT 04-1	SHBT 04-1	NCBT 04-1	ATBT 04-1	SUBT 04-1
RBBT 04-2	SHBT 04-2	NCBT 04-2	ATBT 04-5	SUBT 04-
RBBT 04-3	SHBT 04-6	NCBT 04-3	ATBT 04-6	SUBT 04-3
RBBT 04-4	SHBT 04-7	NCBT 04-4	ATBT 04-10	SUBT 04-4
RBBT 04-5	SHBT 04-11	NCBT 01	ATBT 04-12	SUBT 04-5
RBBT 04-6	SHBT 04-12	NCBT 02	ATBT 04-13	SUBT 04-6
RBBT 04-7	SHBT 04-18		ATBT 04-14	SUBT 04-7
RBBT 04-8	SHBT 04-22		ATBT 04-17	SUBT 04-8
RBBT 04-11	SHBT 04-25		ATBT 04-24	SUBT 04-10
RBBT 04-13	SHBT 04-37		ATBT 04-26	SU 01
RBBT 04-14	SHBT 04-40		ATBT 04-27	SU 02
RBBT 05-2	SHBT 04-41		ATBT 04-28	
RBBT 05-4	SHBT 04-42		ATBT 04-30	
RBBT 05-7	SHBT 04-44		ATBT 04-31	
RBBT 05-11	SHBT 04-45		ATBT 04-32	
RBBT 05-12	SHBT 04-46		ATBT 04-33	
RBBT 05-14	SHBT 04-47		ATBT 04-36	
RBBT 05-17	SHBT 04-49		ATBT 04-38	
RBBT 05-10	SHBT 04-50		ATBT 04-40	
RB 05	SH 01		ATBT 04-41	
	SH 02		ATBT 04-43	
			ATBT 04-44	
			ATBT 04-45	
			ATBT 04-47	
			AT 01	
			AT 02	
			AT 03	
			AT 04	

Appendix 4
List of potato lines genetically modified to express high solids, trial
 # 92-004.

RUSSET BURBANK

RBHS 01-5
RBHS 01-23
RBHS 01-
RBHS 01-
RBHS 01-
RBHS 01-
RBHS 02-2
RBHS 02-5
RBHS 03-3
RBHS 03-
RBHS 03-
RBHS 03-
RBHS 03-
RBHS 05-
RBHS 06-
RBHS 07-
RBHS 09-
RBHS 10-
RBHS 12-
RBHS 13-
RBHS 14-

RB CONTROL

ATLANTIC

ATMT 01-
ATMT 01-
ATMT 01-
ATMT 01-
ATMT 01-
ATMT 01-
ATMT 01-

AT CONTROL

1992 CPB RESISTANT POTATO TEST
RIVERHEAD, NEW YORK
(USDA PERMIT # 92-002-02) (Monsanto # 91-100)

FINAL REPORT

Jennifer Feldman
HybriTech Seed International
Monsanto Agricultural Group

The purpose of field release #92-002-02 was to confirm the efficacy of eight Russet Burbank potato clones which had been genetically modified to express the *B.t.t.* delta endotoxin. Natural CPB infestations were allowed to occur, and insect pressure was compared in both transgenic and non-transgenic plots. Leaf and tuber samples were shipped to Monsanto Co. in Chesterfield, MO for analysis.

Experimental layout:

Eight transgenic *B.t.t.*-expressing Russet Burbank potato clones and a standard clone of Russet Burbank were shipped from the Maine Seed Potato Board to [CBI DELETED] [CBI DELETED] in Riverhead, NY. The seed tubers arrived on April 16, 1992 and were planted on May 5, 1992.

All plots consisted of a single 25 ft row, with potato plants spaced 12 inches apart in the row, for a total planting density of 15,557 plants per acre. Each transgenic plot was paired with a similar sized plot of standard Russet Burbank potatoes, with plots arranged lengthwise down the experimental field. Two or three rows of standard Russet Burbanks flanked each of the experimental plots. A 10m buffer area surrounding the entire plot was kept fallow during the season. The transgenic potatoes occupied 0.013 acres, and the entire plot area, including non-transgenic controls and buffers, occupied 0.48 acres.

No insecticides were applied for control of the Colorado potato beetles. The entire experimental area was sprayed once with Monitor and Lannate to control potato and melon aphids. Colorado potato beetle eggs, adults, and larvae were counted and defoliation was estimated weekly throughout the season in each plot. All plots were harvested for yield on September 29, 1992.

Plant growth and results:

Potato plants in the southern end of the experimental field emerged slightly later than those on the northern end. This difference was attributed solely to the position in the field, since both transgenics and non-transgenics were affected. All plots reached 90-

100% emergence by June 12, 1992.

All of the transgenic clones had a high degree of resistance to every stage of CPB. Transgenic potatoes had fewer CPB eggs, larvae, and adults season long (Table 1). These plants suffered minimal defoliation despite the movement of CPB adults into the plots from surrounding non-transgenic controls, which were totally defoliated by mid-July. The transgenic clones were not noticeably different from standard Russet Burbank except for the marked resistance to CPB. No differences in susceptibility to other disease or insect pests were observed .

The differences in CPB feeding damage were reflected in total plot yields (Table 1). Standard Russet Burbank plots which became completely defoliated produced an extremely low yield, in most cases less than the amount of potato seed planted. The yield of all of the transgenic Russet Burbank clones was extremely high in comparison.

Response to specific issues:

1) Horizontal movement

No evidence of the movement of the CPB resistance trait to surrounding plants was observed.

2) Changes in survival characteristics

There was no change in the ability of the transgenic potatoes to survive as compared to non-transgenics except in the plants' ability to withstand attack from the Colorado potato beetle. No volunteers were observed in the plot area in 1993.

3) Expression level of the genes

All transgenic clones appeared to express the *B.t.t.* gene at a high level, as each one provided season-long control of the CPB. The *B.t.t.* expression level was measured through leaf and tuber tissue samples which were analyzed by Monsanto Co., Chesterfield, MO.

4) Stability and inheritance of the new genes

Because potatoes are vegetatively propagated, no Mendelian analysis of inheritance was performed. The trait has been shown to be stable over 3 field generations of potato production. Tubers from this trial were not saved for further field studies.

5) Published data

Data from this trial has not been published.

Schedules of major activities:

April15-16, 1992

[CBI DELETED

Seed tubers were shipped to] Riverhead, NY. USDA permit number 92-007-03M.

May 5, 1992

Seed tubers were planted.

September 29, 1993

All plots were harvested for yield, and excess tubers were returned to the field for disposal.

October 23, 1993

Plot area was disced to destroy remaining tubers and to prepare the field for the following year.

1992 WINTER POTATO TRIALS IN FLORIDA AND HAWAII

(USDA PERMIT # 92-262-02) (MONSANTO # 92-089)

FINAL REPORT

David F. Hammond
HybriTech Seed International
Monsanto Co.

This releasese of genetically modified Russet Burbank, Atlantic and Shepody potatoes, expressing a gene conferring resistance to feeding and damage by Colorado Potato Beetle (CPB) was a post-harvest test of tuber samples from seed lots grown in Maine, New York and Wisconsin, to complete the seed potato certification requirements of the individual states for these seed potatoes. The plots of transgenic Shepody plants transformed to resist infection by PVY comprised a preliminary field screen of these lines to evaluate the resistance and the experimental protocol. This test was conducted by HybriTech in collaboration with the Maine Seed Potato Board from October 1992 through February 1993 at the Florida Farm of the Maine Seed Potato Board. Field testing was not conducted in Hawaii as planned and requested in the permit application.

Experimental Layout

FL-1:

The planting materials were tubers collected as samples from seed lots of potatoes harvested in the fall of 1992 in Maine, New York and Wisconsin. The tubers were shipped from the production sites to the Maine Seed Potato Board in Presque Isle, ME to be treated to break dormancy and subsequently shipped to the Florida Farm of the Maine Seed Potato Board for the field trial(Monsanto# 92-078, USDA# 92-174-06M). The samples from the seed lots consisted of up to 400 tubers / lot and these were planted the first week of December in an isolated plot at the Florida Farm. At least 10 meters separated the transgenic potatoes from any adjacent potatoes. The tubers were planted at 10" spacing in rows 36 inches apart. Following emergence of the plants and growth to a height of 6-12 inches the plants were observed to detect symptoms of virus infection that would have arisen from the mother plants becoming infected during the 1992 growing season in the respective states.

The trial occupied an area of less than 0.25 acre and included 3625 plants. All of the plants were transgenic for resistance to CPB. Agronomic practices including pest control, fertility and irrigation were typical for potato production in the area. A sprout inhibitor (maleic hydrazide) approved by the APHIS representative who inspected the site was applied to the plots during the growing season to prevent germination of any tubers that might have developed before the vines were destroyed. At the conclusion of the test the plants were destroyed by an herbicide routinely used for potato vine desiccation. The plot was disked immediately following vine death and left fallow for 4-6 weeks before a rotation crop of a sorghum-sudan grass hybrid was planted for summer cover. The cover crop was plowed in September 1993 and the area left fallow until planting of transgenic potatoes in a subsequent post-harvest seed potato trial in November 1993.

FL-2:

The experiment was not replicated and consisted of five rows. Each of the nine plots

consisted of up to 10 transgenic plants of the Shepody variety transformed to resist infection by PVY. Up to three PVY infected Shepody tubers were planted at the end of each plot. The tubers were planted at 10" spacing in rows 36 inches apart. At the end of January the plots were evaluated for visible symptoms of PVY infection. Leaves from each of the trangenic plants were collected and tested by ELISA to detect infection by PVY. Tubers from each plant were collected and tested by ELISA to detect PVY infection. Infection by PVY was detected in at least one plant of each transgenic line.

The total area occupied by these plots did not exceed 0.1 acre. Agronomic practices including pest control, fertility and irrigation were typical for potato production in the area except that the plots were not treated to control aphids. A sprout inhibitor approved by the APHIS representative who inspected the site was applied to the plots during the growing season to prevent germination of any tubers that might develop before the vines were destroyed. At the conclusion of the test the plants were destroyed by an herbicide routinely used for potato vine desiccation. The plot was disked immediately following vine death and left fallow for 4-6 weeks before a rotation crop of a sorghum-sudan grass hybrid was planted for summer cover. The cover crop was plowed in September 1993 and the area left fallow until planting of transgenic potatoes in a subsequent post-harvest seed potato trial in November 1993.

Plant Growth and General Observations

Emergence and growth were rapid and excellent. No virus infection was noted in the samples from the seed lots. Symptoms of infection were observed in the plots with the plants exposed to aphids and PVY inoculum from the infector plants. Several lines were considered to have abnormal vines in terms of less vigorous vine growth and abnormal leaf morphology. These lines were discarded from subsequent seed potato production in 1993. Tuber development was normal for the respective varieties and the time the plants were allowed to grow. No symptoms of infection were observed during monitoring that could be attributed to *Agrobacterium* .

Responses to Specific Issues

1) Horizontal movement

No evidence of movement of the CPB or PVY resistance traits was observed.

2) Changes in survival characteristics

There was no change in the ability of the trangenic potatoes to survive as compared to non-transgenics grown in the same field. No volunteers were observed during the fallow period in the spring following vine desiccation and none have been observed during the fall fallow period in 1993.

3) Expression level of the genes

The expression levels of the Btt and PVY coat protein genes were not measured in this trial. Field trials conducted in the summer of 1992 indicated that the plants resisted attack by CPB in a manner comparable to that observed in 1991.

4) Stability and inheritance of the new genes

Because potatoes are vegetatively propagated no Mendelian analysis of inheritance was performed. The trait has been stable over 3 field generations of potato production.

5) Published data

Data from this trial has not been published.

Table 1. Schedule of Major Activities - Presque Isle, ME and Homestead, FL

October, 1992 - Samples were shipped to Presque Isle for dormancy breaking treatment and to Homestead, FL for field planting

December 1, 1992 - Samples were planted at the Florida Farm

January 29-30, 1993 - Samples were evaluated visually for disease. Leaf samples were collected and assayed by ELISA for virus infection.

February, 1993 - Leaf samples were tested to confirm freedom from disease or infection, sprout inhibitor was sprayed on plots, vines were destroyed, plots were disked, plot area was left fallow until April, 1993

1993 TRIAL OF POTATOES RESISTANT TO INSECTS, DISEASES AND THAT CONTAIN HIGH LEVELS OF SOLIDS

(USDA PERMIT # 92-363-05) (MON # 92-159R)

FINAL REPORT

John Cudnohufsky, Hybritech Seed International, Inc.

USDA permit #92-363-05 allowed the field evaluation of genetically modified potatoes expressing either singly or in combination, a gene providing resistance to feeding by the Colorado potato beetle (CPB), resistance to Potato Leaf Roll Virus (PLRV), resistance to Potato Virus Y (PVY), or an increase in tuber solids content. Under this permit, thirty separate experiments were performed. The experiments were conducted by Monsanto and HybriTech Seed International, Inc. in collaboration with academic and private cooperators at the following locations:

Center, Colorado
Aberdeen, Idaho
Ashton, Idaho
Caldwell, Idaho (2 locations)
Grace, Idaho
Parma, Idaho
Island Falls, Maine
Monticello, Maine
Presque Isle, Maine
St. David, Maine
St. Agatha, Maine
Beltsville, Maryland
Lakeview, Michigan
Stanton, Michigan
Manhattan, Montana
Ronan, Montana
Freeville, New York
Lake Placid, New York
Beach, North Dakota
Rollette, North Dakota
Grand Forks, North Dakota
Lisbon, North Dakota
Wooster, Ohio
Echo, Oregon
Hermiston, Oregon
Rock Springs, Pennsylvania
Othello, Washington
Prosser, Washington

The proposed trials at Kimberly, Idaho and one of the Othello, Washington locations were not initiated.

EXPERIMENTAL PROGRAM

Trial 93-01-01: Efficacy, horticultural and processing evaluation of first year CPB resistant lines.

Purpose:

To evaluate the horticultural and processing characteristics of Russet Burbank potatoes genetically modified to express the insecticidal protein from *Bacillus thuringiensis var. tenebrionis* (*B.t.t.*) for control of the CPB.

Summary:

This experiment was a field evaluation of the efficacy of CPB resistant potatoes. The experiment was conducted in Freeville, NY in association with Cornell University. Transformed potato lines were grown from minitubers in plots 3 ft wide by 10 ft long, with each line replicated twice and arranged in a randomized complete block design. The total area occupied by transgenic plants did not exceed 0.15 acres. The entire plot area, including buffers and borders, was less than 1 acre in size. The plot was surrounded on all sides by a 10 meter buffer in which no potatoes were grown. A list of the vector constructs and line numbers evaluated is in Appendix 1. The schedule of major operations is contained in Table 1.

Since the purpose of the study was to evaluate the insect control properties of the transgenic lines, no insecticides were applied for the control of CPB except in protected control plots. Insecticides were applied to all plots for the control of potato leafhoppers when insect populations reached the treatment threshold.

The potatoes in most plots emerged uniformly and similarly to the control. Some lines were late emerging, which was probably related to the size and dormancy conditions of the minitubers. All experimental lines demonstrated good control of the Colorado potato beetle with no defoliation evident at any time during the season. Unprotected non-transgenic controls were defoliated by mid-season.

All transgenic lines demonstrated outstanding CPB control throughout the season. With the exception of their resistance to CPB, no other differences in susceptibility to diseases (PLRV, PVY, early or late blight, verticillium leaf spot or rusts) or insects (aphids, leafhoppers, mites, or cutworms) were observed between transgenic and non-transgenic potatoes. Some differences in plant vigor and size were observed. Those lines with abnormal growth characteristics were not selected for further evaluation. There were no unusual agronomic characteristics that would pose a threat to the environment.

All potatoes in this trial were harvested and graded for yield. Potatoes saved for post-harvest analyses were disposed of by spreading on the test site and allowing them to freeze. The plot area will not be planted to potatoes in 1994 and will be monitored for volunteers. Volunteers will be chemically or mechanically destroyed.

Trial 93-01-03: Efficacy and agronomic evaluation of CPB resistant and PVY resistant lines.

Purpose:

To evaluate the efficacy and agronomic characteristics of lines containing genes mediating both CPB resistance and PVY resistance.

Summary:

This experiment was conducted in Presque Isle, Maine in association with the University of Maine. Transgenic Russet Burbank, Snowden, and Shepody, lines were grown from plantlets for evaluation of PVY resistance. Each plot consisted of 8 transgenic plants and 4 non-transgenic Shepody plants which originated from a known 100% PVY infected seedlot. A list of the vector constructs evaluated is in Appendix 2. The plants were arranged in the row such that every transgenic plant was next to a non-transgenic infector plant on one side, in the following fashion:

x o x x o x x o x x o x

where x=transgenic plant, and o=non-transgenic infector plant. The plot rows were separated by 6 ft to allow human access throughout the season. The transgenic plants occupied an area of less than 0.15 acres, and the entire plot area was less than 1.6 acres in size. The trial was situated in an isolated field, with no other potatoes in the surrounding 10 meter area. Every non-transgenic infector plant was infested with green peach aphids in early July. The plants were evaluated by ELISA and rated twice during the season for PVY symptoms.

Most transgenic lines were comparable to the non-transgenic controls in growth habit. A few lines were slightly stunted or less vigorous or had different leaf shape than the controls of the same variety. These lines were not selected for further evaluation. Those lines with normal growth characteristics and exhibiting resistance to PVY will be further evaluated. No differences in susceptibility to diseases, other than PVY (PLRV, early or late blight, verticillium, leaf spot or rusts) or insects other than CPB (aphids, leafhoppers, mites, or cutworms) were observed between transgenic and non-transgenic potatoes.

All of the lines that exhibited resistance to PVY were lifted by hand, bagged, and saved for further testing. Non-transgenic potatoes were also lifted and saved. The potatoes were placed in a secure storage facility at the test location. Potatoes that were not saved for further testing were left in the field to freeze over the winter. The plot area will not be planted to potatoes in 1994 and will be monitored for volunteers. Volunteers will be chemically or mechanically destroyed. The schedule of major events are shown in Table 2.

Trial 93-01-05: Agronomic evaluation of CPB resistant potato lines.

Purpose:

To evaluate the agronomic traits of selected CPB resistant potato plants to ensure that the best experimental lines will meet or exceed current requirements of commercial growers and potato processors.

Summary:

Two plot designs were used in this experiment, one for evaluating second year seed pieces and one for third year seed pieces. A list of the vector constructs evaluated is in Appendix 1.

1) **Second year trial.**

This trial was conducted in Lakeview, MI, Hermiston, OR, and Presque Isle, ME and Aberdeen, ID. In all locations, the potatoes were grown using conventional irrigation, fertility, and pest management practices. Appendix 1B lists the vector constructs and plant lines evaluated at each site.

MICHIGAN

In Michigan, in cooperation with Michigan State University, CPB resistant potato lines were grown from minitubers. Each plot consisted of 30 minitubers spaced 8" apart in the row. The trial was replicated 4 times and arranged in a randomized complete block design. The transgenic potatoes occupied less than 0.09 acres, and the entire trial area, including buffers, borders, and non-transgenic controls, was less than 0.5 acres in size. The plot was surrounded by a 10 meter buffer in which no potatoes were grown. The schedule of major events is shown in Table 3.

The potatoes were slightly delayed in emergence, due to cool weather after planting. However, potatoes in all plots emerged eventually and final plant stand was excellent. Some plots, both transgenic and non-transgenic, experienced herbicide damage, which seemed to be caused by a combination of cool weather and sprout length at the time of application. All affected plants outgrew the damage within several weeks. The transgenic lines were judged to be of good type and vigor, and comparable to the controls in appearance and productivity. With the exception of their resistance to CPB, no other differences in susceptibility to diseases (PLRV, PVY, early or late blight, verticillium, rusts, or leaf spot) or insects (aphids, leafhoppers, mites or cutworms) were observed between transgenic and non-transgenic potatoes. The plot area will not be planted to potatoes in 1994 and will be monitored for volunteers. Volunteers will be chemically or mechanically destroyed.

OREGON

Oregon State University at the Hermiston Agricultural Research and Extension Center, evaluated Shepody and Russet Burbank CPB resistant potato lines. Both minitubers and tuber seed pieces were grown in plots 20 ft long and 3 ft wide. Each plot was replicated

6 times and arranged in a randomized complete block design. The area occupied by transgenic potatoes was 0.16 acres. The two varieties were maintained as separate blocks, but the total area of both, including buffers and borders, was less than 1.25 acre in size. The plot was surrounded by a 10 meter buffer in which no potatoes were grown. The schedule of major events is listed in Table 4.

The transgenic Shepody lines were substantially smaller than non-transgenic controls and emerged later. This difference is attributed solely to the size of the respective minitubers, since non-transgenic control minitubers were grown in a different greenhouse crop and were significantly larger in size. The transgenic plants caught up in size eventually, and only minor differences in growth habit were evident after mid-season.

Some leaf distortion was evident in transgenic and non-transgenic Russet Burbank plants grown from seed pieces, which was attributed to high soil temperatures during emergence. One line in particular, RBBT04-14, appeared to be particularly susceptible. This line was not selected for further evaluation. All plants outgrew the symptoms within two weeks, and it did not appear to have a lasting effect on growth habit or vigor. Yields of the transgenic lines were comparable to the controls.

With the exception of their resistance to CPB, no other differences in susceptibility to diseases (PLRV, PVY, early or late blight, verticillium, rusts, or leaf spot) or insects (aphids, leafhoppers, mites or cutworms) were observed between transgenic and non-transgenic potatoes. The plot area will not be planted to potatoes in 1994 and will be monitored for volunteers. Volunteers will be chemically or mechanically destroyed.

MAINE

This trial was conducted in cooperation with the University of Maine, and was carried out at the Aroostook Farm in Presque Isle. Atlantic, Shepody, and Russet Burbank CPB resistant potato lines grown from mini tubers were evaluated. Each plot was 20-25 ft long, and replicated four times. Each variety was grown in a separate block, and arranged in a randomized complete block design. The total area with transgenic potatoes was 0.15 acres and the entire trial area was less than 0.75 acres in size. The trial was separated from all other potatoes by at least 10 meters.

Some differences in plant size and vigor were evident as a result of field variability and slope. However, this did not appear to be a result of line-specific differences.

Because of its proximity to experimental disease (late blight) trials, the entire plot was heavily infected with late blight by the end of the season, which interfered with harvest. There did not appear to be any difference in susceptibility to this or any other diseases (PLRV, PVY, early blight, verticillium, rusts, or leaf spot) or insects (aphids, leafhoppers, mites or cutworms) between transgenic and non-transgenic potatoes. The schedule of major events is listed in Table 2.

No plant characteristics were observed in any of the trials that would suggest that the transgenic potatoes pose a threat to the environment. The plot area will not be planted to potatoes in 1994 and will be monitored for volunteers. Volunteers will be chemically or

mechanically destroyed.

IDAHO

The University of Idaho Experiment Station in Aberdeen, evaluated Atlantic, Shepody, and Russet Burbank CPB resistant potato lines. Each plot in this trial was a single row 25 ft long, and all lines were replicated 6 times. The transgenic potatoes occupied 0.33 acres, and the entire trial area was 1.5 acres in size. The plot was surrounded by a 10 meter buffer area in which no commercial potatoes were grown. The schedule of major events is shown in Table 5.

The Shepody and Atlantic plants were herbicide damaged in their early growth as a result of cool, wet conditions following application of the pesticide. Most plants recovered from the damage and resumed vigorous growth after about 2 weeks. There was no difference in the susceptibility of transgenic or nontransgenic lines to the herbicide. With the exception of their resistance to CPB, no other differences in susceptibility to diseases (PLRV, PVY, early or late blight, verticillium, rusts, or leaf spot) or insects (aphids, leafhoppers, mites or cutworms) were observed between transgenic and non-transgenic potatoes. The plot area will not be planted to potatoes in 1994 and will be monitored for volunteers. Volunteers will be chemically or mechanically destroyed.

2) Third year trial.

This trial was conducted at each of five U.S. sites: 1) Caldwell, ID on a private research farm; 2) Monticello, ME on a private farm, 3) Stanton, MI on a private farm, 4) Lisbon, ND on a private farm and, 5) a private farm in Echo, Oregon. The experiment in every location consisted of two row plots with 20 seed pieces each (25 ft maximum). Seven transgenic Bt-expressing lines (RBBT02-06, RBBT02-10, RBBT02-12, RBBT02-16, RBBT02-17, RBBT02-18, and RBBT02-23) were evaluated in relation to three control plots. The experiment was replicated 6 times (4 times in ME) and arranged in a randomized complete block design. The plots were separated within rows by 3-5 hills of red potatoes to serve as markers at harvest. The trial area was located within commercial potato fields in all sites except Caldwell, which was on a private research farm. In each site, the transgenic potatoes were surrounded by a 10 meter buffer area. In Michigan, Idaho, and Oregon, the buffer area contained potatoes which were maintained as part of the plot area. In North Dakota, the plot was separated from other potatoes by more than 10 meters. The transgenic potatoes covered 0.15 acres, and each trial in total was less than 1 acre in size.

The potatoes were grown with conventional fertility, pest, and irrigation management. All pests, including the CPB, were controlled. At harvest, the experimental potatoes were dug, bagged, and transported to the associated research farm nearby for grading. The potatoes were held in a secure storage facility for grading. When grading was completed the potatoes were disposed of. In some cases, the potatoes remain in storage for future studies.

With the exception of their resistance to CPB, no other differences in susceptibility to diseases (PLRV, PVY, early or late blight, verticillium, rusts, or leaf spot) or insects (aphids, leafhoppers, mites or cutworms) were observed between transgenic and non-

transgenic potatoes. All transgenic lines were similar to non-transgenic controls in terms of emergence, vigor, uniformity, and yield. No growth characteristics were observed that would be a threat to the environment. The plot areas will not be planted to potatoes in 1994 and will be monitored for volunteers. Volunteers will be chemically or mechanically destroyed. The schedule of major events for each site are shown in Tables 6a through 10.

Trial 93-01-06: Performance confirmation trial.

Purpose:

To test selected CPB resistant potato lines under commercially representative production conditions and identify those with the best CPB resistance, horticultural and processing characteristics.

Summary:

This trial was conducted at six locations: 1) University of ID-Caldwell Experiment Station, 2) University of ME Aroostook Farm, Presque Isle, ME, 3) Red River Valley Potato Growers' Experiment Station, Grand Forks, ND, 4) Pennsylvania State University Experiment Station at Rock Springs, PA, and 5) University of WA Experiment Station in Othello, WA. The vector constructs and plant lines evaluated at these locations are listed in Appendix 1C. The plots in ID, ME, PA and WA consisted of two 15' rows which were unreplicated and alternated with nontransgenic potatoes. No CPB control was provided. In the ND trial, each plot was one row of 20 ft. Transgenic CPB resistant potatoes were compared with other conventional and experimental insecticides in this trial, as well as with unprotected controls. The transgenic potatoes in all locations covered less than 0.2 acres, and the entire trial areas were less than 0.6 acres in size in all sites. The plots in all locations were separated from other potatoes by a distance of at least 10 meters.

At all locations, the transgenic lines demonstrated outstanding CPB control throughout the season. With the exception of their resistance to CPB, no other differences in susceptibility to diseases (PLRV, PVY, early or late blight, verticillium, rusts, or leaf spot) or insects (aphids, leafhoppers, mites or cutworms) were observed between transgenic and non-transgenic potatoes. In addition, all transgenic lines were similar to non-transgenic controls in terms of emergence, vigor, uniformity, and yield. The plot areas will not be planted to potatoes in 1994 and will be monitored for volunteers. Volunteers will be chemically or mechanically destroyed. The schedule of major events at each site are found in Tables 6A, 2, 11, 12 and 13.

Trial 93-01-07: CPB population dynamics.

Purpose:
To evaluate the effect of CPB resistant potatoes on beneficial and pest arthropods and to determine the need for supplementary insecticide applications

Summary:

This study represents the second year of a long-term effort to develop pest management practices for transgenic CPB resistant potatoes. The experiment was conducted at the Hermiston Agricultural Research and Extension Center (HAREC) in Hermiston, Oregon and at the Ohio State University Experiment Station in Wooster, OH. The transgenic potatoes grown in these trials were an assortment of lines containing the PV-STBT02 construct (RBBTO2-06, RBBT02-10, and RBBT02-12). The potatoes were mixed together before planting so that the plots had uniform planting material throughout. The two locations had similar, but not identical, experimental designs.

Each treatment represented experimental pest management regimes, simulating conventional (insecticidal) control of insect pests and experimental, "low impact" techniques utilizing beneficial arthropods. In Hermiston, each plot was 54' X 54'. Each treatment was replicated 6 times, and arranged in a Latin Square design. Two of the 6 treatments (0.8 acres) were planted to CPB resistant potatoes, while the remaining 4 treatments were non-transgenic Russet Burbank potatoes. The entire experimental area of this study was 3.4 acres. The block was separated from commercial potatoes by 10 meters.

In the Wooster, OH study, plots were 48' X 48' in size, replicated 4 times, and arranged in a randomized complete block. In this experiment, 3 treatments were composed of CPB resistant potatoes (0.63 acres), and three treatments were non-transgenic potatoes. The entire area of transgenic potatoes was less than 0.7 acres. The entire trial, including buffers and borders, was less than 2.0 acres in size. The trial area was surrounded by at least 10 meters of a non-potato crop on all sides.

All arthropods were monitored in the plots throughout the season to determine the effect of management practices on populations of beneficial and pest insects. The highest numbers of beneficial arthropods (lady beetles, big-eyed bugs, nabids, minute pirate bugs, and spiders) were collected in transgenic CPB resistant potatoes which did not receive supplemental insecticides. In Oregon, aphid populations in transgenic plots were maintained at a stable level throughout the season. In contrast, the aphid populations in the foliar insecticide (permethrin) plots, increased exponentially as natural enemies were eliminated. This same result occurred in Ohio, which demonstrates the importance of natural enemies in pest control. With the exception of CPB control and the resulting impact on pest management practices, no other differences in susceptibility to diseases (PLRV, PVY, early or late blight, verticillium, rusts, or leaf spot) were observed between transgenic and non-transgenic potatoes. The species of arthropods in transgenic plots was the same as that in untreated non-transgenic plots or those treated with a systemic insecticide. In addition, the transgenic plots were similar to non-transgenic controls in terms of emergence, vigor, and uniformity.

The plot areas will not be planted to potatoes in 1994 and will be monitored for volunteers. Volunteers will be chemically or mechanically destroyed. The schedule of major events are in Tables 4 and 14.

Trial 93-01-09: CPB resistance management - II.

Purpose:

A number of different pest management strategies are under consideration. This trial concentrated on using a mixed planting of CPB resistant and control potatoes to provide a refuge for susceptible individuals to dilute the frequency of resistance genes in the CPB population and, thereby, delay the development of CPB resistance.

Summary:

Atlantic potatoes transformed with the vector PV-STBT04 (ATBT04-06, ATBT04-14, and ATBT04-17) were grown at the Cornell University Vegetable Experiment Station at Freeville, NY. Plots were 2 rows wide and 15 ft long. Yields in plots with seed mix ratios (transgenic:non-transgenic) of 85:15, 70:30, and 55:45 were compared with 100% transgenic and 100% non-transgenic yields. Each treatment was replicated 6 times and arranged in a randomized complete block design. The total transgenic plot area was 0.05 acres, and the entire trial acreage was less than 0.5 acres. The trial was separated from all other potatoes by a distance of 10 meters. The schedule of major events appears in Table 1.

Natural CPB infestations were allowed to occur. Some CPB damage occurred to non-transgenic plants in mixed plantings, but feeding was generally suppressed by the presence of any transgenic plants. No other differences in susceptibility to diseases (PLRV, PVY, early or late blight, verticillium, rusts, or leaf spot) or insects (aphids, leafhoppers, mites or cutworms) were observed between transgenic and non-transgenic potatoes. In addition, the transgenic potatoes were similar to the non-transgenic potatoes in terms of emergence, vigor, and uniformity. No significant yield loss was detected in any of the mixed plantings, while the unprotected control produced very small yields due to CPB feeding. The plot area will not be planted to potatoes in 1994 and will be monitored for volunteers. Volunteers will be chemically or mechanically destroyed.

Trial 93-01-10: Seed production.

Purpose:

To increase the quantity of selected potato lines, while evaluating yield potential and phenotypic uniformity.

Summary:

Tubers or plantlets were planted in multi-row, non-replicated blocks and grown with production practices that are standard to each site location. All pests, including the Colorado potato beetle were controlled. There was a 10 meter buffer separating the transgenic potatoes from adjacent potatoes. The acreage planted and vector constructs evaluated at each location are listed in Appendix 3. All major operations for each site are listed in Tables 15 - 25.

The transgenic plants grew identically to the non-transformed controls at all locations. Observations by cooperators detected no differences in phenotypic traits of the foliage or tubers. Yields were comparable. In addition, no differences in susceptibility to diseases (PLRV, PVY, early or late blight, verticillium, rusts, or leaf spot) or insects (aphids, leafhoppers, mites or cutworms) were observed between transgenic and non-transgenic potatoes. There were no unusual or unexpected results which would pose a threat to the environment.

All tubers that met the selection criteria for certified seed were lifted, harvested and stored for use in 1994. Tubers not meeting the selection criteria were spread back on the surface of the plot area to freeze over the winter. The plot areas will not be planted to potatoes in 1994 and will be monitored for volunteers. Volunteers will be chemically or mechanically destroyed.

Trial 93-01-16: Efficacy comparison of CPB resistant potatoes and conventionally bred potato hybrids.

Purpose:

To evaluate the efficacy of CPB resistant potatoes in comparison to conventionally bred potato hybrid varieties.

Summary:

CPB resistant potatoes, cultivar Atlantic, containing plasmid vector PV-STBT04 (line number ATBT04-17) were compared to various non-transgenic potatoes for natural resistance to feeding by CPB in Beltsville, Maryland. The seed pieces were planted 1 foot apart in rows 42 inches wide. Each plot of 5 plants was separated by 2 feet in a row. There were 6 to 20 replications depending on seed piece variability. The area planted to transgenic potatoes was 0.2 acres and the entire plot area planted to both transgenics and borders was less than 1.0 acre. Conventional fertilizer and weed control practices were followed except that neither fungicides nor insecticides were applied. The transgenic Atlantic potatoes were planted nineteen days later than the other varieties. The major operations for the site are listed in Table 26.

Counts of all stages of CPB were made once, early in the season. Defoliation ratings were taken four times, to better indicate plant damage and plant resistance. With the exception of their resistance to CPB, no other differences in susceptibility to diseases (PLRV, PVY, early or late blight, verticillium, rusts, or leaf spot) or insects (aphids, leafhoppers, mites or cutworms) were observed between transgenic and non-transgenic potatoes. All transgenic lines were similar to non-transgenic controls in terms of emergence, vigor, and uniformity. No growth characteristics were observed that would be a threat to the environment. The plot area will not be planted to potatoes in 1994 and will be monitored for volunteers. Volunteers will be chemically or mechanically destroyed.

Trial 93-02-01: First year efficacy and agronomic evaluation of high solids lines.

Purpose:

To evaluate the efficacy, agronomic and processing characteristics of lines containing genes mediating increased solids production in tubers.

Summary:

First year efficacy trials were conducted at Aberdeen and Parma, Idaho. Transformed Russet Burbank, Atlantic, Norchip and Norkotah potatoes were examined in these trials. Plantlets were observed for vigor, plant type, growth uniformity, and maturity. Standard agronomic practices with regards to field preparation, fertilization, irrigation and pest control were conducted with these trials. Specific tuber evaluations included tuber type, yield, external tuber quality and bruising susceptibility. Plots were single rows, 12 feet long, replicated two times. Rows were spaced 34 - 36 inches apart. The area planted to transgenics at Aberdeen was 0.66 acres and at Parma 0.86 acres. The entire plot area planted to both transgenics and borders was less than 3.6 acres at either location. See Table 5 for schedule of major operations at Aberdeen and Table 27 for schedule of operations at Parma, ID.

Fourteen different constructs, representing numerous lines were evaluated. See Appendix 4A for list of constructs tested. 400 transgenic lines were examined at Aberdeen and 523 lines were examined at Parma. Several lines at each location were judged to offer advantages over the controls and will be observed in future tests. Lines not offering advantages over the controls were not selected for further evaluation.

Three agronomic evaluations were conducted at Parma, ID. They consisted of measuring the impact of potassium on the bruising potential of high solids potatoes; a comparison of the agronomic characteristics of plantlets, mini-tubers and seed pieces, and an evaluation of solids distribution in seed pieces. Each plot was a single row, 12 feet long. The different trials were replicated either four, seven or three times, respectively. Plants were also monitored for differences in vine type, growth uniformity, vigor, number of tubers, tuber weight, tuber grade and specific gravity and susceptibility to disease and insects.

No differences in susceptibility to diseases (PLRV, PVY, early or late blight, verticillium, rusts, or leaf spot) or insects (aphids, leafhoppers, mites or cutworms) were observed between transgenic and non-transgenic potatoes at either location. There were no unusual or unexpected results which would pose a threat to the environment. At harvest, all tubers were lifted and placed on the soil surface. Those lines which were selected for post-harvest tests were collected and stored. The trial areas will be observed for volunteers, which will be destroyed. The field will not be planted to potatoes in 1994.

Trial 93-02-02: Second year efficacy and agronomic evaluation of high solids lines

Purpose:
In second year trials, evaluate the efficacy, agronomic and processing characteristics of lines containing genes mediation increased solids production in tubers.

Summary:

Trials were conducted at Hermiston, Oregon and Presque Isle, Maine. All agronomic practices, with respect to field preparation, fertilization, irrigation and pest control, to grow potatoes were conducted standard to the region of the testing site. See Appendix 4B for a list of vector constructs and plant lines tested. See Table 2 for schedule of major operations at Presque Isle and Table 4 for schedule of operations at Hermiston.

Two different experiments were conducted at the Hermiston, Oregon site, one with plantlets and the second with mini-tubers. The plantlet plot design was 1 row, 20 feet long, with plantlets 12 inches apart in the row, replicated four times. 22 transgenic Russet Burbank lines were compared to two control lines. The second experiment compared mini-tubers of 13 transgenic potato lines to one control. The plot design was 1 row, 20 feet long, mini-tubers planted 12 inches apart in the row and one replication. The area planted to transgenic potatoes was 0.13 acres and the entire area was less than 2.0 acres.

The experiment at Maine compared mini-tubers of seven transgenic Russet Burbank lines to one control. Plot design was 1 row, 25 feet long, with mini-tubers planted 16 inches apart in the row, replicated four times. The area planted to transgenic potatoes was 0.06 acres and the entire area was less than 2.0 acres.

Plants were observed for emergence and phenotypic characteristics and disease and insect susceptibility throughout the season. No differences in susceptibility to diseases (PLRV, PVY, early or late blight, verticillium, rusts, or leaf spot) or insects (aphids, leafhoppers, mites or cutworms) were observed between transgenic and non-transgenic potatoes at either location.

Tuber samples were collected and observed for type, bruising potential, internal defects and frying evaluations. Several lines at each location were judged to offer advantages over the controls and will be observed in future tests. Tubers from these lines are being evaluated for long term storage characteristics. Lines not meeting this criteria were not selected for further evaluation.

There were no unusual or unexpected results which would pose a threat to the environment. The trial areas will be observed for volunteers, which will be destroyed. The field will not be planted to potatoes in 1994.

Trial 93-03-01: Field release for first year screening of potato leafroll virus (PLRV) resistance

Purpose:

To evaluate the effect of inserted genes conferring resistance to PLRV and assess the agronomic and processing characteristics of transformed lines. A secondary objective for those lines also containing the *Btt* gene was to assess the efficacy of this gene in controlling CPB.

Summary:

The plot design was two replicates arranged in a randomized block. Each replicate was one row (36") wide by 10 feet long at both Prosser, Washington and Parma, Idaho. There were 177 transgenic and 4 non-transgenic Russet Burbank lines planted at each location. See Appendix 5A for a list of vector constructs and plant lines evaluated.

The section of the trial containing multi-trait (MT) lines transformed with a construct with genes for both CPB resistance and PLRV resistance were separated from the rest of the trial area by a 10 meter fallow buffer. At each location, the area planted to transgenic potatoes was 0.3 acres and the entire area was less than 2.5 acres. All plants were inoculated with 10-15 PLRV infected aphids as described in permit protocol. After one week, the entire plot was sprayed with a registered aphicide to kill aphids.

All control lines and the transgenic lines in which less than 25% of the plants showed PLRV symptoms were harvested, stored for observation of net necrosis in tubers, and plant back PLRV indexing. All other lines were lifted and left on the soil surface to freeze.

No differences in susceptibility to diseases other than to PLRV (PVY, early or late blight, verticillium, rusts, or leaf spot) or insects other than CPB and aphids, after the aphicide application (leafhoppers, mites or cutworms) were observed between transgenic and non-transgenic potatoes at either location. In addition, the transgenic plots were similar to non-transgenic controls in terms of emergence, vigor, and uniformity. There were no unusual or unexpected results which would pose a threat to the environment.

Harvested tubers were held in a locked storage prior to examination of tuber net necrosis and plant back for PLRV indexing. All tuber and plant material was disposed of according to conditions of the field release permit. The plot areas will not be planted to potatoes in 1994 and will be observed for volunteers in spring 1994. Any volunteers will be chemically or mechanically destroyed. See Tables 27 and 28 for the schedule of major operations at Parma and Prosser, respectively.

Trial 93-03-02: Field release for second year screening of PLRV resistance

Purpose:

To evaluate the effect of inserted genes conferring resistance to PLRV and assess the agronomic and processing characteristics of transformed lines. A secondary objective for those lines also containing the *Btt* gene was to assess the efficacy of this gene in controlling CPB.

Summary:

The plot design was four replications in a randomized block design. Each replicate was one row (36") wide by 20 feet long. 13 transgenic and two non-transgenic control lines were evaluated. The area planted to transgenic potatoes was 0.1 acres and the entire area was less than 0.75 acres. See Appendix 5B for a list of vector constructs and plant lines evaluated.

These lines were evaluated as part of the first year screen and underwent the same inoculation procedure as described above for first year screen. No differences in susceptibility to diseases other than to PLRV (PVY, early or late blight, verticillium, rusts, or leaf spot) or insects other than CPB and aphids, after the aphicide application (leafhoppers, mites or cutworms) were observed between transgenic and non-transgenic potatoes at either location. In addition, the transgenic plots were similar to non-transgenic controls in terms of emergence, vigor, and uniformity. There were no unusual or unexpected results which would pose a threat to the environment. The plot areas will not be planted to potatoes in 1994 and will be monitored for volunteers. Volunteers will be chemically or mechanically destroyed.

Harvest procedures and disposal were the same as described above. See Tables 27 and 28 for schedule of major operations at Parma, Idaho and Prosser, Washington.

Trial 93-03-05: Effect of PLRV resistance on virus spread

Purpose:

To evaluate the effect of inserted genes conferring resistance to PLRV on plant to plant spread of PLRV in potato and subsequent impact on the agronomic and processing characteristics of transformed lines.

Summary:

The plot was arranged in a randomized block design with four replications per treatment (line) at Prosser, Washington. Each replication was one row (36") by 21 ft long, with 9 ft of fallow ground between each row. Three non-transgenic control lines (2 Russet Burbank and 1 BelRus) and 7 transgenic lines were tested. The area planted to transgenic potatoes was 0.10 acres and the entire area, including buffer rows, was less

than 0.5 acres. Appendix 5C lists the vector constructs and plant lines evaluated.

Planting material for this trial were rooted cuttings propagated at the Prosser experiment station by the primary cooperator. A single central spreader plant in each row was inoculated with viruliferous aphids, 3-4 weeks after planting, and caged for one week. Virus was then allowed to spread within the row and several measurements were made on the rate of virus spread within each line.

No differences in susceptibility to diseases other than to PLRV (PVY, early or late blight, verticillium, rusts, or leaf spot) or insects (CPB, aphids, leafhoppers, mites or cutworms) were observed between transgenic and non-transgenic potatoes at either location. In addition, the transgenic plots were similar to non-transgenic controls in terms of emergence, vigor, and uniformity. There were no unusual or unexpected results which would pose a threat to the environment.

All control lines and the transgenic lines were harvested, stored for observation of net necrosis in tubers, and plant back PLRV indexing. Harvested tubers were held in a locked storage facility prior to examination of tuber net necrosis and plant back for PLRV indexing. All tuber and plant material was disposed of according to conditions of the field release permit. The plot areas will not be planted to potatoes in 1994 and will be monitored for volunteers in spring 1994. Volunteers will be chemically or mechanically destroyed. A summary of the major operations involved this trial are listed in Table 28.

Trial 93-03-06: Field exposure to natural PLRV strains

Purpose:

To evaluate the resistance of genetically modified potato lines to naturally occurring strains of potato leaf roll virus (PLRV) and the subsequent impact on the agronomic and processing characteristics of the lines.

Summary:

The trial was conducted at Hermiston, Oregon. The plot design was a randomized block with 4 replications. Each replication was 4 rows (12 ft) wide by 10 ft long and were separated from adjacent replications by 4 ft on each end and 4 rows on each side. The area planted to transgenic potatoes was 0.12 acres and the entire area, including buffer rows, was less than 1.0 acre. The vector constructs evaluated are listed in Appendix 5D. The border area was planted with non-transgenic BelRus, a PLRV resistant variety, to facilitate the objectives of the trial.

No differences in susceptibility to diseases other than to PLRV (PVY, early or late blight, verticillium, rusts, or leaf spot) or insects (CPB, aphids, leafhoppers, mites or cutworms) were observed between transgenic and non-transgenic potatoes at either location. In addition, the transgenic plots were similar to non-transgenic controls in terms of emergence, vigor, and uniformity. There were no unusual or unexpected results which would pose a threat to the environment. The trial area will be observed

for volunteers, which will be destroyed. The field will not be planted to potatoes in 1994.

All tubers and plant material generated in the trial area, including the BelRus border areas were treated as part of the trial and destroyed. All handling, harvest, storage, and disposal of the tubers and plant material from was carried out in compliance with the conditions of the field release permit. A summary of the major operations involved this trial are listed in Table 4.

Trial 93-03-07: Field release for plant-back PLRV study

Purpose:

To determine the effects of chronic seed infection on foliar symptoms and tuber yield and quality of PLRV resistant transgenic lines.

Summary:

The trial was conducted at Prosser, Washington. Plots were arranged in a randomized complete block design with two replications. Each replication consisted of a single 20 ft row. The area planted to transgenic potatoes was 0.05 acres and the entire area, including buffer rows, was less than 0.5 acres. Four non-transgenic control lines and 16 transgenic lines, which were included in a 1992 PLRV field test at the Prosser experiment station, constituted the treatments. Planting material for this trial was tubers held over in storage from the 1992 field trial at the Prosser experiment station. See Appendix 5C for a list of vector constructs and plant lines evaluated.

Secondary virus symptoms were observed on these plants from 6-12 weeks after planting. Plots were harvested, weighed and stored for net necrosis evaluation. After evaluations were complete, all tubers and plant material was disposed of per the conditions of the permit.

No differences in susceptibility to diseases other than to PLRV (PVY, early or late blight, verticillium, rusts, or leaf spot) or insects (CPB, aphids, leafhoppers, mites or cutworms) were observed between transgenic and non-transgenic potatoes at either location. In addition, the transgenic plots were similar to non-transgenic controls in terms of emergence, vigor, and uniformity. There were no unusual or unexpected results which would pose a threat to the environment.

The plot areas will not be planted to potatoes in 1994 and will be observed for volunteers in spring 1994. Volunteers will be chemically or mechanically destroyed. A summary of the major operations involved this trial are listed in Table 28.

Plant growth and general observations for all trials

Potato plants developing from either plantlets or tubers developed normally throughout the season. All plants were examined closely for such common growth parameters as emergence, vigor, growth habit, leaf type, color, disease susceptibility, insect susceptibility, flowering, maturity, tuber type, yield, storage and processing characteristics. Even though there were minor individual differences between some plants, at some locations, the field results indicate that the potatoes transformed to control CPB, have resistance to PLRV, resistance to PVY, or have a higher solids content, or combinations of the above traits, have only these specific trait differences from the non-transformed controls.

Cooperators at all locations were required to observe the transgenic plants for *any differences* from the controls and record any possible adverse environmental effects. The results of the field trials showed that the transformed potatoes have similar horticulture traits to the non-transformed control potatoes and do not demonstrate any other competitive advantages in the environment. Lines which showed vine abnormalities were characterized by less vigorous vine growth and atypical vine and leaf morphology. These abnormal lines were not selected for further evaluation. Yield, specific gravity, and processing characteristics of selected RB-*Btt* lines were within the range of variability of non-transgenic Russet Burbank lines in the trial.

Responses to specific issues

1) Horizontal movement:

No evidence of movement of the CPB tolerance trait, virus resistance, high solids or combinations of these traits was observed.

2) Changes in survival characteristics:

There was no evidence of changes in the survival characteristics of the transgenic potato plants. All plot sites will be monitored for volunteer potato plants during the next growing season.

3) Expression level of the genes:

The expression level of the *Btt* gene was assessed by comparing defoliation caused by larval and adult CPB in transgenic lines to that in non-transgenic lines, as well as by Western blot analysis. The expression level of the virus resistance gene was assessed by comparing disease expression to non-transgenic lines. The high solids gene was assessed by comparing solids levels of transformed and control lines in post harvest evaluations

4) Stability and inheritance of the new genes:

Because potatoes are vegetatively propagated, no Mendelian analysis of inheritance was performed. Our experience to date indicates trait stability over several generations of vegetative propagation.

5) Published data:

At this point, we are not aware of any published data by Monsanto for these specific tests.

SCHEDULE OF MAJOR OPERATIONS

Tables 1 through 28 contain the information for each site listed in USDA field release permit # 92-363-05.

Table 1. Schedule of major operations, Freeville, NY.

DATE	OPERATION
5/12/93	Received tubers under USDA permit number 93-036-07M.
5/18/93	Transplanting to field completed
8/31/93	Harvested 25 pounds of tubers
9/14/93	Harvested 60 pounds of tubers
9/30/93	All tubers disposed of by spreading on the surface of the field plot to freeze over the winter.
3/9/94	All tubers in samples not destroyed as part of analytical procedures have been destroyed in accordance with conditions of these movement permits.

Table 2. Schedule of major operations, Presque Isle, ME.

DATE	OPERATION
6/7/93	Received plantlets/tubers intrastate. Plants placed in greenhouse in accordance with protocol, awaiting field planting.
6/7/93	Transplanting to field completed.
9/27/93	Harvested 1296 pounds of tubers.
10/22/93	All tubers disposed of by field spreading and discing.
	All tubers in samples not destroyed as part of analytical procedures have been destroyed in accordance with conditions of these movement permits.

Table 3. Schedule of major operations, Lakeview, MI.

DATE	OPERATION
5/11/93	Received plantlets under USDA permit number 93-036-07M.
5/12/93	Transplanting to field completed.
10/5/93	Harvested 3,275 pounds of tubers. After post-harvest analysis all tubers disposed of by field spreading and discing.
	300 lbs. were shipped to HybriTech in Boise, ID, for further analyses under USDA notification #93-279-01N. All tubers in samples not destroyed as part of analytical procedures have been destroyed in accordance with conditions of these movement permits.

Table 4. Schedule of major operations, Hermiston, OR.

DATE RECEIVED 5/5/93, 5/12/93, and 5/25/93

Received tubers under USDA permit numbers 93-036-03M and 93-036-04M. Plants stored in greenhouse in accordance with protocol, prior to field planting

DATE	OPERATION
5/17/93	Transplanting to field completed
10/4/93	Harvested 1440 pounds of tubers for virus trial
10/21/93	Harvested 5,670 pounds of tubers for insect control trial
10/22/93	Harvested 2,100 pounds of tubers for solids trial
	Tubers stored at University of Oregon, Hermiston research farm.
3/11/94	All tubers not saved for further analysis disposed of by field spreading and discing or placed on soil surface to freeze.
	Samples were shipped to Monsanto in St. Louis , for further analysis under USDA permit # 93-069-03M. All tubers in samples not destroyed as part of analytical procedures have been destroyed in accordance with conditions of these movement permits.

Table 5. Schedule of major operations, Aberdeen, ID.

DATE	OPERATION
5/1/93	Received plantlets under USDA permit numbers 93-036-03M and 93-036-04M.
	Plants placed in greenhouse in accordance with protocol, awaiting field preparation. Seed pieces also utilized from 1992 trials.
5/11/93	Transplanting to field completed.
9/27/93	Harvested 16,164 pounds of tubers. Tubers stored at University of Idaho, Aberdeen storage.
11/5/93	All tubers not saved for further tests were disposed of by spreading on the surface of the soil and discing.
	Samples were shipped to Monsanto in St. Louis, for further analysis under USDA permit # 93-069-03M.
	All tubers in samples not destroyed as part of analytical procedures have been destroyed in accordance with conditions of these movement permits.

Table 6a. Schedule of major operations, Caldwell, ID.

DATE	OPERATION
5/1/93	Received tubers under USDA permit #93-036-04M.
	Plants placed in greenhouse in accordance with protocol, awaiting field planting.
5/5/93	Transplanting to field completed
	Harvested 5,280 pounds of tubers. 4,800 lbs tubers stored in secure facility.
	Samples were shipped to Monsanto in St. Louis , for further analysis under USDA permit # 93-069-03M. All tubers in samples not destroyed as part of analytical procedures have been destroyed in accordance with conditions of these movement permits.

Table 6b. Schedule of major operations, Caldwell, ID.

DATE	OPERATION
4/18/93	Received tubers under USDA permit #93-036-04M.
5/12/93	Transplanting to field completed
9/7/93	Harvested 125 pounds of tubers
	All tubers disposed of by field spreading and discing.
	Samples were shipped to Monsanto in St. Louis , for further analysis under USDA permit # 93-069-03M. All tubers in samples not destroyed as part of analytical procedures have been destroyed in accordance with conditions of these movement permits.

Table 7. Schedule of major operations, Monticello, ME.

DATE	OPERATION
6/7/93	Received plantlets intrastate.
6/9/93	Transplanting to field completed
9/27/93	Harvested 648 pounds of tubers
10/22/93	All tubers not saved for further testing were disposed of by field spreading, discing, and allowing them to freeze over the winter.
	All tubers in samples not destroyed as part of analytical procedures have been destroyed in accordance with conditions of these movement permits.

Table 8. Schedule of major operations, Stanton, MI.

DATE	OPERATION
5/12/93	Received plantlets/tubers under USDA permit number 93-036-07M.
5/12/93	Transplanting to field completed.
	Harvested 4,700 pounds of tubers
	All tubers disposed of by field spreading and discing.
	560 lbs. were shipped to HybriTech in Boise, ID, for further analysis under USDA notification # 93-279-01N. All tubers in samples not destroyed as part of analytical procedures have been destroyed in accordance with conditions of these movement permits.

Table 9. Schedule of major operations, Lisbon, ND.

DATE	OPERATION
5/1/93	Received tubers under USDA permit number 93-036-04M.
5/13/93	Planting to field completed
9/29/93	Harvested 315 pounds of tubers
11/15/93	All tubers disposed of by spreading on the field from where they were harvested and discing. All tubers in samples not destroyed as part of analytical procedures have been destroyed in accordance with conditions of the movement permit.

Table 10. Schedule of major operations, Echo, OR.

DATE	OPERATION
4/18/93	Received tubers under USDA permit number 93-036-04M.
5/6/93	Transplanting to field completed.
10/4/93	Harvested 2100 pounds of tubers
	Tubers stored at University of Oregon, Hermiston farm facility.
	All tubers in samples not destroyed as part of analytical procedures have been destroyed in accordance with conditions of these movement permits.

Table 11. Schedule of major operations, Grand Forks, ND.

DATE	OPERATION
5/1/93	Received plantlets under USDA permit number 93-036-02M.
5/11/93	Planting to field completed
9/9/93	Harvested 336 pounds of tubers
9/14/93	All tubers disposed of by spreading on the field from where they were harvested and discing. All tubers in samples not destroyed as part of analytical procedures have been destroyed in accordance with conditions of the movement permit.

Table 12. Schedule of major operations, Rock Springs, PA.

DATE	OPERATION
4/30/93	Received tubers from under USDA Permit number 93-036-04M.
5/29/93	Transplanting to field completed.
10/13/93	Harvested 600 pounds of tubers.
10/27/93	All tubers disposed of by field spreading and allowing to freeze.
	All tubers in samples not destroyed as part of analytical procedures have been destroyed in accordance with conditions of these movement permits.

Table 13. Schedule of major operations, Othello, WA.

DATE	OPERATION
4/30/93	Received tubers from under USDA Permit number 93-036-04M. Plants placed in greenhouse in accordance with protocol, prior to planting.
5/13/93	Transplanting to field completed.
8/4/93	Harvested 824.6 pounds of tubers
	All tubers disposed of by spreading on the surface of the field and discing.

Table 14. Schedule of major operations, Wooster, OH.

DATE	OPERATION
4/30/93	Received tubers under USDA permit number 93-036-04M.
5/17/93	Transplanting to field completed.
9/24/93	Harvested 842.5 pounds of tubers.
	Tubers stored in a secure storage facility. Tubers not needed for post-harvest storage were frozen prior to disposal.

Table 15. Schedule of major operations, Center, Colorado.

DATE	OPERATION
5/1/93	Received tubers under USDA permit # 93-036-08M. Minitubers placed in locked storage in prior to planting.
5/24/93	Planting in field completed
3/15/94	Tubers disposed of by composting.
	Samples were shipped to Monsanto in St. Louis , for further analysis in contained facilities under USDA permit # 93-069-03M. All tubers in samples not destroyed as part of analytical procedures have been destroyed in accordance with conditions of these movement permits.

Table 16. Schedule of major operations, Ashton, ID.

DATE	OPERATION
5/1/93	Received minitubers under USDA Permit # 93-036-08M.
	Minitubers placed in locked storage in accordance with protocol, awaiting field planting.
6/24/93	Transplanting to field completed.
9/30/93	Harvested and stored 680 cwt of tubers.
	Samples were shipped to Monsanto in St. Louis , for further analysis under USDA permit # 93-069-03M. All tubers in samples not destroyed as part of analytical procedures have been destroyed in accordance with conditions of these movement permits.

Table 17. Schedule of major operations, Grace, ID.

DATE	OPERATION
5/1/93	Received tubers under USDA permit #93-036-03M.
	Minitubers placed in locked storage in accordance with protocol, awaiting field preparation
5/20/93	Transplanting to field completed
9/15/93	Harvested and stored 1150 cwt of tubers
	Samples were shipped to Monsanto in St. Louis, for further analysis under USDA permit # 93-069-03M. All tubers in samples not destroyed as part of analytical procedures have been destroyed in accordance with conditions of these movement permits.

Table 18. Schedule of major operations, Island Falls, ME.

DATE RECEIVED 6/12/93, 6/14/93, 6/15/93, 6/18/93, and 6/20/93.

Received plantlets intrastate.

DATE	OPERATION
6/20/93	Transplanting to field completed.
9/25/93	Harvested and stored 875.5 cwt of tubers.
	Samples were shipped to Monsanto in St. Louis , for further analysis under USDA permit # 93-069-03M. All tubers in samples not destroyed as part of analytical procedures have been destroyed in accordance with conditions of these movement permits.

Table 19. Schedule of major operations, St David, ME.

DATE	OPERATION
5/1/93	Received tubers intrastate. Plants placed in greenhouse in accordance with protocol, awaiting field planting.
6/6/93	Transplanting to field completed.
9/30/93	Harvested and stored 2,161 cwt of tubers. All tubers in samples not destroyed as part of analytical procedures have been destroyed in accordance with conditions of these movement permits.

Table 20. Schedule of major operations, St. Agatha, ME.

DATE RECEIVED 5/1/93, 6/6/93, 6/8/93, and 6/10/93

Received tubers intrastate. Tubers locked in storage in accordance with protocol, awaiting field preparation. Plantlets were planted as received.

6/12/93	Transplanting to field completed.
9/15/93	Harvested and stored 1,911 cwt of tubers.
	All tubers in samples not destroyed as part of analytical procedures have been destroyed in accordance with conditions of these movement permits.

Table 21. Schedule of major operations, Manhattan, MT.

DATE	OPERATION
6/1/93	Received plantlets intrastate.
	Plants placed in greenhouse in accordance with protocol prior to planting.
6/13/93	Transplanting to field completed.
9/15/93	Harvested and stored 70 cwt of tubers.
	All tubers in samples not destroyed as part of analytical procedures have been destroyed in accordance with conditions of these movement permits.

Table 22. Schedule of major operations, Ronan, MT.

DATE	OPERATION
2/25/93	Received plantlets intrastate.
	Plants were multiplied in the lab and grown in the greenhouse in accordance with protocol prior to planting.
6/13/93	Transplanting to field completed.
10/1/93	Harvested and stored 307 cwt of tubers.
	All tubers in samples not destroyed as part of analytical procedures have been destroyed in accordance with conditions of these movement permits.

Table 23. Schedule of major operations, Lake Placid, NY.

DATE	OPERATION
	Tubers used for transplanting were stored on-site from 1992 field trial.
6/1/93	Transplanting to field completed.
9/25/93	Harvested and stored 24 cwt of tubers.
	All tubers in samples not destroyed as part of analytical procedures have been destroyed in accordance with conditions of these movement permits.

Table 24. Schedule of major operations, Beach, ND.

DATE	OPERATION
5/18/93	Received tubers under USDA Permit 93-036-08M.
5/29/93	Transplanting to field completed.
9/30/93	Harvested and stored 553 cwt of tubers.
	All tubers in samples not destroyed as part of analytical procedures have been destroyed in accordance with conditions of these movement permits.

Table 25. Schedule of major operations, Rollette, ND.

DATE	OPERATION
6/12/93	Received plantlets under USDA permit number 93-036-08M.
6/12/93	Transplanting to field completed.
9/30/93	Harvested and stored 270 cwt of tubers.
	All tubers in samples not destroyed as part of analytical procedures have been destroyed in accordance with conditions of these movement permits.

Table 26. Schedule of major operations, Beltsville, MD.

DATE	OPERATION
May-93	Received plantlets under USDA permit number 93-036-04M.
6/2/93	Transplanting to field completed.
8/18/93	Harvested 4.5 pounds of tubers. Tubers destroyed by being held for 24 hours at 90 degrees centigrade.
	All tubers in samples not destroyed as part of analytical procedures have been destroyed in accordance with conditions of these movement permits.

Table 27. Schedule of major operations, Parma, ID.

DATE RECEIVED 4/23/93, 5/18/93, 5/25/93, 5/27/93, 5/28/93, 6/2/93, and 6/3/93

Received plantlets under USDA permit numbers# 93-036-04M and 93-036-03M. Plantlets placed in greenhouse in accordance with protocol, awaiting field planting.

DATE	OPERATION
6/12/93	Transplanting to field completed
9/27/93	Harvested 3,150 pounds of tubers. Tubers stored in a secure location on site.
	All tubers disposed of by field spreading and tilling.
	Samples were shipped to Monsanto in St. Louis, MO for further analysis under USDA permit #93-069-03M.
	All tubers in samples not destroyed as part of analytical procedures have been destroyed in accordance with conditions of these movement permits.

Table 28. Schedule of major operations, Prosser, WA.

DATE	OPERATION
5/17/93	received plantlets under USDA permit number 93-036-03M.
	Plants placed in greenhouse in accordance with protocol, awaiting field planting.
6/11/93	Transplanting to field completed.
9/28/93	Harvested 1,405 pounds of tubers. Tubers stored in secure facility at Prosser research facility.
	Samples were shipped to Monsanto in St. Louis , for further analysis under USDA permit #93-069-03M. All tuber samples not saved for further analysis or destroyed as part of analytical procedures have been disposed of in accordance with conditions of these movement permits.

Appendix 1A. List of vector constructs and plant lines tested in 1993 first year CPB resistant trials at Freeville, New York.

VARIETY	VECTOR	LINE NUMBER
Russet Burbank	PV-STBT02	RBBT02-06
		RBBT02-10
		RBBT02-12
		RBBT02-16
		RBBT02-17
		RBBT02-18
		RBBT02-23
		RUSSET BURBANK CONTROL
Norchip	PV-STBT02	NCBT02-01
		NCBT02-02
	PV-STBT04	NCBT04-05
		NCBT04-06
		NCBT04-07
		NCBT04-08
		NCBT04-09
		NCBT04-10
		NCBT04-11
		NCBT04-12
		NCBT04-13
		NCBT04-14
		NCBT04-15
		NCBT04-16
		NCBT04-17
		NCBT04-18
		NORCHIP CONTROL
Superior	PV-STBT02	SUBT02-01
		SUBT02-02
		SUBT02-03
		SUBT02-04
		SUBT02-05
		SUBT02-07
		SUBT02-08
		SUBT02-09
		SUBT02-10
		SUBT02-11
		SUBT02-12
		SUBT02-13
		SUPERIOR CONTROL

Appendix 1B. Location and list of vector constructs and plant lines tested in 1993 second year agronomic CPB resistant trials.

LOCATION	VARIETY	VECTOR	LINE NUMBER
Lakeview, MI	Atlantic	PV-STBT04	ATBT04-01
			ATBT04-06
			ATBT04-17
			ATBT04-24
			ATBT04-26
			ATBT04-27
			ATBT04-28
			ATBT04-30
			ATBT04-32
			ATBT04-33
			ATBT04-40
			ATBT04-43
			ATBT04-44
			ATBT04-47
			ATLANTIC CONTROL
Hermiston, OR	Russet Burbank	PV-STBT04	RBBT04-01
			RBBT04-02
			RBBT04-03
			RBBT04-04
			RBBT04-05
			RBBT04-06
			RBBT04-07
			RBBT04-10
			RBBT04-11
			RBBT04-14
			RB CONTROL
	Shepody	PV-STBT04	SHBT04-01
			SHBT04-02
			SHBT04-07
			SHBT04-12
			SHBT04-18
			SHBT04-41
			SHBT04-47
			SHEPODY CONTROL

Appendix 1B (Contd.). Location and list of vector constructs and plant lines tested in 1993 second year agronomic CPB resistant trials.

LOCATION	VARIETY	VECTOR	LINE NUMBER
Presque, Isle, ME	Russet Burbank	PV-STBT04	RBBT04-04
			RBBT04-05
			RBBT04-06
	Atlantic	PV-STBT04	ATBT04-10
			ATBT04-17
			ATBT04-24
			ATBT04-26
			ATBT04-27
			ATBT04-32
			ATBT04-33
			ATBT04-36
			ATBT04-40
			ATBT04-43
			ATBT04-47
			ATLANTIC CONTROL
	Shepody	PV-STBT04	SHBT04-01
			SHBT04-02
			SHBT04-07
			SHBT04-12
			SHBT04-18
			SHBT04-41
			SHBT04-47
			SHEPODY CONTROL
Aberdeen, ID	Russet Burbank	PV-STBT04	RBBT04-01
			RBBT04-02
			RBBT04-03
			RBBT04-04
			RBBT04-05
			RBBT04-06
			RBBT04-07
			RBBT04-08
			RBBT04-10
			RBBT04-11
			RBBT04-14
		PV-STBT05	RBBT05-01
			RBBT05-02

Appendix 1B (Contd.). Location and list of vector constructs and plant lines tested in 1993 second year agronomic CPB resistant trials.

LOCATION	VARIETY	VECTOR	LINE NUMBER
Aberdeen, ID	Russet Burbank	PV-STBT05	RBBT05-04
			RBBT05-06
			RBBT05-07
			RBBT05-11
			RBBT05-12
			RBBT05-15
			RBBT05-16
			RBBT05-17
			RBBT05-18
			RB CONTROL
	Atlantic	PV-STBT04	ATBT04-01
			ATBT04-06
			ATBT04-12
			ATBT04-13
			ATBT04-14
			ATBT04-17
			ATBT04-24
			ATBT04-26
			ATBT04-27
			ATBT04-28
			ATBT04-30
			ATBT04-31
			ATBT04-32
			ATBT04-36
			ATBT04-40
			ATBT04-43
			ATBT04-44
			ATBT04-47
			ATLANTIC CONTROL
	Shepody	PV-STBT04	SHBT04-01
			SHBT04-02
			SHBT04-07
			SHBT04-11
			SHBT04-12
			SHBT04-18
			SHBT04-34
			SHBT04-41
			SHBT04-42
			SHBT04-47
			SHEPODY CONTROL

Appendix 1C. Location and list of vector constructs and plant lines tested in 1993 CPB resistant performance confirmation trials.

LOCATION	VARIETY	VECTOR	LINE NUMBER
Caldwell, ID	Russet Burbank	PV-STBT02	RBBT02-06 RBBT02-10 RBBT02-12 RBBT02-16 RBBT02-17 RBBT02-23 RB CONTROL
Presque Isle, ME	Russet Burbank	PV-STBT04	RBBT04-04 RBBT04-06 RBBT04-10 RBBT04-11 RB CONTROL
	Shepody	PV-STBT04	SHBT04-01 SHBT04-02 SHBT04-07 SHBT04-18 SHBT04-41 SHBT04-42 SHBT04-47 SHEPODY CONTROL
Grand Forks, ND	Russet Burbank	PV-STBT02	RBBT02-06 RBBT02-10 RBBT02-23 RB CONTROL
	Shepody	PV-STBT04	SHBT04-01 SHBT04-02 SHBT04-41 SHBT04-42 SHEPODY CONTROL
Rock Springs, PA	Atlantic	PV-STBT04	ATBT04-17 ATBT04-26 ATBT04-32 ATBT04-36 ATBT04-43 ATLANTIC CONTROL

Appendix 1C (Contd.). Location and list of vector constructs and plant lines tested in 1993 second year CPB resistant performance confirmation trials.

LOCATION	VARIETY	VECTOR	LINE NUMBER
Othello, WA	Russet Burbank	PV-STBT04	RBBT04-04
			RBBT04-06
			RBBT04-10
			RBBT04-11
			RB CONTROL
	Shepody	PV-STBT04	SHBT04-01
			SHBT04-02
			SHBT04-07
			SHBT04-41
			SHBT04-42
			SHBT04-47
			SHEPODY CONTROL

Appendix 2. List of vector constructs and plant lines tested in 1993 insect control and PVY trials at Presque Isle, ME.

VARIETY	VECTOR	LINE NUMBER
Russet Burbank	PV-STMT15	RBMT15-01
		RBMT15-02
		RBMT15-04
		RBMT15-06
		RBMT15-07
		RBMT15-09
		RBMT15-10
		RBMT15-11
		RBMT15-15
		RBMT15-23
		RBMT15-24
		RBMT15-28
		RBMT15-30
		RBMT15-32
		RBMT15-33
		RBMT15-34
		RBMT15-35
		RBMT15-36
		RBMT15-38
		RBMT15-39
		RBMT15-41
		RBMT15-47
		RBMT15-48
		RBMT15-49
		RBMT15-123
		RBMT15-138
		RBMT15-139
		RBMT15-142
Shepody	PV-STMT15	SHMT15-48
		SHMT15-66
		SHMT15-69
		SHMT15-74

Appendix 2 (Contd.). List of vector constructs and plant lines tested in 1993 insect control and PVY trials at Presque Isle, ME.

VARIETY	VECTOR	LINE NUMBER
Snowden	PV-STMT15	SNMT15-13
		SNMT15-26
		SNMT15-36
		SNMT15-37
		SNMT15-38
		SNMT15-39
		SNMT15-40
		SNMT15-43
		SNMT15-44
		SNMT15-47
		SNMT15-48
		SNMT15-98
		SNMT15-157
		SNMT15-160
		SNMT15-191

Appendix 3. Acreage of trangenic potatoes and vector constructs and plant lines tested in 1993 seed production trials.

LOCATION	TRANSGENIC ACREAGE	TOTAL ACREAGE	VECTOR	LINE NUMBER
Center, CO	0.45	1.0	PV-STBT02	RBBT02-18
Ashton, ID	2.5	9.0	PV-STBT02	RBBT02-06
				RBBT02-10
				RBBT02-12
				RBBT02-17
Grace, ID	5.0	9.0	PV-STBT02	RBBT02-06
				RBBT02-10
				RBBT02-12
				RBBT02-16
				RBBT02-18
				RBBT02-23
Island Falls, ME	9.1	15	PV-STBT02	RBBT02-06
				RBBT02-10
				RBBT02-12
				RBBT02-16
				RBBT02-18
				RBBT02-23
			Plus all lines evaluated in all trials	
St. David, ME	8.0	9.0	PV-STBT02	RBBT02-06
				RBBT02-17
St. Agatha, ME	13	15	PV-STBT02	RBBT02-06,
				RBBT02-10
				RBBT02-12
				RBBT02-18
				RBBT02-23
Manhattan, MT	1.3	1.5	PV-STBT02	RBBT02-06
Ronan, MT	2.7	3.0	PV-STBT02	RBBT02-06
				RBBT02-12
Lake Placid, NY	0.1	1.5	PV-STBT04	RBBT04-01
				RBBT04-06
				RBBT04-17
				RBBT04-26
Beach, ND	3.0	5.0	PV-STBT02	RBBT02-06
				RBBT02-10
				RBBT02-12
				RBBT02-17
Rollette, ND	3.5	6.0	PV-STBT02	RBBT02-06,
				RBBT02-10
				RBBT02-12
				RBBT02-18
				RBBT02-23

Appendix 4A. List of vector constructs and plant lines tested in 1993 first year high solids trials at Aberdeen, ID and Parma, ID.

VARIETY	VECTOR	LINE NUMBER
Russet Burbank	PV-STMT01	RBMT01-03 through RBMTO1-93
Norchip	PV-STMT01	NOMT01-01, NOMT01-05, NOMT01-19
Atlantic	PV-STMT01	ATMT01-15 through ATMTO1-40
Russet Burbank	PV-STHS01	RBHS01-06 through RBHS01-25
	PV-STHS02	RBHS02-04 through RBHS02-05
	PV-STHS03	RBHS03-03 through RBHS03-51
	PV-STHS07	RBHS07-02 through RBHS07-31
	PV-STHS10	RBHS10-10 through RBHS10-39
	PV-STHS13	RBHS13-02 through RBHS13-245
	PV-STHS14	RBHS14-06
	PV-STHS15	RBHS15-01 through RBHS15-336
	PV-STHS16	RBHS16-01 through RBHS16-128
	PV-STHS17	RBHS17-06 through RBHS17-15
	PV-STHS21	RBHS21-01 through RBHS21-100
	PV-STHS22	RBHS22-01 through RBHS22-128
	PV-STHS23	RBHS23-01 through RBHS23-24
	PV-STHS24	RBHS24-01 through RBHS24-30
	PV-STHS27	RBHS27-01 through RBHS27-61
	PV-STHS29	RBHS29-144 through RBHS29-218
	PV-STHS31	RBHS31-30 through RBHS31-275

Appendix 4B. List of vector constructs and plant lines tested in 1993 second year high solids trials at Presque Isle, ME and Hermiston, OR.

VARIETY	VECTOR	LINE NUMBER
Russet Burbank	PV-STMT01	RBMT01-03 through RBMTO1-93
Norchip	PV-STMT01	NOMT01-01, NOMT01-05, NOMT01-19
Atlantic	PV-STMT01	ATMT01-15 through ATMTO1-40
Russet Burbank	PV-STHS01	RBHS01-06 through RBHS01-25
	PV-STHS02	RBHS02-04 through RBHS02-05
	PV-STHS03	RBHS03-03 through RBHS03-51
	PV-STHS07	RBHS07-02 through RBHS07-31
	PV-STHS10	RBHS10-10 through RBHS10-39
	PV-STHS13	RBHS13-02 through RBHS13-245
	PV-STHS14	RBHS14-06

Appendix 5A. List of vector constructs and plant lines tested in 1993 first year PLRV trials at Prosser, WA and Parma, ID.

VARIETY	VECTOR	LINE NUMBER
Russet Burbank	PV-STMT04	RBMT04-01 through RBMT04-76
	PV-STMT10	RBMT10-02 through RBMT10-07
	PV-STMT11	RBMT11-01 through RBMT11-25
	PV-STMT12	RBMT12-01 through RBMT12-25
	PV-STMT13	RBMT13-01 through RBMT13-03
	PV-STLR07	RBLR07-27 and RBLR07-34
	PV-STLR08	RBLR08-47
	PV-STLR09	RBLR09-04 through RBLR09-22
	PV-STLR12	RBLR12-01 through RBLR12-24
	PV-STLR14	RBLR14-01 through RBLR14-25

Appendix 5B. List of vector constructs and plant lines tested in 1993 second year PLRV trials at Prosser, WA and Parma, ID.

VARIETY	VECTOR	LINE NUMBER
Russet Burbank	PV-STLR02	RBLR02-05
	PV-STLR03	RBLR03-04
	PV-STLR05	RBLR05-25
	PV-STLR09	RBLR09-04 through RBLR10
	PV-STLR11	RBLR11-02 through RBLR11-16

Appendix 5C. List of vector constructs and plant lines tested in 1993 PLRV plantback and virus spread trials at Prosser, WA.

VARIETY	VECTOR	LINE NUMBER
Russet Burbank	PV-STLR02	RBLR02-05
	PV-STLR03	RBLR03-04 through RBLR03-09
	PV-STLR05	RBLR05-02 through RBLR05-25
	PV-STLR07	RBLR07-33
	PV-STLR08	RBLR08-47
	PV-STLR09	RBLR09-04 through RBLR10
	PV-STLR11	RBLR11-02 through RBLR11-22

Appendix 5D. List of vector constructs and plant lines tested in 1993 PLRV natural exposure trials at Hermiston, OR.

VARIETY	VECTOR	LINE NUMBER
Russet Burbank	PV-STLR02	RBLR02-05
	PV-STLR03	RBLR03-04
	PV-STLR07	RBLR07-33
	PV-STLR08	RBLR08-33
	PV-STLR09	RBLR09-04
	PV-STLR11	RBLR11-04 through RBLR11-18

1993 TRIAL OF POTATOES RESISTANT TO INSECTS, DISEASES, HIGH SOLIDS AND COMBINATIONS OF THE ABOVE TRAITS

(USDA PERMIT # 93-004-01) (MON # 92-161R) (WI Permit # 93-32)

FINAL REPORT

John Cudnohufsky, HybriTech Seed International, Inc.

INTRODUCTION

This trial was a field evaluation of genetically modified potatoes expressing either a gene providing tolerance to feeding by the Colorado potato beetle (CPB) or providing resistance to Potato Virus Y or increasing the solids levels in tubers or combinations of the above traits. It consisted of eleven separate experiments.

The experiments were conducted by Monsanto and HybriTech Seed International, Inc. in collaboration with academic and private cooperators at the following Wisconsin locations:

Antigo, Langlade County
Coloma, Coloma County
Hancock, Waushara County (2 locations)
Harrison, Marathon County
Rhinelander, Oneida County

EXPERIMENTAL LAYOUT

Trial 93-01-01: Efficacy, horticultural and processing evaluation of first year CPB resistant lines.

Purpose:

Evaluate the horticultural and processing characteristics of Russet Burbank and other potato varieties genetically modified to express the insecticidal protein from *Bacillus thuringiensis* subsp. *tenebrionis* (*B.t.t.*).

Summary:

This trial was a field evaluation of the efficacy of CPB resistant potatoes producing the *B.t.t.* protein for CPB control. The experiment was conducted at the University of Wisconsin Hancock Research Station. Transformed potato lines of plasmid vectors PV-STBT02 and PV-STBT04 were grown from minitubers in plots 3 ft wide by 10 ft long. Each clone was replicated twice and arranged in a randomized complete block. See Appendix 1 for a list of lines evaluated. The total area occupied by transgenic plants did not exceed 0.15 acres. The entire plot area, including buffers and borders, was less than

1.0 acre in size. The plot was surrounded on all sides by a 10 meter buffer in which no commercial potatoes were grown. The schedule of major operations throughout the season is displayed in Table 1.

Since the purpose of the study was to evaluate the insect control properties of the transgenic lines, no insecticides were applied for the control of CPB except in protected control plots. Insecticides were applied to all plots for the control of potato leafhoppers when insect populations reached the treatment threshold.

The potatoes in most plots emerged uniformly and similarly to the control. Some lines were late emerging, which was probably related to the size and dormancy conditions of the minitubers. All experimental lines demonstrated good control of the Colorado potato beetle with no defoliation evident at any time during the season. Unprotected non-transgenic controls were defoliated by mid-season.

With the exception of their resistance to CPB, no other differences in susceptibility to diseases (PLRV, PVY, early or late blight, verticillium leaf spot or rusts) or insects (aphids, leafhoppers, mites, or cutworms) were observed between transgenic and non-transgenic potatoes. Some differences in plant vigor or size were observed, but these off-type lines were not selected for further evaluation. There were no unusual agronomic characteristics that would pose a threat to the environment.

All potatoes in this trial were harvested and graded for yield. These potatoes remain in a secure storage facility at the Hancock Experiment Station. The plot area will not be planted to potatoes in 1994 and will be monitored for volunteers. Volunteers will be chemically or mechanically destroyed.

Experiment # 93-01-03: Efficacy and agronomic evaluation of CPB resistant and PVY resistant lines.

Purpose:

To evaluate the efficacy and agronomic characteristics of lines containing genes mediating both CPB and PVY resistance.

Summary:

Transgenic Russet Burbank, Snowden, Shepody, and Superior lines were grown from plantlets for evaluation of PVY resistance at University of Wisconsin, Hancock Experiment Station in Hancock, Wisconsin. Each plot consisted of 8 transgenic plants, and 4 non-transgenic Shepody plants which originated from a known 100% PVY infected seedlot. The plants were arranged in the row such that every transgenic plant was next to a non-transgenic infector plant on one side, in the following fashion:

x o x x o x x o x x o x

where x=transgenic plant, and o=non-transgenic infector plant. The plot rows were separated by 6 ft to allow human access throughout the season. The transgenic plants

occupied an area of less than 0.15 acres, and the entire plot area was less than 1.5 acres in size. Every non-transgenic infector plant was infested with green peach aphids in early July. The plants were evaluated by ELISA test and rated twice during the season for PVY symptoms.

Because of excessive rain and low fertility, all plantlets exhibited relatively poor growth. Most transgenic lines were comparable to the non-transgenic controls in growth habit. A few lines were slightly stunted or less vigorous or had different leaf shape than the controls of the same variety. These lines were not selected for further evaluation. Those lines with normal growth characteristics and exhibiting resistance to PVY will be further evaluated. No differences in susceptibility to diseases, other than PVY (PLRV, early or late blight, verticillium, leaf spot or rusts) or insects other than CPB (aphids, leafhoppers, mites, or cutworms) were observed between transgenic and non-transgenic potatoes.

All of the lines that exhibited resistance to PVY were lifted by hand, bagged, and saved for further testing. Non-transgenic potatoes and a representative sample of transgenic PVY-susceptible lines were also lifted and saved. The potatoes were placed in a secure storage facility at the experiment station. Potatoes that were not saved for further testing were left in the field to freeze over the winter. The lines evaluated are listed in Appendix 2 and the schedule of major events are shown in Table 2.

The plot area will not be planted to potatoes in 1994 and will be monitored for volunteers. Volunteers will be chemically or mechanically destroyed.

Trial 93-01-05: Second year agronomic evaluation of CPB resistant potato lines

Purpose:

To evaluate the agronomic traits of selected CPB resistant potato plants to ensure that the best experimental lines will meet or exceed current requirements of commercial growers and potato processors.

Summary:

Both minitubers and tuber seed pieces were grown in plots 20 ft long and 3 ft wide at the Hancock Experiment Station. Each plot was replicated 6 times and arranged in a randomized complete block design. The vector constructs and plant lines evaluated are listed in Appendix 3. The total area occupied by transgenic potatoes was 0.3 acres. The total trial area, including buffers and borders, was less than 1 acre in size. No commercial potatoes were grown in the 10 meter buffer area surrounding these potatoes.

Since the objective of the test was to compare agronomic characteristics of transformed lines with those of nontransgenic controls, all pests were controlled using conventional methods.

With the exception of their resistance to CPB, no other differences in susceptibility to diseases (PLRV, PVY, early or late blight, verticillium leaf spot or rusts) or insects (aphids, leafhoppers, mites, or cutworms) were observed between transgenic and non-transgenic potatoes. There were no unusual agronomic characteristics that would pose a threat to the environment.

Atlantic control plants grown from minitubers were substantially larger than all transgenic lines grown from minitubers. This difference was attributed to the size of the initial propagules, since control minitubers were produced in an earlier greenhouse crop and were much larger in size. The resulting yields reflected this difference, with higher yields observed in mini-tuber control plots which originated from larger sized minitubers. Yields of transgenic lines in plots grown from seed pieces were all comparable to those of the nontransgenic controls. The plot area will not be planted to potatoes in 1994 and will be monitored for volunteers. Volunteers will be chemically or mechanically destroyed. The schedule of major events is listed in Table 3.

Experiment # 93-01-05: Third year agronomic evaluation of CPB resistant potato lines

Purpose:

To evaluate the horticultural and processing characteristics of Russet Burbank, CPB resistant potatoes.

Summary:

This trial was conducted at Coloma, Wisconsin and consisted of seven CPB resistant potato lines and three control lines, replicated 6 times and arranged in a randomized complete block. The lines evaluated in this test are listed in Appendix 4. Each plot was two rows of 20 seed pieces spaced 12" apart in the row. The plots were separated within the row by 4 hills of red potatoes which served as plot markers at harvest. The trial area was located in a commercial potato field, but was surrounded on each side by 10 meters of soybeans. The transgenic potatoes covered 0.15 acres, and the entire trial was less than 0.7 acres in size.

The potatoes were grown with conventional fertility, pest, and irrigation management. All pests, including the CPB, were controlled. At harvest, the experimental potatoes were dug, bagged, and transported to the Hancock Research Station for grading.

With the exception of their resistance to CPB, no other differences in susceptibility to diseases (PLRV, PVY, early or late blight, verticillium leaf spot or rusts) or insects (aphids, leafhoppers, mites, or cutworms) were observed between transgenic and non-transgenic potatoes. All transgenic lines were similar to non-transgenic controls in terms of emergence, vigor, uniformity, and yield. No growth characteristics were observed that would be a threat to the environment. The plot area will not be planted to potatoes in 1994 and will be monitored for volunteers. Volunteers will be chemically or mechanically destroyed. The schedule of major events are given in Table 4.

Experiment # 93-01-06 Performance confirmation trial

Purpose:

To test selected CPB resistant potato lines under commercially representative production conditions and identify those with the best insect resistance and horticultural and processing characteristics.

Summary:

Potato plots in this trial conducted at the Hancock Experiment Station consisted of two rows of 15 feet each. Transgenic potato plots were alternated with non-transgenic potatoes so that each transgenic plot was bordered on both sides by two rows of non-transgenic potatoes. The plot was unreplicated. Conventional irrigation, weed, and disease management practices were used to grow the potatoes, however, no CPB control was provided. The transgenic potatoes occupied less than 0.03 acres. The entire trial area, including control plots, buffers, and borders, was less than 0.4 acres in size. The area immediately surrounding the potatoes was unplanted.

The transgenic lines emerged uniformly and similarly to the non-transgenic controls, with comparable type and vigor. All transgenic potato lines demonstrated a high level of CPB control. Very few egg masses or larvae were found on the plants at any time during the season. Defoliation was very minimal, even after control plots were defoliated and summer adults moved into the plots in large numbers. With the exception of their resistance to CPB, no other differences in susceptibility to diseases (PLRV, PVY, early or late blight, verticillium leaf spot or rusts) or insects (aphids, leafhoppers, mites, or cutworms) were observed between transgenic and non-transgenic potatoes. No growth characteristics were observed that would be a threat to the environment.

The potatoes in this plot were not harvested. They were left in the field to freeze over the winter, and will be tilled up in the spring. The plot area will not be planted to potatoes in 1994 and will be monitored for volunteers. Volunteers will be chemically or mechanically destroyed. The lines evaluated are in Appendix 5 and the schedule of major events are found in Table 5.

Experiment # 93-01-07: CPB population dynamics

Purpose:

To evaluate the effect of CPB resistant potatoes on beneficial and pest arthropods and to determine the need for supplementary insecticide applications.

Summary:

Non-transgenic and transgenic potatoes were planted at the Hancock Experiment Station in plots 48' by 48' in size, replicated 4 times, and arranged in a randomized complete block design. Each of six treatments received an experimental pest management regime which simulated conventional or reduced-pesticide ("low impact") management

practices. Three of the six treatments were planted to transgenic potatoes, which represented less than 0.7 acres. The entire trial, including non-transgenic treatments, buffers, and borders, was less than 2.0 acres in size. The trial are was surrounded by at least 10 meters of a non-potato crop on all sides. Arthropods were monitored in the plots throughout the season to determine the effect of management practices on populations of beneficial and pest insects.

The highest numbers of beneficial arthropods (lady beetles, big-eyed bugs, nabids, minute pirate bugs, and spiders) were collected in transgenic CPB resistant potatoes which received "low impact", short residual insecticides for potato leafhopper control. Aphid populations remained low in all plots except those treated with permethrin. The aphid populations in the permethrin treated plots increased exponentially as the natural enemies of this pest were eliminated.

Potato fleabeetles, which are closely related to CPB and also a pest of potatoes, were significantly lower in transgenic plots than in non-transgenic controls. It was concluded that *B.t.t.* provided some suppression of this pest, but the life stage of potato fleabeetle susceptible to *B.t.t.* is undetermined. The level of flea beetle control, however, does not appear to be great enough to be of commercial importance. With the exception of their resistance to CPB and activity on potato flea beetle, no other differences in susceptibility to diseases (PLRV, PVY, early or late blight, verticillium leaf spot or rusts) or insects (aphids, leafhoppers, mites, or cutworms) were observed between transgenic and non-transgenic potatoes. No growth characteristics were observed that would be a threat to the environment. The plot area will not be planted to potatoes in 1994 and will be monitored for volunteers. Volunteers will be chemically or mechanically destroyed.

The transgenic potatoes grown in this trial were a combination of Russet Burbank lines RBBT02-06, RBBT02-10 and RBBT02-12 that contain the plasmid vector PV-STBT02. The potatoes were mixed together before planting so that the planting material was uniform throughout. The schedule of major events are in Table 6.

Experiment # 93-01-08: CPB resistance management - I

Purpose:

A number of different pest management strategies are under consideration and this trial concentrated on using a refuge for susceptible individuals as a method to dilute resistance genes in a population and thereby, delay the development of CPB resistance to the *B.t.t.* protein.

Summary:

The experiment consisted of individual potato plots enclosed in large (12' X 12') field cages. Each treatment represented a different resistance management option including various spatial arrangements such as trap crops and mixed transgenic and non-transgenic seed, and alternative applications such as foliar microbial *B.t.t.* Each treatment was replicated three times.

The cages were arranged in three long "banks", each containing one replicate. The transgenic potatoes grown in this trial were a combination of Russet Burbank lines RBBT02-06, RBBT02-10 and RBBT02-12 that contain the plasmid vector PV-STBT02. The potatoes were mixed together before planting so that the planting material was uniform in all cages.

The experiment, including non-transgenic controls, covered less than 0.2 acres. The entire area, with borders and a 10 meter buffer surrounding the cages on all sides, covered less than 0.75 acres. The field cages were surrounded by grass, with no other potatoes within 10 meters.

Colorado potato beetle movement and behavior was monitored on individual plants throughout the season. This information will help to determine the optimal deployment strategy for reducing the risk of resistance development in the CPB.

With the exception of their resistance to CPB, no other differences in susceptibility to diseases (PLRV, PVY, early or late blight, verticillium leaf spot or rusts) or insects (aphids, leafhoppers, mites, or cutworms) were observed between transgenic and non-transgenic potatoes. No growth characteristics were observed that would be a threat to the environment.

The potatoes were not harvested, but were left in the soil to freeze over the winter. They will be tilled up in the spring after the cages are removed. The plot area will not be planted to potatoes in 1994 and will be monitored for volunteers. Volunteers will be chemically or mechanically destroyed. The schedule of major events during the season is shown in Table 7.

Trial 93-01-10: Seed production

Purpose:

To increase the quantity of selected potato lines, while evaluating yield potential and phenotypic uniformity.

Summary:

Tubers or plantlets were planted in single row, non-replicated blocks and grown with production practices that are standard to each site location. All pests, including the CPB were controlled. There was a 10 meter buffer separating the transgenic potatoes from adjacent potatoes. The acreage and lines that were evaluated at each location are listed in Appendix 6. All major operations for each site are listed in Tables 8, 9 and 10.

The transgenic plants grew identical to the non-transformed controls. Observations by cooperators detected no differences in phenotypic traits of the foliage or tubers. With the exception of resistance to CPB, no other differences in susceptibility to diseases (PLRV, PVY, early or late blight, verticillium leaf spot or rusts) or insects (aphids, leafhoppers, mites, or cutworms) were observed between transgenic and non-transgenic potatoes. No growth characteristics were observed that would be a threat to the

environment. Yields were comparable to or greater than the controls.

All tubers that met the selection criteria for certified seed, were lifted, harvested and stored for use in 1994. All other tubers were left on the surface of the soil to freeze over the winter. These trial areas will not be planted to potatoes in 1994 and all volunteers will be destroyed.

Trial 93-02-02: Second year efficacy and agronomic evaluation of high solids lines.

Purpose:

In second year trials, evaluate the efficacy, agronomic and processing characteristics of lines containing genes mediation increased solids production in tubers.

Summary:

Trials were conducted at the Hancock Research Farm. All agronomic practices, with respect to field preparation, fertilization, irrigation and pest control, to grow potatoes were conducted standard to the region.

The plot design was 1 row, 15 feet long, with plants 12 inches apart in the row, replicated four times, in a randomized complete block design. The transgenic potatoes, represented less than 0.4 acres and the entire trial was less than 1.0 acre in size. The trial are was surrounded by at least 10 meters of a non-potato crop on all sides. Transgenic Russet Burbank, Atlantic and Norchip potato lines were compared to their respective control lines. See Appendix 7 for a list of the lines tested.

Plants were observed for emergence and phenotypic characteristics and disease and insect susceptibility throughout the season. No differences in susceptibility to diseases (PLRV, PVY, early or late blight, verticillium, rusts, or leaf spot) or insects (aphids, leafhoppers, mites or cutworms) were observed between transgenic and non-transgenic potatoes

Tuber samples were collected and observed for type, bruising potential, internal defects and frying evaluations. Several lines at each location were judged to offer advantages over the controls and will be observed in future tests. Tubers from these lines are being evaluated for long term storage characteristics. Lines not meeting this criteria were not selected for further evaluation.

There were no unusual or unexpected results which would pose a threat to the environment. The trial areas will be observed for volunteers, which will be destroyed. The field will not be planted to potatoes in 1994. The schedule of major events during the season is shown in Table 11.

Plant growth and general observations for all trials

Transplant and seed piece survival was good to excellent, depending on weather conditions and care early in the season. Plants developed normally during the season. Most lines were indistinguishable from non-transgenic controls. The lines which showed vine abnormalities were characterized by less vigorous vine growth (chlorosis) and atypical vine and leaf morphology. These abnormal lines were not selected for further evaluation. Most lines produced tubers of a size and shape consistent with those of their respective non-transgenic controls grown from transplants. Yield, specific gravity, and processing characteristics of selected lines were within the range of variability of non-transgenic control lines in the trial.

Responses to specific issues

1) Horizontal movement:

No evidence of movement from the test site of the CPB tolerance or virus resistance or high solids traits were observed.

2) Changes in survival characteristics:

There was no evidence of changes in the survival characteristics of the transgenic potato plants. Volunteer potato plants will be monitored and destroyed at all locations during the next growing season.

3) Expression level of the genes:

The expression level of the *B.t.t.* gene was assessed by comparing defoliation caused by larval and adult CPB in transgenic lines to that in non-transgenic lines, as well as by Western blot. Expression of the gene resulted in virtually complete protection against feeding by all stages of the CPB despite heavy insect pressure during the growing season. The expression of the virus resistance genes was assessed by measuring disease susceptibility and with ELISA tests. The expression of the solids genes were measured by dry matter increases during and at the end of the season.

4) Stability and inheritance of the new genes:

Because potatoes are vegetatively propagated, no Mendelian analysis of inheritance was performed. Our experience to date indicates trait stability over several generations of vegetative propagation.

5) Published data:

At this point, we are not aware of any published data for this specific test.

SCHEDULE OF MAJOR OPERATIONS

Tables 1 through 11 contain the information for each site listed in the USDA field release permit # 93-004-01 and Wisconsin permit # 93-32.

Table 1. Schedule of major operations for trial # 93-01-01, Hancock, WI.

DATE	OPERATION
5/12/93	Received 640 minitubers (less than 15 lbs) under USDA permit #93-036-07M).
5/12/93	All minitubers received planted in the field.
10/4/93	1050 lbs of potatoes harvested. 300 lbs of potatoes were pulverized and composted. The rest of the potatoes remain in secure storage at Hancock.

Table 2. Schedule of major operations for trial # 93-01-03, Hancock, WI.

DATE	OPERATION
6/3/93	3600 transgenic plantlets received under USDA permit #93-036-03M. Plantlets were stored in secure facility at night and moved outdoors under supervision during the day for hardening off, as per the protocol.
6/7/93	All plots were planted in the field. Remaining plantlets were held in a secure greenhouse facility until planting was completed.
6/30/93	Excess plant material was destroyed by pulverization and composting.
10/6/93	Potato plots of no further research interest were left in the soil to freeze over the winter. They will be tilled in spring to enhance decomposition. Selected plots were hand harvested, bagged, and placed into a secure storage facility at the Hancock Experiment Station. Samples of these potatoes were shipped to Presque Isle, ME (permit #93-253-01N) for winter testing. The remaining potatoes were transported to Madison, WI (after notifying the WI Dept. of Agriculture State Biotechnologist) where they were grown in the greenhouse and reevaluated for PVY resistance using ELISA techniques. This plant material was autoclaved before being discarded.

Table 3. Schedule of major operations for trial # 93-01-05, Hancock, WI.

DATE	OPERATION
4-30-93	340 lbs of Russet Burbank and Atlantic tuber seed received under USDA permit #93-036-04M. The seed was stored at the Hancock Experiment Station in a secure storage until planting.
5/12/93	1283 Atlantic minitubers (about 18 lbs) received under USDA permit #93-036-07M.
5/5/93	Atlantic seed pieces planted. Remaining seed was returned to storage until planting was completed.
5/13/93	Atlantic minitubers and Russet Burbank seed tubers planted. All minitubers were planted. Remaining Russet Burbank seed was returned to storage until planting was completed.
6/30/93	Excess seed destroyed by pulverization and composting.
10/5/93	A total of 2600 lbs of Russet Burbank and 5020 lbs of Atlantic potatoes harvested from both mini-tuber and seed tuber plots. 2238 lbs of potatoes disposed of by pulverization and composting.
11/19/93	60 Russet Burbank tubers were removed from storage and planted at the Hancock Research station as per USDA permit #93-260-02N. All remaining potatoes from this trial are currently being held in a secure potato storage at Hancock, WI.

Table 4. Schedule of major operations for trial # 93-01-05, Coloma, WI.

DATE	OPERATION
4/30/93	245 lbs transgenic seed potatoes received under USDA permit #93-036-04M.
5/1/93	Seed tubers planted [CBI DELETED] Remaining seed was returned to secure storage facility at the Hancock Experiment Station until planting was completed.
6/30/93	Excess seed destroyed by pulverization and composting.
9/16/93	6580 lbs of potatoes harvested and transported to the Hancock Experiment Station for grading and storage. 1530 lbs were disposed of by pulverization and composting.

Table 4 (Contd.).

9/22/93	225 lbs of potatoes were sent to Monsanto Co., St. Louis, MO, and 1000 lbs to University of ID, Aberdeen, ID (both movements were made under permit #93-253-01N). The potatoes that were not destroyed or shipped to other locations remain in storage at the Hancock Experiment Station.

Table 5. Schedule of major operations for trial # 93-01-06, Hancock, WI.

DATE	OPERATION
4/30/93	75 lbs transgenic seed potatoes received under USDA permit # 93-036-04M.
5/1/93	Transgenic seed potatoes planted at the Hancock Experiment Station, Hancock, WI. Remaining potatoes were stored in a secure facility until planting was completed.
6/30/93	Remaining seed potatoes destroyed by pulverization and composting.
10/5/93	760 lbs of potatoes harvested and destroyed by pulverization and composting.

Table 6. Schedule of major operations for trial # 93-01-07, Hancock, WI.

DATE	OPERATION
4/30/93	1320 lbs of seed tubers received under USDA permit #93-036-04M.
5/5/93	Planting completed at the Hancock Experiment Station, Hancock, WI. Remaining seed placed in a secure storage facility until planting was completed.
6/30/93	Excess seed disposed of by pulverization and composting.
10/4/93	2310 lbs of potatoes harvested from the central portion of each plot (6 rows X 14 plants). The outer portion of each plot was left in the field to freeze over the winter, and will be tilled up in the spring to enhance decomposition.

Table 7. Schedule of major operations for trial # 93-01-08, Hancock, WI.

DATE	OPERATION
4/30/93	200 lbs of transgenic seed potatoes received under USDA permit #93-036-04M. Tubers were stored in secure facility at the Hancock Experiment Station.
5/5/93	Seed tubers were hand planted in field cages. Remaining seed was returned to storage until the planting season was over.
6/30/93	Excess seed destroyed by pulverization and composting. Potatoes left in the field to freeze over the winter, and will be tilled up in the spring to enhance decomposition.

Table 8. Schedule of major operations for trial # 93-01-10, Antigo, WI.

DATE	OPERATION
	Received plantlets USDA permits # 93-036-02M and 93-036-03M. on 6/10/93 and 6/13/93.
6/15/93	Transplanting to field completed.
10/1/93	Harvested 679 cwt of tubers. Tubers stored on site in secure facility. Samples were shipped to Maine, for winter testing in contained facilities under USDA permit # 93-253-01N.

Table 9. Schedule of major operations for trial # 93-01-10, Harrison, WI.

DATE	OPERATION
6/3/93	Received plantlets under USDA permit # 93-036-03M on 6/3/93 and 6/10/93.
6/15/93	Transplanting to field completed.
10/6/93	Harvested 408 cwt of tubers. Tubers stored at Antigo, WI facility. Samples were shipped to Maine for winter testing in contained facilities under USDA permit # 93-253-01N.

Table 10. Schedule of major operations for trial # 93-01-10, Rhinelander, WI.

DATE	OPERATION
3/93	Plantlets shipped intrastate. Mini-tubers stored on site also used for planting.
5/14/93	Transplanting to field completed.
9/20/93	Harvested 427 cwt of tubers. Tubers stored on site. Samples were shipped to Maine, for winter testing in contained facilities under USDA permit # 93-253-01N.

Table 11. Schedule of major operations for trial # 93-02-02, Hancock, WI.

DATE	OPERATION
6/3/93	Received plantlets under USDA permit # 93-036-03M. Plants placed in greenhouse in accordance with protocol prior to field planting.
6/7/93	Transplanting to field completed.
10/6/93	Harvested 5,000 pounds of tubers. 500 pounds were stored at the Hancock facility.
10/13/93	Shipped 500 pounds of tubers to Boise, ID for analysis under USDA permit # 92-353-01N.
3/25/94	All remaining tubers disposed of by field spreading and discing.

Appendix 1. List of vector constructs and plant lines evaluated in first year CPB resistant potato efficacy evaluation at Hancock, WI.

VARIETY	VECTOR	LINE NUMBER
Norchip	PV-STBT02	NCBT02-01
		NCBT02-02
	PV-STBT04	NCBT04-05
		NCBT04-06
		NCBT04-07
		NCBT04-08
		NCBT04-09
		NCBT04-10
		NCBT04-11
		NCBT04-12
		NCBT04-13
		NCBT04-14
		NCBT04-15
		NCBT04-16
		NCBT04-17
		NCBT04-18
		NCBT04-19
		NORCHIP CONTROL
Superior	PV-STBT02	SUBT02-01
		SUBT02-02
		SUBT02-03
		SUBT02-04
		SUBT02-05
		SUBT02-09
		SUBT02-10
		SUBT02-11
		SUBT02-13
		SUPERIOR CONTROL

Appendix 2. List of vector constructs and plant lines evaluated in efficacy and agronomic evaluation of CPB resistant and PVY resistant potatoes at Hancock, WI.

VARIETY	VECTOR	LINE NUMBER
Russet Burbank	PV-STMT15	RBMT15-01
		RBMT15-04
		RBMT15-06
		RBMT15-07
		RBMT15-09
		RBMT15-10
		RBMT15-11
		RBMT15-15
		RBMT15-23
		RBMT15-24
		RBMT15-28
		RBMT15-30
		RBMT15-34
		RBMT15-35
		RBMT15-36
		RBMT15-38
		RBMT15-39
		RBMT15-41
		RBMT15-47
		RBMT15-48
		RBMT15-49
		RBMT15-72
		RBMT15-123
		RBMT15-138
		RBMT15-139
		RBMT15-142
		RBMT15-149
		RBMT15-154
Shepody	PV-STMT15	SHMT15-48
		SHMT15-69
		SHMT15-74

Appendix 2 (Contd.). List of vector constructs and plant lines evaluated in efficacy and agronomic evaluation of CPB resistant and PVY resistant potatoes at Hancock, WI.

VARIETY	VECTOR	LINE NUMBER
Snowden	PV-STMT15	SNMT15-26
		SNMT15-36
		SNMT15-37
		SNMT15-38
		SNMT15-39
		SNMT15-40
		SNMT15-43
		SNMT15-44
		SNMT15-47
		SNMT15-48
		SNMT15-157
		SNMT15-160
		SNMT15-191
Superior	PV-STMT15	SUMT15-07
		SUMT15-08
		SUMT15-10
		SUMT15-11
		SUMT15-12
		SUMT15-13
		SUMT15-20
		SUMT15-23
		SUMT15-24
		SUMT15-25

Appendix 3. Vector constructs and plant lines evaluated in second year agronomic trial, Hancock, WI.

VARIETY	VECTOR	LINE NUMBER
Russet Burbank	PV-STBT04	RBBT04-01
		RBBT04-02
		RBBT04-03
		RBBT04-04
		RBBT04-05
		RBBT04-06
		RBBT04-07
		RBBT04-10
		RBBT04-11
		RBBT04-14
		RB CONTROL
Atlantic	PV-STBT04	ATBT04-01
		ATBT04-06
		ATBT04-12
		ATBT04-13
		ATBT04-14
		ATBT04-17
		ATBT04-24
		ATBT04-26
		ATBT04-27
		ATBT04-28
		ATBT04-32
		ATBT04-33
		ATBT04-40
		ATBT04-43
		ATBT04-44
		ATLANTIC CONTROL

Appendix 4. Vector constructs and plant lines evaluated in third year agronomic trial at Coloma, WI.

VARIETY	VECTOR	LINE NUMBER
Russet Burbank	PV-STBT02	RBBT02-06
		RBBT02-10
		RBBT02-12
		RBBT02-16
		RBBT02-17
		RBBT02-18
		RBBT02-23
		RUSSET BURBANK CONTROL

Appendix 5. Vector constructs and plant lines evaluated in performance confirmation trial at Hancock, WI.

VARIETY	VECTOR	LINE NUMBER
Russet Burbank	PV-STBT02	RBBT02-06
		RBBT02-10
		RBBT02-12
		RBBT02-16
		RBBT02-23
	PV-STBT04	RBBT04-06
		RUSSET BURBANK CONTROL
Atlantic	PV-STBT04	ATBT04-01
		ATBT04-06
		ATBT04-14
		ATBT04-17
		ATBT04-24
		ATBT04-26
		ATBT04-36
		ATLANTIC CONTROL

Appendix 6. Acreage of trangenic potatoes and vector constructs and plant lines tested in 1993 seed production trials.

LOCATION	TRANSGENIC ACREAGE	TOTAL ACREAGE	VECTOR	LINE NUMBER
Antigo, WI	4.9	8.0	PV-STBT02	RBBT02-06 RBBT02-10 RBBT02-18 RBBT02-23
Harrison, WI	3.0	5.0	PV-STBT02	RBBT02-06 RBBT02-10 RBBT02-12 RBBT02-16 RBBT02-17 RBBT02-18 RBBT02-23
Rhinelander, WI	1.5	5.0	PV-STBT02	RBBT02-06 RBBT02-10 RBBT02-12 RBBT02-17

Appendix 7. Vector constructs and plant lines evaluated in high solids trial at Hancock, WI.

VARIETY	VECTOR	LINE NUMBER
Russet Burbank	PV-STHS01	RBHS01-06
		RBHS01-10
		RBHS01-17
		RBHS01-25
		RBHS02-04
		RBHS02-05
		RBHS03-03
		RBHS03-05
		RBHS03-17
		RBHS03-20
		RBHS03-27
		RBHS03-32
		RBHS03-41
		RBHS03-49
		RBHS03-51
		RBHS07-09
		RBHS10-10
		RBHS10-25
		RBHS10-36
		RBHS10-39
		RBHS13-02
		RBHS13-03
		RBHS13-13
		RBHS13-23
		RBHS13-34
		RBHS13-51
		RBHS13-68
		RBHS13-70
		RBHS14-06
	PV-STMT01	RBMT01-03
		RBMT01-10
		RBMT01-11
		RBMT01-30
		RBMT01-37
		RBMT01-50
		RBMT01-65
		RBMT01-81
		RBMT01-82
		RBMT01-93

Appendix 7 (Contd.). Vector constructs and plant lines evaluated in high solids trial at Hancock, WI.

VARIETY	VECTOR	LINE NUMBER
Atlantic	PV-STMT01	ATMT01-15
		ATMT01-31
		ATMT01-33
		ATMT01-40
Norchip	PV-STMT01	NOMT01-01
		NOMT01-05
		NOMT01-19

1993 Potato Trial in Queenstown, Maryland

(USDA PERMIT # 93-056-03) (MONSANTO # 93-038R)

FINAL REPORT

Terry B. Stone
Monsanto Agricultural Group

This release of potatoes genetically modified to be resistant to Colorado potato beetle (CPB) was performed at the Asgrow research farm in Queenstown, Maryland. The plot was established as a demonstration of the efficacy of CPB resistant potatoes in controlling this pest. The trial was performed by Monsanto in cooperation with the Asgrow seed company from June 7, 1993 through November 11, 1993.

Experimental Layout

The planting materials were tubers shipped from Hybritech Seed International in Island Falls, ME to the Asgrow Agricultural Research Farm for the field trial (Monsanto# 93-038A, USDA# 93-063-05M). Approximately 20 tubers each of CPB resistant Russet Burbank potato line RBBT02-06 line and control potatoes were planted on June 7, 1993. The tubers were planted by hand at 10" to 12" spacing in rows 36 inches apart. The potatoes were artificially infested with Colorado potato beetle larvae to induce a high level of infestation.

The trial occupied an area of less than 0.1 acre and included less than 0.03 acres of CPB resistant plants. Agronomic practices including, fertility and irrigation were typical for potato production in the area. The field was not treated with insecticides in accordance with the purpose of the test. At the conclusion of the test the plants and tubers were disked and buried in the plot area. The plot will not be planted to potatoes in 1994.

Plant Growth and General Observations

Emergence and growth of the 20 transgenic potato plants was no different than that of the 20 nontransgenic potato plants. No virus or other disease infection was noted. The transgenic potatoes were resistant to CPB, while the control potatoes were severely defoliated. There were no differences in susceptibility to other insect pests between the two plant types. No symptoms of infection were observed during monitoring that could be attributed to *Agrobacterium*.

Responses to Specific Issues

1) Horizontal movement

No evidence of movement of the CPB resistance trait was observed.

2) Changes in survival characteristics

There was no change in the ability of the trangenic potatoes to survive as compared to non-transgenic potatoes grown in the same field. No volunteers were observed during the fallow period following harvest.

3) Expression level of the genes

The expression level of the Btt gene was not measured in this trial. Field trials conducted in the summer of 1993 indicated that the plants resisted attack by CPB in a manner comparable to that observed in 1992.

4) Stability and inheritance of the new genes

Because potatoes are vegetatively propagated no Mendelian analysis of inheritance was performed. The trait has been stable over 3 field generations of potato production.

5) Published data

Data from this trial has not been published.

Table 1. Schedule of Major Activities

April 18, 1993 - Samples were shipped from Island Falls, Maine to Queenstown, Maryland for field planting under USDA movement permit #93-063-05M.

June 7, 1993 - Tubers were planted.

November 10, 1993 - Plants were destroyed by disking and the tubers were buried in the plot area.

APPENDIX 6

EXAMPLE FIELD MONITORING FORMS

Hancock, 6/23/92
Agronomic Trial

AGRONOMIC TRAITS OF TRANSGENIC POTATOES
Check-off list

Visually evaluate the agronomic traits of transgenic potatoes compared to control plants. Ask the questions: do the transgenic plants look different from the controls and, are the transgenics more susceptible to disease or insect attack compared to the controls? Make two observations during the season.

Line #: 107 Control - 1

Agronomic Traits:	Visual differences from controls: None	Yes	Describe
Physical characteristics			
Plant vigor	o	o	
Stunted growth, shorter plants	o	o	
Chlorotic color	o	o	
Leaflet shape	o	o	
Flowering	o	o	
Others?	o	o	
Disease susceptibility			
Early blight	o	o	
Late blight	o	o	
Leaf spot	o	o	
Rusts	o	o	
Verticillium	o	o	
Mildew	o	o	
Others?	o	☒	upward curling of leaves, possibly virus
Insect susceptibility			
Aphids	o	o	
Colorado potato beetle	o	☒	adults + egg masses finding damage
Cutworms	o	o	
Leafhoppers	o	o	
Spider mites	o	o	
Others?	o	o	

([CBI DELETED] ___ Date 6/23/92

92AGRON
(3/18/92)

AGRONOMIC TRAITS OF TRANSGENIC POTATOES
Check-off list

Visually evaluate the agronomic traits of transgenic potatoes compared to control plants. Ask the questions: do the transgenic plants look different from the controls and, are the transgenics more susceptible to disease or insect attack compared to the controls? Make two observations during the season.

Line #: 104 BT-12

Agronomic Traits:	Visual differences from controls: None	Yes	Describe
Physical characteristics			
Plant vigor	ø	o	
Stunted growth, shorter plants	ø	o	
Chlorotic color	ø	o	
Leaflet shape	ø	o	
Flowering	ø	o	
Others?	ø	o	
Disease susceptibility			
Early blight	ø	o	
Late blight	ø	o	
Leaf spot	ø	o	
Rusts	ø	o	
Verticillium	ø	o	
Mildew	ø	o	
Others?	o	ø	<1% leaf curl
Insect susceptibility			
Aphids	o	o	
Colorado potato beetle	o	ø	Adult/larval minimal feeding
Cutworms	o	o	
Leafhoppers	o	o	
Spider mites	o	o	
Others?	o	o	

Ot [CBI DELETED] Date 6/23/92

92AGRON
(3/18/92)

AGRONOMIC TRAITS OF TRANSGENIC POTATOES
Check-off list

Visually evaluate the agronomic traits of transgenic potatoes compared to control plants. Ask the questions: do the transgenic plants look different from the controls and, are the transgenics more susceptible to disease or insect attack compared to the controls? Make two observations during the season.

Line #: 218 BT-18

Agronomic Traits:	Visual differences from controls: None	Yes	Describe
Physical characteristics			
Plant vigor	o	o	
Stunted growth, shorter plants	o	o	
Chlorotic color	o	o	
Leaflet shape	o	o	
Flowering	o	o	
Others?	o	o	
Disease susceptibility			
Early blight	o	o	
Late blight	o	o	
Leaf spot	o	o	
Rusts	o	o	
Verticillium	o	o	
Mildew	o	o	
Others?	o	o	
Insect susceptibility			
Aphids	o	o	
Colorado potato beetle	o	o	less feeding
Cutworms	o	o	
Leafhoppers	o	o	
Spider mites	o	o	
Others?	o	o	

[CBI DELETED] ___ Date 6/23/92

92AGRON
(3/18/92)

322

Staunton, Va

USDA Agronomic Check-off

– = normal cultivar characteristics

O = off-type, resistant, or otherwise different from cultivar in any way.

		Physical character.							Disease Suscept.					Insect Suscept.					
Clone/ plot	Rep	Plant vigor	Stunted growth	Chlorotic color	Leaflet shape	Flowering (early, late)	Others?	% Plants affected (if any)	Blight	Verticillium	Virus (describe)	Others?	% Plants affected (if any)	Aphids	Colorado potato beetle	Leafhoppers	Others?	% Plants affected (if any)	Comments/description
STO2-6																			Normal
STO2-10																			Normal
STO2-12																			Normal
STO2-16																			"
STO2-17																			"
STO2-18																			"
STO2-13																			"
Control 1																			"
Control 2																			"
Control 3																			"

date: 6-11-93 Location: Staunton, VA 1

[CBI DELETED]

College of
Agricultural Sciences
Experiment Station

Hermiston Agricultural
Research & Extension Center
P.O. Box 105
Hermiston, Oregon 97838 .

January 14, 1994

Terry Stone
The Monsanto Agricultural Company
700 Chesterfield Parkway North
St. Louis, Missouri 63198

Dear Terry,

During 1992, eight transgenic lines with the Btt mechanism for Colorado potato beetle resistance (pMON10547 plant vector): BT06, BT10, BT16, BT18, BT23, and BT28 were compared with normal "Russet Burbank" (RBID) for levels of protein. The study was planted May 19, 1992, as seed pieces. These plots were observed each Wednesday beginning May 27, 1992 and ending August 19, 1992 (13 times) for abnormal growth or agronomic condition. The characteristics observed were (1) plant vigor, (2) stunted growth, (3) chlorotic color, (4) leaflet shape, (5) flowering, (6) early blight, (7) late blight, (8) leaf spot, (9) rusts, (10) verticillium, (11) mildew, (12) aphids, (13) CPB, (14) cutworms, (15) leafhoppers, (16) leafhoppers, and (17) spider mites. No difference between the transgenic plants and the normal check plants was observed.

Sincerely,

[CBI DELETED]

Field Monitoring for Disease/Insect/Weediness Characteristics

- Make observations at least once every 4 weeks during the growing season
- Compare control vs. transgenic lines for obvious differences by the following criteria:
  - Disease (resistance/susceptibility to diseases not specifically engineered to resist).
  - Insects (resistance/susceptibility to insect feeding not specifically engineered to resist).
  - Weediness (less susceptibility to herbicides not specifically engineered to resist, unusual proliferation, etc.).
- Record observations below.

Disease none

Insects none

Weediness none

[CBI DELETED]

1-18-93
2-14-93
Date

HOMESTEAD FLORIDA
Study Location

1992/1993 WINTER SEED EVAL.
Study Title

Appendix 5

41-001 325

AGRONOMIC TRAITS CHECKOFF

X= normal cultivar characteristics.
O= off-type-,resistant, or otherwise different from cultivar in any way.

	Physical character.						Disease Susceptibility								Insect Suscept.							
Line #	Plant vigor	Stunted growth	Chlorotic color	Leaflet shape	Flowering	Others?	Early Blight	Late Blight	Leaf spot	Rusts	Verticillium	Mildew	Others?	Aphids	Colorado potato beetle	Cutworms	Leafhoppers	Spider mites	Others?	Comments/description
																				Aberdeen Second year Bt
B+2	X	X	X	X	X															
B+4	X	X	X	X	X															
B+6	X	X	X	X	X															
B+9	X	X	X	X	X															
B+10	X	X	X	X	X															
B+11	X	X	X	X	X															
B+12	X	X	X	X	X															
B+13	X	X	X	X	X															
B+16	X	X	X	X	X															
B+17	X	X	X	X	X															
B+18	X	X	X	X	X															
B+19	X	X	X	X	X															
B+20	X	X	X	X	X															
B+21	X	X	X	X	X															
B+23	X	X	X	X	X															
B+25	X	X	X	X	X															
B+26	X	X	X	X	X															
B+28	X	X	X	X	X															

All Normal

date: 29 July 93

[CBI DELETED]

AGRONOMIC TRAITS CHECKOFF

X= normal cultivar characteristics.
O= off-type-,resistant, or otherwise different from cultivar in any way.

	Physical character.						Disease Susceptibility							Insect Suscept.						
Line #	Plant vigor	Stunted growth	Chlorotic color	Leaflet shape	Flowering	Others?	Early Blight	Late Blight	Leaf spot	Rusts	Verticillium	Mildew	Others?	Aphids	Colorado potato beetle	Cutworms	Leafhoppers	Spider mites	Others?	Comments/description
BT06	X*	X*	⊠	X	X	← None observed								→		← None →				*Slow emergence associated
BT10	X	X	⊠	X	X															with South portion of
BT12	X*	X*	X	X	X															the field.
BT16	X	X	X	X	X															
BT17	X	X	X	X	X															
BT18	X*	X*	X	X	X															
BT23	X	X	X	X	X↔															** Buds observed 6/18/92
BT28	X*	X*	X	X	X															
RB	X	X	X	X	X		↓			↓				↓	See Data		↓			

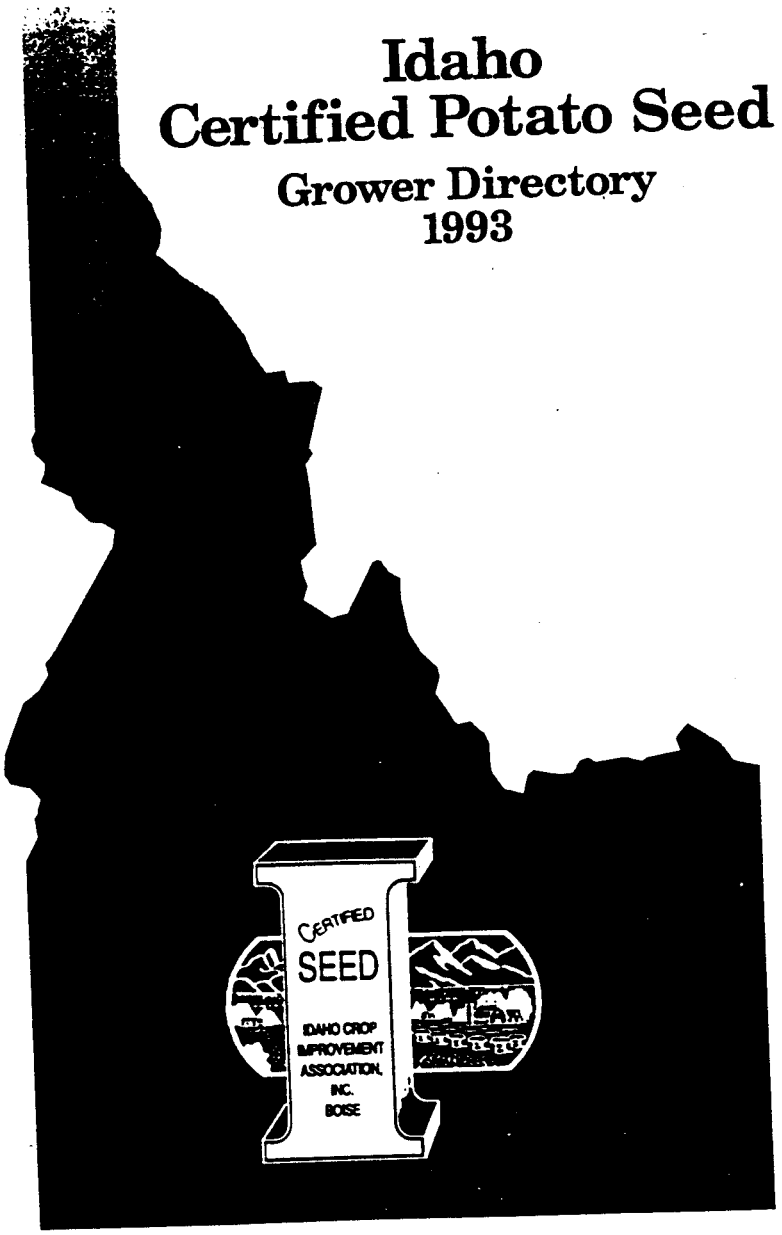
date: 11/16/92

[CBI DELETED]

APPENDIX 7

STATE SEED CERTIFICATION DIRECTORIES

Idaho
Certified Potato Seed
Grower Directory
1993



Idaho Crop Improvement Association, Inc.

Idaho Falls

HOW TO USE THIS DIRECTORY

1. Grower's name and address are listed alphabetically by county and according to variety.

2. Certification Number - Specific seed lot identification number assigned by Idaho Crop Improvement Association that follows the seed lot throughout the entire certification process. Seed buyers should refer to this number when making inquiries about a seed lot.

3. Acres Entered Column - This indicates the number of acres entered for certification.

4. Acres Accepted Column - This figure indicates the acreage that passed the two summer field inspections.

5. An (X) under the "Acres Accepted" column means that the potatoes did not pass field inspection requirements and are disqualified from certification. The following codes explain the reasons for rejection:

   L = Leafroll — W = Excessive weeds
   H = Herbicide Injury — R = Bacterial Ring Rot
   Y = PVY-Mosaic — V = Variety mix
   A = Administrative (See grower inspection report or contact the Idaho Falls office for an an explanation.)

6. The numbers under the PVY-Mosaic and Leafroll columns indicate the frequency at which these diseases were identified in a particular seed lot during the 1st and 2nd field inspections.

7. Explanations of readings:

   (. . . .) indicates that no PVY-Mosaic or Leafroll plants were found in the standard plant count of 200 plants per acre.
   (*) means a trace, or a frequency of less than 0.0001.
   0.0001 - a frequency of 1 plant per 10,000 plants counted.
   0.0002 - a frequency of 2 plants per 10,000 plants counted.
   0.0003 - a frequency of 3 plants per 10,000 plants counted.
   0.0010 - a frequency of 10 plants per 10,000 plants counted.
   0.0020 - a frequency of 20 plants per 10,000 plants counted.

8. PVX - This designation indicates that this lot was laboratory tested for potato virus X by ELISA and passed the PVX requirement. See the Idaho Rules of Certification for information on the % of PVX allowed in each seed class. PVX testing of G4-G6 seed classes is optional. Absence of the PVX designation in these classes could mean that the lot was not PVX tested.

9. Limited Generation seed class terminology:

   Nuc (1st field generation) — G-4 (5th field generation)
   G-1 (2nd field generation) — G-5 (6th field generation)
   G-2 (3rd field generation) — G-6 (7th field generation)
   G-3 (4th field generation)

10. Abbreviations/Notations:

    "See Field Report" - PVX Nuc and PVX G1 seed classes receive observational inspections. The buyer must check field inspection reports for full information concerning inspection results for these classes.
    "Pending Lab Test" - Due to adverse local environmental conditions, these lots could not receive an adequate 2nd field inspection. Laboratory testing of tuber samples will be conducted on these lots immediately after harvest. This will not replace the normal post-harvest growout test in California.
    Exp - Experimental variety maintained by the University of Idaho.
    EXP 1 - 1st year grown after an experimental variety has been named. Non-generational class seed.
    EXP 2 - 2nd year after naming. Non-generational class seed.
    EXP 3 - 3rd year after naming. Non-generational class seed.
    EXP 3 is not eligible for recertification.
    LS - Clonal line selection (See the Idaho Rules of Certification).

11. Disease tolerances for specific limited generation seed classes are listed in the current year Idaho Rules of Certification published in February by the Idaho Crop Improvement Association. Copies are available by contacting the Idaho Falls office at (208) 522-9198.

NOTICE TO ALL BUYERS

ALL SEED BUYERS should be aware of the following:

1. **This grower directory lists all seed potatoes entered for certification by the Idaho Crop Improvement Association in June of 1993. Some lots were disqualified for certification during the two summer field inspections - such lots are denoted by an (X) in the "Acres Accepted" column of the directory. BUYERS ARE ENCOURAGED TO CHECK inspection reports for additional information.**

2. **SEED BUYERS SHOULD ASK TO SEE A COPY OF ALL ICIA INSPECTION RECORDS for any seed they plan to purchase. Additional information about each seed lot, inspections and inspection results are recorded on these reports. These records are available from either the seed grower or the Idaho Falls ICIA Office at (208) 522-9198.**

3. **Seed lots listed in this directory must pass ICIA STORAGE INSPECTIONS in order to remain eligible for certification. A SUPPLEMENT to this 1993 grower directory is available in JANUARY 1994 that reports any change in the status of seed lots based upon the results of ICIA Storage Inspections. Contact the Idaho Falls ICIA Office to receive a copy of this Grower Directory Supplement.**

4. **All seed lots must be POST-HARVEST TUBER TESTED in order to be eligible for certification. The post-harvest tuber test involves a grow out and evaluation of plants at Oceanside, California or a laboratory test. Each lot must pass this inspection. (See the Idaho Rules of Certification for specific requirements.) Seed lots that do not pass these requirements cannot be sold as certified seed. RESULTS of the 1993 post-harvest test are available in early March and can be obtained from either the seed grower or the Idaho Falls ICIA Office.**

5. **CERTIFICATION IS NOT COMPLETE until seed potatoes listed in this directory are INSPECTED, GRADED, TAGGED AND SEALED AT SHIPPING by the Federal State Inspection Service, according to the Idaho Rules of Certification. Any seed potatoes listed in this directory that are sold without being inspected, graded, tagged and sealed are REJECTED from certification. They are NOT certified seed potatoes according to the Idaho Rules of Certification. ALL BUYERS ARE ENCOURAGED TO BE SURE THAT THE SEED THEY PURCHASE HAS HAD A SHIPPING POINT INSPECTION AND HAS BEEN PROPERLY TAGGED AND SEALED.**

6. **Seed buyers should refer to the official "CERTIFICATION NUMBER" listed in this directory in all transactions. This unique number follows each seed lot throughout the entire certification process outlined in paragraphs 1-5 listed above. It will also be listed on the official certification tag (Blue, Green or Yellow) that accompanies the seed potatoes purchased.**

7. **All grades of seed (BLUE, GREEN OR YELLOW) must meet the same field, storage and post-harvest testing requirements. The TAG COLOR represents only the physical grade of the potatoes at shipment. Refer to the Idaho Rules of Certification for information on specific grade standards.**

8. **Because potatoes are a perishable product, buyers purchasing certified seed should note the date the potatoes were tagged. Neither the Idaho Crop Improvement Association nor the Federal State Inspection Service can assume responsibility for out-of-grade potatoes except for a reasonable period after they are inspected, tagged and sealed.**

9. **Every reasonable effort has been made to assure the accuracy and completeness of the information contained in this directory. The Idaho Crop Improvement Association disclaims any liability for errors or omissions and encourages persons having questions regarding the information to contact the Idaho Falls office.**

10. **Copies of this directory may be obtained at County Agent offices or by writing the Idaho Crop Improvement Association, P. O. Box 51139, Idaho Falls, Idaho 83405.**

Name/Address	Certification Number	Acres Entered	Inspect.	PVY Mosaic	Leafroll	Acres Accepted	Class

RUSSET BURBANK (H1)

THE DESIGNATION (H1) REFERS TO A HYBRITECH TRANSGENIC POTATO THAT RESISTS ATTACK BY COLORADO POTATO BEETLE

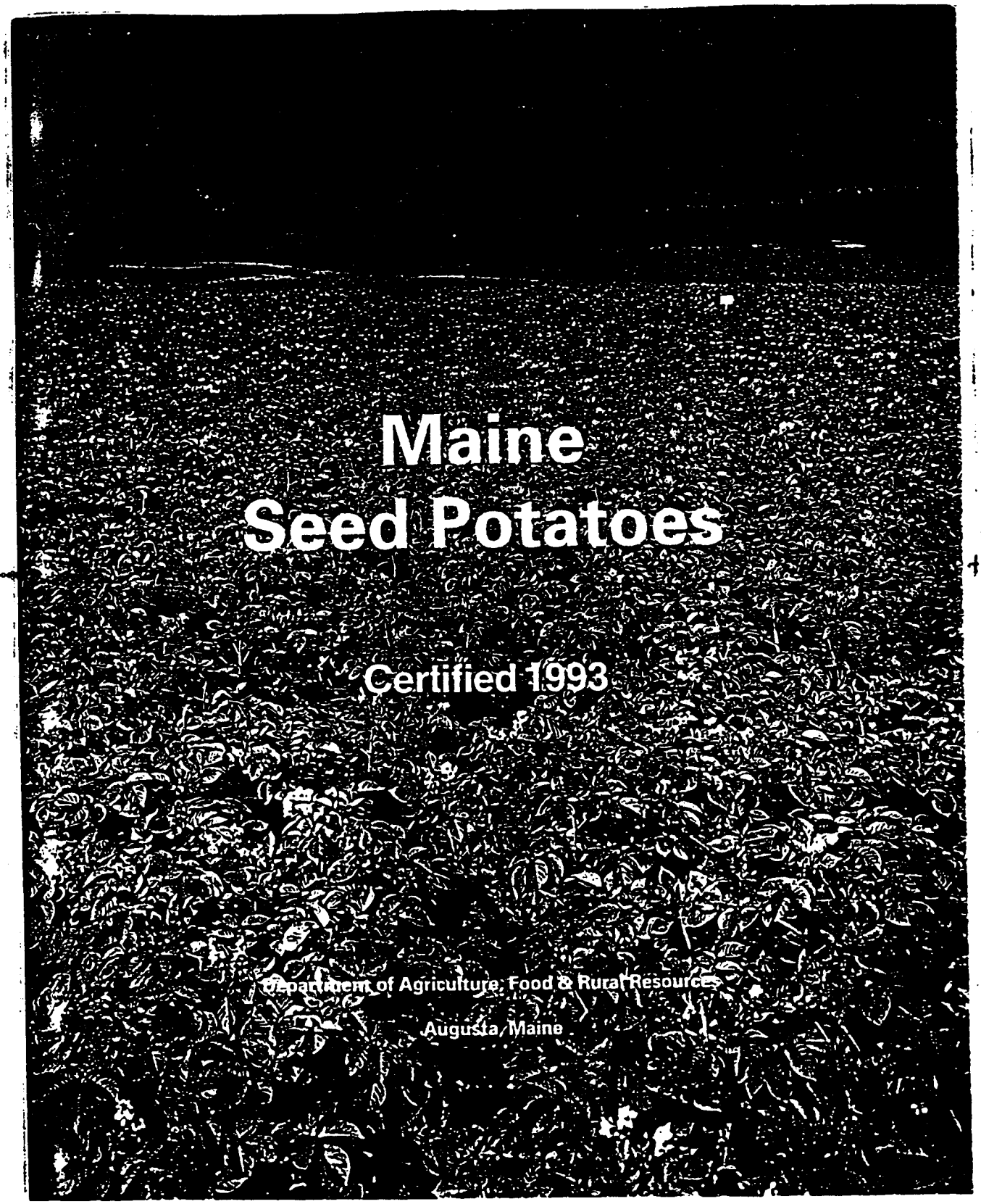
CARIBOU COUNTY

Stoddard Farms &	43158462	4	1st	See Field Report			
HybriTech Seed		4	2nd	See Field Report		4	PVX Nuc
International	43158827	4	1st	See Field Report			
2004 Two Mile Road		4	2nd	See Field Report		4	PVX Nuc
Grace, ID 83241							
(208) 425-3826 Curtis							
(208) 425-3677 Frank							
(208) 425-3645 Office							
(208) 425-3330 Fax							

FREMONT COUNTY

Ashton Hi-Tech	43228818	0.5	1st	See Field Report			
Seed Co. & HybriTech		0.5	2nd	See Field Report		0.5	PVX Nuc
Seed International	43228819	0.5	1st	See Field Report			
4054 E. 1300 N.		0.5	2nd	See Field Report		0.5	PVX Nuc
Ashton, ID 83420	43228820	0.5	1st	See Field Report			
(208) 652-3560		0.5	2nd	See Field Report		0.5	PVX Nuc
	43228821	0.2	1st	See Field Report			
		0.2	2nd	See Field Report		0.2	PVX Nuc
	43228822	0.8	1st	See Field Report			
		0.8	2nd	See Field Report		0.8	PVX Nuc
	43228823	0.4	1st	See Field Report			
		0.4	2nd	See Field Report		0.4	PVX Nuc
	43228824	1	1st	See Field Report			
		1	2nd	See Field Report		1	PVX Nuc
	43228825	0.9	1st	See Field Report			
		0.9	2nd	See Field Report		0.9	PVX Nuc

For Explanation of Directory,
See Pages 1 and 2


Maine
Seed Potatoes
Certified 1993
Department of Agriculture, Food & Rural Resources
Augusta, Maine

CERTIFIED SEED REGULATIONS

FIELD INSPECTION REQUIREMENT:

Fields entered for Certification shall receive at least two field inspections spaced approximately one month apart. A third, and additional inspection for Bacterial Ring Rot will be made after the second inspection and before the completion of harvest.

FIELD INSPECTION TOLERANCES:

Maximum tolerances during field inspection are as follows:

	1st Inspection	2nd Inspection
Leafroll.....	2%	1%
Mosaic.....	3%	2%
Spindle Tuber*.....	1/4%	1/4%
Total Virus.....	3%	3%
Varietal Mixture.....	1%	1/4%

Bacterial Ring Rot or Root Knot Nematode.....No Tolerance

*Any lot in which Spindle Tuber is detected will not be eligible for Foundation class.

Any field which according to the Maine Department of Agriculture's sampling methods exceeds the maximum field inspection tolerances will be rejected from Maine's Seed Potato Certification Program.

POST-HARVEST TEST REQUIREMENT:

All varieties will be required to be tested to meet final certification. All plots of all varieties producing seed to be planted for certification next year MUST be tested, except for seed plots of 1 acre or less to be used for "grower's own" seed. If not tested such seed and its progeny cannot be sold as seed.

a. Certified Seed Class - Each post-harvest test sample of 400 tubers may represent no more than 40 acres. Only lots showing 5% or less total virus (Mosaics and Leafroll) and 1/4% or less Spindle Tuber Viroid will be designated as Certified Class seed potatoes.

b. Foundation Seed Class - Each post-harvest test sample of 400 tubers may represent no more than 15 acres. Only lots showing 1/2% or less total virus (Mosaic and Leafroll) and no Potato Spindle Tuber Viroid will be designated as Foundation Class seed potatoes.

c. Nuclear - All nuclear seed lots must be post-harvest tested. NI seed lots shall consist of 4 tubers per 100 plants for each variety produced. Post-harvest test samples of 400 tubers may represent no more than three acres for Nuclear 2 or 3 and no more than eight acres for Nuclear 4. Only lots showing 1/2% or less total virus (Mosaic and Leafroll) will qualify for the Nuclear designation.

SHIPPING POINT INSPECTION:

In order to receive State of Maine seed potato tags or bulk shipping certificates, Maine Certified and Foundation seed potatoes are required to be inspected by an authorized Maine Seed Potato Specialist at the time of packing and must meet the requirements of the Maine Seed Potato Grade. Maine's seed potato grade is among the strictest in the country, even surpassing grade requirements established by the federal government. Seed potatoes which fail to meet the requirements of the Maine Seed Potato Grade will not be considered certified and will not be issued seed potato tags or bulk shipping certificates.

SEED POTATO CERTIFICATION IN MAINE
1993

Listed below are the varieties and the acres of each which have met all field requirements for certification in Maine as prescribed by the Commissioner of Agriculture. (This does not include growers own seed plots) All lots are subject to a further inspection at time of grading. Certification tags will be issued by the Seed Potato Specialists for lots which meet the official grades for Maine Certified Seed and will receive final certification only after Florida test showing 5% or less virus diseased plants.

Variety		Abbreviation	1993 Acreage Certified	1992 Comparative	Page Index
SUPERIOR	*	SP	6,480.085	6,384.05	42-47
ATLANTIC	*	AT	5,545.164	4,996.44	9-13
NORWIS		NW	3,369.17	4,648.31	29-32
KATAHDIN		KT	2,119.107	1,872.246	21-23
ALLEGANY		AG	1,204.024	2,854.75	8-9
SNOWDEN	*	SD	1,176.15	822.73	40-41
SHEPODY	*	SE	801.98	591.932	39-40
MONONA		MN	739.66	982.3	27-28
GOLDRUSH		GH	600.47	33.75	20
RUSSET BURBANK	*	RB	571.326	795.99	37-38
ONTARIO		OT	567.52	337.427	33-34
KENNEBEC		KN	486.821	403.499	23-24
RANGER RUSSET		RA	368.785	25.55	35
LA CHIPPER		LP	230.10	399.40	25
FRONTIER RUSSET		FR	212.395	28.60	19
RUSSET NORKOTAH	*	RN	185.212	272.6	38
SUNRISE		SR	169.41	312.19	42
WF31-4			160.10	93.10	49
KANONA		KA	157.13	41.90	21
RED LA SODA		RL	156.10	106.101	36
LA ROUGE		LA	138.316	282.31	26
BELRUS		BR	129.0413	129.20	15
B9922-11			109.95	11.80	14
MAINECHIP		MC	97.305	14.53	26-27
YUKON GOLD		YG	94.854	116.842	49
KRANTZ		KZ	91.48	29.237	24
ONAWAY		OY	90.35	46.17	33
CHIPPEWA		CH	77.30	104.70	17
COASTAL RUSSET		CT	64.30	63.53	17
LANGLADE		LN	63.60	61.00	26
NORKING RUSSET		NK	57.01	26.19	29
LA12-59 (FONTENOT)			56.96	40.11	25
CAROLA		CC	50.93	30.93	16
NORLAND		NO	46.59	94.10	29
AF1060-2			46.56	16.00	6
ELBA		EL	46.28	42.59	19
RED CLOUD		RC	45.256	8.21	36
SEBAGO		SB	43.30	40.50	39
SOMERSET		SM	41.30	24.60	42
ALASCLEAR		AL	40.08	15.10	7
NORCHIP	*	NC	39.335	177.32	28
HI-LITE RUSSET		HL	34.60	62.80	21
CHERRY RED		CD	33.982	3.602	16-17
RED PONTIAC		RP	32.58	20.60	36
AF828-5 (ST JOHNS)			29.90	2.20	6-7

Variety	Abbreviation	1993 Acreage Certified	1992 Comparative	Page Index
SPAULDING ROSE	SS	---	0.003	
FRITO-LAY VARIETIES		1,110.40	953.75	
		28,307.85	29,028.745	

ACCURATE ACREAGE AS OF SEPTEMBER 27,1993 NUMBER OF GROWERS: 318

* Denotes that Hybritech Seed International, Inc. has transgenic potatoes of this variety that are modified to confer resistance to Coloardo Potato Beetle (Leptinotarsa-decemlineata (Say)). Seed Potato Producers who have certified transgenic lines of these varieties are denoted by (H1) after the generation number in that varieties' grower listings.

********** I M P O R T A N T N O T I C E **********

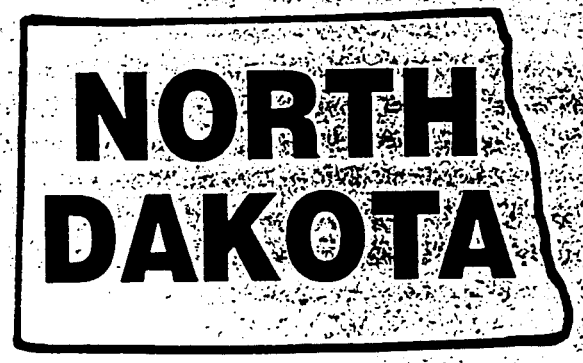
THE # SYMBOL PRINTED AFTER THE GENERATION NUMBER OF A SEED POTATO LOT IN THIS DIRECTORY DENOTES SAID SEED POTATO LOT PASSED ALL THE REQUIREMENTS OF THE MAINE SEED POTATO CERTIFICATION PROGRAM BUT WAS PRODUCED ON A FARMING OPERATION ON WHICH BACTERIAL RING ROT WAS DETECTED IN 1993.

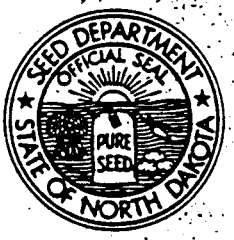
****

LAST NAME	FIRST NAME	TOWN	PHONE#	CERT#	GEN#	#ACRES
		RUSSET BURBANK (CONT'D)				
DOYEN & SONS	WILLARD C.	MAPLETON	769-3561	44U	G2CA	129.
DUFOUR	GIL	ST DAVID	728-7145	453D	G4ND	27.
DUFOUR	GIL	ST DAVID	728-7145	453E	N3ME	3.
DUFOUR	GIL	ST DAVID	728-7145	453F	N4ME	10.
DUREPO	JON	FT FAIRFIELD	473-7022	394B	G1ME	2.
DUREPO	JON	FT FAIRFIELD	473-7022	394A	G2ME	7.
ELLIS FARMS		ASHLAND	435-6448	154E	N4CA	35.5
FALL FARMS		LINCOLN	732-4126	541F	N3ME	0.02
FAR NORTH INC/CARTER SD		WASHBURN	455-8411	224E	G3MN	16.
FLEWELLING & SON	WILMOT	CROUSEVILLE	455-4076	157I	G1CA #	14.5
GOOD FARMS,INC.		MONTICELLO	538-9750	127E	G2CA	10.
HYBRITECH SEED INTERN.		ISLAND FALLS	463-2902	645A	N1ME	0.316
KELLEY FARMS,INC.		CARIBOU	492-3501	37A	G2ME	15.
KINGSBURY	SHANE	BRIDGEWATER	425-6111	58C3	N4ME	1.
LABRIE	DANIEL	ST AGATHA	543-6628	120A	G2ME	8.9
LAPLANTE	RICHARD	VAN BUREN	868-5139	558A	N4ME	4.
LEAVITT	FRED & STEVEN	LIMESTONE	325-4327	338A	N4ME	1.
LOVLEY FARMS,INC.	CARL	MAPLETON	764-3686	266C	G3MN	20.
M. K. BROS.		GRAND ISLE	895-3006	613A	G2ND	36.
MAXWELL	KEVIN	LEE	738-3651	6E	N1CA	0.3
MAXWELL	KEVIN	LEE	738-3651	6U	N2CA	20.
PAGE	JEFFREY	STOCKHOLM	896-5827	426C	N4ME	1.
PHILBRICK	JESSE	LIMESTONE	325-4669	382B	G2CA	5.
PLOURDE	GREGORY	STOCKHOLM	896-5867	501B	G4ND	1.
SCHOOLS	THOMAS A.	HOULTON	538-9448	106G	G4NB	5.
SHAW & SON	WENDALL	FT FAIRFIELD	473-7027	73C	G3MN	17.
SOUTH WADE FARMS, INC.		PRESQUE ISLE	768-3381	56A	G1ME	1.5
SOUTH WADE FARMS, INC.		PRESQUE ISLE	768-3381	56J	G2ME	13.
STAPLES	GREGORY	PRESQUE ISLE	764-4512	186H	N3CA	10.
WARD FARMS,INC.		LIMESTONE	325-4811	297B	N4ME	1.7
WEBB	BRIDGET	FT FAIRFIELD	473-7382	474A	G2CA	10.
WRIGHT	DANA C.	MONTICELLO	538-9449	11C	G4NB	2.
HYBRITECH SEED	GIL &	ST DAVID	728-7145	638B	N2ME (H1)	5.
HYBRITECH SEED	GIL &	ST DAVID	728-7145	638A	N2ME (H1)	3.
HYBRITECH SEED INT.,INC.	BERCE/	ST AGATHA	543-7539	634D	N1ME (H1)	3.97
HYBRITECH SEED INT.,INC.	BERCE/	ST AGATHA	543-7539	634A	N2ME (H1)	7.17
HYBRITECH SEED INT.,INC.	BERCE/	ST AGATHA	543-7539	634B	N3ME (H1)	0.81
HYBRITECH SEED INTERN.		ISLAND FALLS	463-2902	645C	N1ME (H1)	5.38
		RUSSET NORKOTAH				
ATCHESON FARMS		WASHBURN	498-8659	113B	G2WI	18.
BRADBURY FARMS		BRIDGEWATER	425-6301	387C	G2WI	65.
DEAN	DANA	MAPLETON	764-0250	117A	G2WI	30.
GOUGH	LAWRENCE W.	HOULTON	532-2267	128F	G2NEB	26.
MAXWELL	KEVIN	LEE	738-3651	6F	N1CA	0.2
MAXWELL	KEVIN	LEE	738-3651	6V	N2CA	8.
MAXWELL	MAYNARD	LEE	738-3651	7E	N3CA	19.
SHAW	ROBERT W.	EASTON	488-3766	141A	G2WI	19.
HYBRITECH SEED INTERN.		ISLAND FALLS	463-2902	645H	N1ME	(H1)0.012

BULLETIN NO. 95

1993 SEED POTATO GROWERS AND VARIETIES





NORTH DAKOTA STATE SEED DEPT.
University Station
Fargo, North Dakota
Phone: 701-239-7210
Fax: 701-239-7214

The seed potato stocks listed are eligible for final certification based on history, field inspections and certain other preliminary requirements. Representative samples of the plants growing in the field have been inspected for disease and found to be within the tolerance established in Bulletin No. 49, available from the State Seed Department. The fields were given at least three inspections. Disease detections are based upon visible symptoms expressed in the potato plants, except for latent potato virus x, which is detected by serological methods. Blackleg disease detection is based on the plant expressing a rotten, black, slimy appearance to the stem. In no way does this imply the amount of the bacteria, Erwinia, present in the tubers produced by this crop. The zero tolerance for bacterial ring rot is chosen for reasons of convenience and practicality and is not to be construed to mean the lot is free from the disease. It means none of the disease was found during inspection. Final certification is not granted until they are properly graded, identified with North Dakota official tags and passed official grade inspection. You must receive the Federal/State certificate to be assured of certified seed.

In North Dakota certified seed potatoes passing field and storage inspections may be sorted into two grades for out of state shipments.

1. BLUE TAG grade is given to seed lots having the higher standards for physical defects, condition and size that approximate U.S. No. 1.

2. YELLOW TAG grade is given to seed lots that have passed field and storage inspection but do not quite meet the grade standards of Blue Tag.They approximate U.S. Commercial.

3. A WHITE TAG is also used, but only for shipments within the state. Seed Department grade inspection on white tag lots is not compulsory but may be obtained upon request.

Many lots are clonal selection of limited generations, but are not so indicated in the book. Foundation or eligible for recertification list is released in February and is available upon request. Winter testing programs are principally a test for virus diseases, consequently with our low virus incidence and lack of late season infection, only foundation or recertification type seed need be winter tested.

An "*" indicates submitted samples from fields were carefully tested for Latent Virus I by the Enzyme-Linked Immunosorbent Assay (ELISA) test and found to show from zero to 2%. Upon request, certified tags will be printed to show Virus I Tested. To qualify for PVX testing, no bacterial ring rot may be found on the farm.

Acreage includes some stock grown for processing but not the fields rejected for application of a sprout inhibitor.

A SUMMARY OF SEED POTATO REQUIREMENTS

1. Fields must be planted with seed that has been winter tested and approved for recertification.

2. All potatoes in the farming operation must be entered for inspection.

3. All equipment and storages in the farming operation may be used only on the acreage entered for certification.

4. Fields are given three or more inspections.

5. Field tolerances:

Second and all Subsequent Inspections
Disease Tolerances (%)

	Generation						
	0	1	2	3	4	5	Cert.
Varietal mixture	0.0	0.1	0.2	0.3	0.3	0.3	0.3
Spindle tuber viroid	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Severe mosaics (PVY)	0.0	0.1	0.2	0.3	0.3	0.3	0.5
Leafroll (PLRV)	0.0	0.1	0.2	0.3	0.3	0.3	0.5
Total serious virus	0.0	0.1	0.2	0.3	0.3	0.3	0.5
**Bacterial ring rot	0.0	0.0	0.0	0.0	0.0	0.0	0.0

**The zero tolerance means that no amount is permissible when inspected. It does not mean that the seed is absolutely free of disease or disease causing agents, but that none was found during inspection.
Since the blackleg disease may be latent, the inspector will record only the amount observed, however any excessive amount can be cause for rejection.

6. Equipment and storages must be thoroughly cleaned and disinfected at least once annually.

7. Seed potatoes in this list have been field inspected as specified in Bulletin No. 49. The State Seed Department and the inspection staff serve only in an official regulatory manner and do not relieve the grower or owner of his responsibility.

DISCLAIMER

Neither the producer, seller, the North Dakota Seed Commission, the Seed Commissioner or his employees make any warranty of any kind, expressed or implied as to the quantity or quality of the crop produced from certified seed, including merchantability, fitness for a particular purpose or absence of disease. The only representation is that the seed potatoes were produced, graded, packed and inspected under the seed certification rules and regulations of the North Dakota State Seed Department.

Name and Post Office	Gen	Acreage
Mallinger Farms, Oslo, MN	3	11.00
Mallinger Farms, Oslo, MN	6	24.00
*Miller, Guy, Minto	2	60.00
*Miller, Guy, Minto	3	45.00
Miller, Guy, Minto	3	75.00
Miller, Henri M., Cando	0	0.50
*Miller, Henri M., Cando	1	1.50
*Olson, Rodney K., Potato Co., Inc., Adams	3	63.00
Olson, Rodney K., Potato Co., Inc., Adams	3	5.60
Owl Brand Seed Farm, Fargo	2	39.00
*Owl Brand Seed Farm, Fargo	3	405.00
Owl Brand Seed Farm, Fargo	3	356.00
Penner, Ray, Grafton	3	62.00
*Sundberg Farms, Grafton	3	149.00
*Sundberg Farms, Grafton	4	72.00
Sundberg Farms, Grafton	4	7.00
Sundberg Farms, Grafton	5	46.00
TBCS, Inc., Thompson Brothers, Park River	2	6.00
*TBCS, Inc., Thompson Brothers, Park River	4	70.00
*Thompson, T. F. & Sons, Inc., Grafton	3	46.00
*Toews, Larry, Pisek	3	48.00
TOTAL RUSSET BURBANK ACREAGE		4,280.85

RUSSET BURBANK+

*Jorde, Mike, Cando	0	3.50
TOTAL RUSSET BURBANK+ ACREAGE		3.50

RUSSET NORKOTAH

Almen, Richard, Grafton	3	32.00
*Arctic Farms, Walhalla	2	337.00
*Arctic Farms, Walhalla	3	205.00
*Belzer Brothers, Cando,	1	3.40
*Bjornstad Bros, Walhalla	1	8.00
*Bjornstad Bros, Walhalla	2	51.00
*Bjornstad Bros, Walhalla	3	103.00
Bjornstad, Bjorn & Jon, Cando,	1	3.37
Clark Bros., Inc., Walhalla,	3	20.00
*Clemenson Farm, Hoople	3	46.00
*Dakota Select Certified Seed, Cando	0	0.10
Dakota Select Certified Seed, Cando	1	2.00
Drees Farming Ass'n., Grand Forks	1	1.70
Drees Farming Ass'n., Grand Forks	2	46.00
*Eagan Bros., Walhalla	3	44.00
Eagan Bros., Walhalla	3	7.00
*Eppler, Leo & Clifford, Walhalla	4	100.00
*Gilleshammer-Thiele Farms, St. Thomas	2	12.00
*Gilleshammer-Thiele Farms, St. Thomas	4	94.00
*Johnson Farms, Walhalla	3	145.00
*Johnson Farms, Walhalla	4	178.00
Johnson Farms, Walhalla	4	10.00
Johnson, Loren J., East Grand Forks	4	34.00
*Jonk Seed Farm Ass'n., Forest River	0	0.10
*Jonk Seed Farm Ass'n., Forest River	3	13.00
*Jorde, Jim Co., Cando	2	263.20
*Jorde, Mike, Cando	0	3.77
*Jorde, Mike, Cando	1	19.18

Russet Burbank+ = Russet Burbank (H-1) HybrTech Transgenic

Name and Post Office	Gen	Acreage
RANGER RUSSET		
*Ekre, Harlan, Beach	0	0.02
Johnson, Holger Farm, Sentinel Butte	0	0.75
*Raisler, John & Denise, Beach	0	3.50
TOTAL RANGER RUSSET ACREAGE		4.27
RED LASODA		
Johnson, Holger Farm, Sentinel Butte	0	0.75
*Johnson, Holger Farm, Sentinel Butte	1	32.00
TOTAL RED LASODA ACREAGE		32.75
RED NORLAND		
*Ekre, Harlan, Beach	0	0.12
*Ekre, Harlan, Beach	1	2.00
*Ekre, Harlan, Beach	2	3.20
*Johnson, Holger Farm, Sentinel Butte	1	2.75
TOTAL RED NORLAND ACREAGE		8.07
RED PONTIAC		
*Ekre, Harlan, Beach	0	0.10
*Ekre, Harlan, Beach	1	2.00
*Ekre, Harlan, Beach	2	2.00
Johnson, Holger Farm, Sentinel Butte	0	0.25
*Johnson, Holger Farm, Sentinel Butte	1	4.50
*Johnson, Holger Farm, Sentinel Butte	2	2.25
TOTAL RED PONTIAC ACREAGE		11.10
REDSEN		
*Ekre, Harlan, Beach	0	0.02
TOTAL REDSEN ACREAGE		0.02
RUSSET BURBANK		
*Ekre, Harlan, Beach	0	0.10
*Ekre, Harlan, Beach	1	1.00
*Ekre, Harlan, Beach	2	2.00
Johnson, Holger Farm, Sentinel Butte	0	0.20
*Johnson, Holger Farm, Sentinel Butte	1	1.75
*Johnson, Holger Farm, Sentinel Butte	2	9.75
*Raisler, John & Denise, Beach	0	3.75
TOTAL RUSSET BURBANK ACREAGE		18.55
RUSSET BURBANK+		
*Raisler, John & Denise, Beach	0	3.00
TOTAL RUSSET BURBANK+ ACREAGE		3.00

Russet Burbank+ = Russet Burbank (H-1)
HybrTech Transgenic

APPENDIX 8

EFFECTS OF HEAT STRESS ON POTATO EMERGENCE

POTATO COUNTRY, U.S.A.

Spud Topics

A WASHINGTON STATE INDUSTRY REPORT

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High Temperature Potato Injury

by

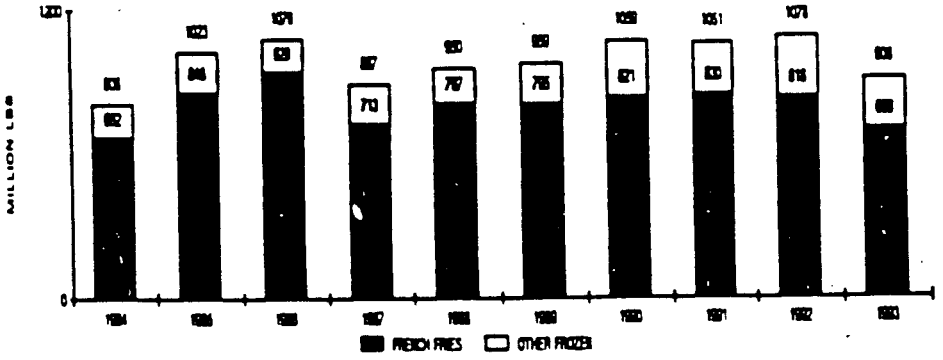
Gary Q. Pelter, Area Extension Agent
WSU Cooperative Extension, Ephrata, WA

High temperature injury to potatoes can occur when soil temperatures are high just prior to plant emergence as occurred in mid-May. Injury seems more prevalent in sandy soils. The symptoms are smaller plants with distorted leaves that may be slightly cupped with irregular margins. Some mild yellowing and mottling of the leaves may also be present. In some cases, the injury may be sufficient to damage the growing point, causing development of several lateral shoots on a single stem, much the same as caused by frost or rhizoctonia injury. Growers and researchers have observed that plants with heat induced lateral shoots tend to set less early tubers, presumably because the plants are devoting energy to the additional shoots. Interestingly, high temperature injury resembles some of the foliar symptoms of low temperature and freezing injury to potatoes. Injured plants generally resume normal aboveground growth within a short period.

• • • • • •

POTATO STOCKS IN COLD STORAGE *

APRIL 30 - FOR YEARS NOTED



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350

College of Agricultural Sciences Experiment Station

Oregon State University

Hermiston Agricultural Research & Extension Center
P.O. Box 105
Hermiston, Oregon 97838

February 22, 1993

To: Jennifer Feldman
HybriTech Seed International, Inc.

From:

[CBI DELETED]

Subject: Heat Stress Symptoms on Emerging Potatoes.

I have often observed morphological difference between plants that emerge by mid-May and those that emerge in early June. The plants emerging in June will often have wavy leave margins and leaflets will have mottled coloration. Usually, all plants will be affected. Symptoms are somewhat similar to those expressed when potatoes are infected with PVY. However, in this situation, the potato plants will out grow these symptoms.

I have always associated these symptoms with late plantings. Quite often during that time of year, we can experience lots of sunshine coupled with bare soils. This can lead to very high soil temperatures while the plants are emerging. Though I have associated these symptoms with high soil temperatures at emergence, the evidence is only circumstantial. This situation has occurred often over the past twelve years and always under the circumstances described above.

[CBI DELETED

]

April 20, 1994

To: Terry Stone
Senior Regulatory Specialist/ BB4D
Monsanto Co.

Subject: Appearance of RBBTO2 foliage

It has been my observation that leaf puckering and distortions at emergence of non-transgenic Russet Burbank is a common occurrence. I have seen it several different years and have related it to weather and soil conditions along with some pre-plant herbicide applications. This condition usually lasts from 7 to 12 days and then is no longer noticeable in the plant.

In the summer of 1993 we grew 5 acres of HybriTech Russet Burbank seed on our farm, but did not observe any abnormalities in the plants. They appeared and developed identical to the non-transgenic Russet Burbank without the condition discussed above.

If I could be of assistance in the future concerning this matter or others, I would be happy to do so.

Sincerely,

[CBI DELETED]

[CBI DELETED

]

TERRY STONE, SENIOR REG. SPECIALIST
MONSANTO CO.
700 CHESTERFIELD VILLAGE PARKWAY
ST. LOUIS, MO. 63198

RE: HEAT STRESS OBSERVATIONS

DURING THE 1993 GROWING SEASON AS WELL AS IN A NUMBER OF YEARS PREVIOUS I HAVE OBSERVED HEAT STRESS OR LEAF PUCKERING IN RUSSET BURBANK POTATOES. THIS IS A COMMON OCCURRENCE THAT WAS SEEN IN BOTH THE TRANSGENIC LINES AS WELL AS THE NON-TRANSGENICS. THE SYMPTOMS GO AWAY AS THE PLANTS GROW.

THANK YOU,

[CBI DELETED]

APPENDIX 9

MANAGEMENT OF INSECT PESTS WITH INSECT RESISTANT PLANTS: RECOMMENDED APPROACHES

Monsanto Agricultural Group

St. Louis, Missouri

MANAGEMENT OF INSECT PESTS WITH INSECT RESISTANT PLANTS: RECOMMENDED APPROACHES

Monsanto Agricultural Group
St. Louis, MO

Abstract

Insect resistant corn, cotton, and potatoes, which exhibit a high level of protection to damage and yield loss by lepidopteran pests (cotton and corn) and the Colorado potato beetle (potatoes) have been developed through the expression of *B.t.* genes in plants. Monsanto has developed recommended approaches to utilize these plants to maximize the utility and durability of these new insect control products. These approaches are being tested and will be optimized in the field prior to commercial introduction of insect resistant crops.

Introduction

Insect resistant crops represent an important new management tool to control crop damage and loss due to insect pests. These plants offer significant benefits to the grower, the consumer and the environment. Insect resistance has been developed through the expression of genes that produce insecticidal proteins from *Bacillus thuringiensis* (*B.t.*) in the cells of the plants. The particular genes being developed by Monsanto for cotton and corn are derived from the *B.t. kurstaki* strain, and for potatoes from *B.t. tenebrionis*. These proteins are the basis of several commercially available microbial insecticides, which have been demonstrated as highly selective for insects, with no activity against other types of living organisms such as mammals, fish, birds or non-insect invertebrates (earthworms, spiders, etc.) (EPA, 1991; EPA, 1988). In addition, these proteins show a remarkable insect specificity (MacIntosh *et al.*, 1990). The *B.t.* genes developed for cotton and corn produce proteins that are active only against certain lepidopteran larvae with no activity against other orders of insects. Importantly, this activity spectrum overlaps with several important pests of these crops which include the tobacco budworm, cotton bollworm or corn earworm, European corn borer, pink bollworm and several others such as cabbage looper, salt marsh caterpillar and cotton leaf perforator. Likewise, the *B.t.t.* gene developed for potatoes produces a protein active only against the Colorado potato beetle (CPB). Because these control agents are proteins, they have been found to break down rapidly in the environment and in mammalian digestive systems.

The use of insect resistant plants will provide important benefits to growers, society and the environment (McGaughey and Whalon, 1992; Gasser and Fraley, 1989; Gould, 1988). First and foremost, these plants offer an alternative to chemical insecticides currently used to control susceptible insect pests with efficacy equal to or better than that of current control methods. The use of insect resistant cotton, corn and potatoes will significantly reduce the application of chemical insecticides directed at these pests. The reduction of insecticide use will have direct benefits to the grower, such as less time and effort spent on insect control and reduced exposure to chemical insecticides.

Insect resistant crops are also likely to produce secondary benefits in pest control as an indirect result of the reduction in use of chemical insecticides. Chemical insecticides like pyrethroids are relatively non-specific and have the effect of killing beneficial predatory and parasitic insects (Roush and Tingey, 1993; Van den Bosch and Stern, 1962). Because the *B.t.* proteins produced by insect resistant plants are not active against these beneficial insects, populations have been shown to rise significantly in fields planted with insect resistant cotton and CPB resistant potatoes (Feldman, *et al.*, 1993; Reed *et al.*, 1992; Luttrell *et al.*, 1994). Preserving the beneficial insect population should enhance the biological control of both target pests and non-target pests such as mites, aphids, and leafhoppers, which increase as problems as their natural predators are removed. In addition, insect resistant cotton and corn and CPB resistant potatoes are equally capable of controlling target pest populations, which are beginning to lose their sensitivity to chemical insecticides (Everich, 1994; Stone and Sims, 1993), thus filling a need that is likely to grow in coming years.

The use of insect resistant plants will provide important benefits to growers, society and the environment. To achieve these benefits, it is important that insect resistant plant strategies be implemented and managed properly. In this respect, these plants are no different than any other pesticide. There are two aspects of this management. First, is the development of pest management techniques that allow the farmer to maximize the ability of these plants to control target pests. In essence, this is the development of a total insect management package that will be centered around a new tool, insect resistant cotton, corn or potatoes. Second, is the development of appropriate strategies to maximize the product durability and the utility of insect resistant crops. Part of this management program is the development and implementation of strategies targeted to prevent the development of insect resistance to the *B.t.* proteins produced by these plants. Because both management aspects can affect the way in which insect resistant plants are used by the grower, these two types of management, total pest management and insect resistance management, are interconnected.

Resistance management is not an issue particular to insect resistant plants, given the development of insect resistance to chemical insecticides. Monsanto scientists have addressed insect resistanece for several years in laboratory and field studies and with outside collaborators we have examined nearly every suggestion that has been made for resistance management in insect resistant plants (Sachs, 1993; Stone and Sims, 1993; Everich, 1994; Roush, 1994). As the following discussion will demonstrate, promising strategies for resistance management for insect resistant plants are available and can be recommended. These strategies have been developed in consultation with an expert advisory panel established for each crop taking into account existing research and an understanding of crop production and agronomic practices. Consequently, these strategies may be specific for each crop and target pest. It is evident, however, that insect resistant plants offer some unique options in pest and resistance management that are not available with traditional pesticides.

Integrated Pest and Resistance Management with Insect Resistant Plants

As part of a package to provide economic control of insect loss and damage in cotton, corn, and potatoes, these insect resistant crops will provide a central focus around which other insect management practices will be applied. In many areas lepidopteran pests are the primary damaging insects of cotton and corn, so the use of these insect resistant plants to control these pests will be a major portion of total insect control. The primary pest in potato production is

the CPB. Its control impacts the populations of other pests such as aphids and leafhoppers. By substituting genetically modified cotton, corn or potatoes for chemical pesticides directed at their target pests, a positive impact on overall insect management will result. Many of the details of pest management with insect resistant plants can only be determined by multi-year large scale field tests designed to incorporate these genetically modified crops into current production practices. Such field trials are in progress and are providing the data needed for developing a pest and resistance management program for these crops. These trials involve collaborations between Monsanto, HybriTech Seed International (a wholly owned subsidiary of Monsanto), seed company partners, and academic and extension entomologists. They are examining the impact of insect resistant plants on populations of beneficial and pest insects endemic to the crops and the impact on the use of conventional insecticides for controlling non-target pests (Feldman, *et al.*, 1993; Reed *et al.*, 1992; Luttrell *et al.*, 1994), the establishment of the baseline susceptibility of our insect targets to *B.t.t.* (Stone and Sims, 1993; Everich, 1994; Luttrell,1994) and the impact of mixtures of resistant and non-resistant plants on yield loss (Roush, 1994).

Insect resistant cotton, corn and CPB resistant potatoes will be important additions to the available methods of controlling insect pests. The implementation of these plants is fully consistent with the goals of integrated pest management because:

a) the *B.t.* protein produced by the plants is insect specific, affecting only a few targeted pest species

b) the *B.t.* protein is active only against insects feeding on the plant and thus doing damage

c) use of the plants will reduce the application of chemical insecticides

d) use of the plants will preserve beneficial insects, which will enhance the biological control of non-target pests

Because pest and resistance management are interconnected it is important to develop both of these approaches in tandem for each insect resistant crop.

Combination of Insect Resistant Plants with Chemical Insecticides

One aspect of the use of insect resistant plants for integrated pest management in cotton, potatoes and corn is the continued use of chemical insecticides. Some insecticides will continue to be used in these crops for non-target pests. If possible, these insecticides need to be chosen so as to not negatively impact beneficial arthropods, which are integral in the biological control of non-susceptible species. The combination of insect resistant crops with chemical insecticides, while part of a total insect control package, is not a resistance management option for insect resistant plants per se. Chemical insecticides can reduce the population size of insects selected for resistance to *B.t.* but cannot alter the gene frequencies within this population (Roush, 1989). Alternatively, insect resistant plants should positively impact current chemical insecticides by helping slow resistance development and prolonging the life of these important agricultural chemicals.

Resistance Management for Insect Resistant Plants

As described above, part of managing the implementation of insect resistant plants is the design and implementation of appropriate strategies to delay or prevent the development of insect resistance to *B.t.* in cotton, corn or potatoes. Described below are approaches that will help manage resistance development in these crops. It is important to note that: 1) as insect resistance development is a biological phenomenon, the rate of development is difficult if not impossible to predict and consequently, the efficacy of a strategy to delay or prevent its development may be impossible to demonstrate; 2) because of the available technology, biology of the pest, and the production practices of the crop, implementation of these strategies will be dependent on the crop and the target pest; and 3) field research must be conducted to determine the practical implementation of these strategies within current crop production practices. These strategies have been recommended by several researchers (Gould, 1988; Stone *et al.*, 1991; McGaughey and Whalon, 1992) and are summarized briefly below and then expanded in greater detail in the next section.

Summary of Considered Resistance Management Strategies for Insect Resistant Cotton, Corn and Potatoes

- High dose expression of *B.t.* in plants to control insects heterozygous for resistance alleles.
- Refugia as hosts for sensitive insects provided through non-insect resistant plants or other non-modified hosts.
- Monitoring of insect populations for susceptibility to *B.t.*
- Agronomic practices that minimize insect exposure to *B.t.*
- Integrated pest management (as described above).
- Combination of multiple genes within the same cotton plant, both of which are active against targeted inscets but with different sites/modes of action.
- Incorporation of host plant resistance traits into insect resistant cotton and corn as they are proven effective.
- Incorporation of novel proteins that provide effective control of targeted pests.

Details of Resistance Management Strategies

High Dose Expression

High dose expression for resistance management is based on three assumptions:

1) Resistance will most likely be controlled by one major locus with recessive resistance alleles McGaughey and Beeman, 1988; MacIntosh *et al.*, 1991; Sims and Stone, 1991).

2) Insects developing resistance to *B.t.* will be rare initially and will almost always mate with susceptible insects giving rise to heterozygous progeny (Gould, 1986).

3) More than 95% of the heterozygous progeny will be disabled or killed by insect resistant plants with the same dose as the homozygous susceptible larvae.

The high dose expression strategy uses plant expression of *B.t.* in quantities sufficient to kill those insects heterozygous for resistance to *B.t.* (McGaughey and Whalon, 1992; Roush, 1989). This resistance strategy fits nicely with the fact that high dose expression is essential for commercial efficacy of CPB resistant potatoes and insect resistant cotton and corn because of the range of sensitivity to *B.t.* in cotton and corn insect targets (e.g., at least a 10-fold difference between tobacco budworm and European corn borer and cotton bollworm). High dose expression is also necessary to maintain consistent control across environments and genotypes. We plan to evaluate and develop the high dose expression strategy.

Refugia for Sensitive Insects

Refugia means providing a refuge for sensitive insects within a population so they will not be exposed to *B.t.* and not be selected for resistance. As a resistance management technique, refugia is based on the concept that control failure due to resistance is a population genetics phenomenon. Control failures are observed when the frequency of resistant insects in the population reaches a critical level. Refugia supply susceptible non-selected individuals to the general population. With adequate refugia, the frequency of resistance genes will be very low and spread only very slowly through the population. Refugia is an important component of our insect resistant crop resistance management strategies.

Refugia can be provided either within the crop or outside it. The refuge can also be planted specifically as such or exist naturally. In all of these approaches, the effectiveness of the refuge is based on those insects that survive on the refuge crop rather than its total acreage. This is an important point because, if the refuge is chemically treated, the refuge population is reduced and the amount of acreage required is increased. Examples of refugia that can be utilized are:

1) Refuge outside of the crop: Non-insect resistant cotton, corn or potatoes.

This type of refuge will exist in all the acres not covered by these insect resistant plants. This area will be substantial in the early years after introduction and could supply a sufficient refuge for several years. As insect resistant seed becomes more available and widely grown, this refuge will be reduced. Consequently, over time, reliance on non-insect resistant cotton, corn or potato fields for refugia may not be adequate.

2) Refuge outside of the crop: Non-modified crop hosts.

The European corn borer and the cotton bollworm or corn earworm have many non-cotton or corn hosts including other crops in all locations, which may provide an adequate refuge. The tobacco budworm and Colorado potato beetle have fewer alternatives and the pink bollworm has none. In some locations cotton, corn and potatoes may be the only host for at least one insect generation per season. The use of *B.t.* microbials or transgenic *B.t.* plants on other crops will

also impact their utility as a refuge for insect resistant plants. This option must be evaluated carefully based on the crop, pest biology, and growing regions.

3) Refuge within the crop: Non-insect resistant plants.

In certain cases a likely solution is to provide an "in crop" refuge of non-insect resistant plants. For this in crop refuge, the choices are: a) random mixture of seed of insect resistant and non-resistant plants or b) non-insect resistant plants planted within the same field. The optimum refuge area required must be determined for each crop.

Mixed seed lines (*B.t.* and non-insect resistant seed within the same bag) have a certain appeal due to the "automatic" implementation. A possible problem with mixed seed arises from larvae that survive on a non-insect resistant plant and migrate to a modified plant where they may be less sensitive to *B.t.* because of their size. This could compromise insect control and increase selection pressure for resistance. The likelihood of this occurring is being investigated experimentally before this strategy is implemented.

There may also be economic and logistical problems if a mixed seed strategy is implemented. However, Monsanto, HybriTech and our seed company partners are interested in determining the viability of the mixed seed approach. It is clear that field research is required to determine the percentage of non-insect resistant plants needed as a refuge, and what the impact of this percentage on over all yield, quality and seed company economics.

Another in-crop refuge could be non-insect resistant plants planted specifically by the farmer. Besides providing a refuge, such planting of separate indicator rows of non-insect resistant plants could potentially make scouting easier. Field research is needed to determine the optimum type of planting regime.

Agronomic Practices

Certain agronomic practices may need to be recommended for insect resistant plants. In particular, plow down dates to eliminate unnecessary insect exposure to *B.t.* from cotton regrowth or rotating CPB resistant potatoes with non-resistant potatoes may need to be recommended. The recommendation of these strategies will be determined on a regional basis, if necessary.

Monitoring Insect Resistance

Insect resistance monitoring is an important component of any insect resistance management strategy. A baseline frequency is in development. Resistance of major target pests to *B.t.* has not been detected in the field (Stone and Sims, 1991; Luttrell, 1994; Everich, 1994). Baseline information should be collected on all *B.t.* products (engineered plants and *B.t.* microbials) to know when the frequency of resistant genotypes have increased within the population. This information must be developed on regional bases over several years so that susceptibility changes in populations can be identified and validated.

Pyramiding Traits

A set of strategies for the medium and long term focus on combining multiple insecticidal agents. The rationale is essentially the same for all of these: Expose the insects to two or more active agents with distinct modes of action at the same time, and the probability of any one insect being selected for resistance to both agents simultaneously is extremely low.

1) Combination with a Second Insect Resistance Gene

A second gene within the same plant possessing a different mode of action will significantly reduce the frequency of resistant individuals (Peferoen, 1992; Stone *et al.*, 1991; Van Rie, 1991). Population models indicate that other alternative uses of a second gene such as seed mixture or using single genes in rotation, may be as effective as two genes within the same plant (Gould, 1988a; Gould 1986). Assuming initial gene frequencies for *B.t.* resistance are low, initial introduction of a product with a single *B.t.* gene should not negatively compromise a second gene because the single gene product will be planted on limited acres in the first few years. In the medium term the best choice of second gene is an unrelated *B.t.* gene. In the long term, the use of novel, non-*B.t.* insecticidal genes holds great promise. This area is under active research.

2) Combination with Host Plant Resistance Traits

This is a long term strategy to be implemented by seed companies or public breeders. Host plant resistance traits (HPR) used in combination with insect resistant cotton or corn need to be insecticidally effective and not negatively impact quality or yield. For example, Monsanto currently has funded research on HPR to help set direction on HPR traits that alone or in combination are useful in protecting the plant from lepidopteran insects in cotton (Sachs, 1993). Cotton seed companies are interested in incorporating these traits if they are effective and have no negative effects on yield or quality. Similar work is planned with insect resistant corn. This strategy may have limited application to potatoes, however, as there are few varieties available that provide adequate CPB control and have desirable yield and quality characteristics.

Summary

Insect resistant cotton, corn and potatoes will offer great benefits in overall insect control in these crops. These plants will be developed to fit within existing pest management practices. Research programs for each crop have been in place for several years and will continue. With proper management and implementation, the development of insect resistance to *B.t.* will not be a technical or commercial problem that will limit the value or efficacy of these products. Monsanto has developed a package of strategies that will help effectively manage the potential development of insect resistance. The details of this program and its incorporation into existing pest management programs will be further developed and optimized in the field in the coming years.

Many aspects of the use of insect resistant plants in pest management and the implementation of resistance management strategies are unique to these products as compared to traditional chemical or microbial insecticides. For example, the use of refugia and the

incorporation of multiple resistance traits through molecular biology or plant breeding are aspects that are ideally suited to insect resistant plants. This ability to utilize new methods in pest and resistance management make genetically modified insect resistant plants a critical component for successfully managing insect pests in the future.

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APPENDIX 10

GENETICALLY MODIFIED COLORADO POTATO BEETLE RESISTANT POTATO PLANTS, TOPICALLY-APPLIED MICROBIAL *B.t.t.*, AND CONVENTIONAL INSECTICIDES: COMPARATIVE IMPACT ON COLLEMBOLA

Genetically Modified Colorado Potato Beetle Resistant Potato Plants, Topically-applied Microbial *B.t.t.*, and Conventional Insecticides: Comparative Impact on Collembola

by

Gary L. Reed[1], Andrew S. Jensen[1], Dale T. McCreary[1], Karl A. Puls[1], Jenni L. Cena[1], and Jennifer Feldman[2]

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ABSTRACT

During two years of research, potato plants genetically modified for resistance to Colorado potato beetle were compared with other mechanisms of pest control to determine their impact on the potato ecosystem. One facet of that research involved a measurement of the impact on detritivorus insects including collembola. Plots with genetically modified plants had collembola populations equal to those which would occur without the disruptive impact of insecticides and populations substantially higher than observed where conventional systemic insecticides were used. Higher collembola populations only occurred in permethrin treated potato after predator populations were dramatically reduced. Genetically modified plants also had higher collembola populations than observed in the unprotected plots, an effect of early defoliation and death of the unprotected plants. The genetically modified CPB resistant plants had no measurable deleterious effect on populations of collembola and would appear to be less disruptive of detritivor populations than currently used insecticides.

INTRODUCTION

Past experience has taught us that the introduction of new technology without thorough investigation can lead to detrimental effects on the environment. When The Monsanto Agricultural Company proceeded with the development of genetically modified plant resistance to Colorado potato beetle (CPB) they chose to use the CryIIIA gene from *Bacillus thuringiensis* var. *tenebrionis* (*B.t.t.*) (Perlak et.al., 1993). This gene produces an insect control protein which is insecticidal to CPB and additionally to a very limited number of other coleopteran species. This specificity suggested that the product should have little deleterious impact on the potato ecosystem. In order to corroborate this hypothesis, research has been conducted at the Hermiston Agricultural Research & Extension Center, Hermiston, Oregon. since 1991 on a wide base of organisms inhabiting the potato field. One aspect of this research has focused on organisms which inhabit the decaying vegetation of the potato field. Collembola feed on decaying plant material, fungi, and bacteria (Borer et.al., 1992); and because of their abundance play a very important role in the decomposition of potato foliage at the end of the year.

The potato plant, as grown in the Pacific Northwest, develops a dense canopy by early July most years. Soon after, heavily shaded lower leaves begin to yellow and eventually die and fall to the soil surface. This dead leaf material provides a source of nutrition for fungi and bacteria which in turn are fed upon by a complex of arthropods which very rapidly decompose the dead vegetation. It was important to determine whether the decayed leaves from plants that had the crystal protein would deleteriously impact organisms involved in the decomposition of potato plant debris. This report specifically details a comparison of the impacts of the genetically modified plants with those normally occurring in insecticide treated fields on the collembola, a major inhabitant of the decaying vegetation.

METHODS AND MATERIALS

The genetically modified plant resistance to Colorado potato beetle (Tg) was compared to M-Trak, a biological insecticide with the *B.t.t.* crystal protein (Mtrak); di-syston, a conventional systemic insecticide (Sys); permethrin, a foliar pyrethroid insecticide (Perm); and an unprotected check (None) to determine effect on arthropods in the potato ecosystem. These treatments were compared using a Latin square experimental design in 1993 and as part of a larger experiment using a randomized complete block design in 1994.

Potato was grown in 1993 and 1994 under conditions duplicating as near as possible those used in commercial production in the Columbia Basin of Oregon. A fall application of vapam fumigant was made prior to the 1994 research season. Soil preparation included discing, deep ripping, and formation of hills using a dammer-diker - including shallow ripping between rows and in the hill. Potato seed pieces were planted using a tuber unit planter with a 9" spacing between tubers in 34" rows on May 17, 1993 and May 22 & 23, 1994. Dikes were constructed at the 6" rosette stage of plant growth.

Fertilization was based on both soil and petiole samples and recommendations made by a commercial consultant[3]. Preplant fertilizer excluding nitrogen was applied before field preparation. A side-dressed application of nitrogen was made at planting. During the season. fertilizers were water-run through the center pivot irrigation system. A single application of eptam 7E (3# ai/a) and sencor 4 (0.5 # ai/a) herbicides was water-run at ca. 6" rosette stage of plant growth. Irrigation was applied through a low pressure center pivot irrigation system using spinner sprinklers. Irrigation was applied following a schedule provided by the Northwest Irrigation Network. This schedule is modelled on plant growth stage and evapo-transpiration data from the Agrimet station on the center.

Insecticides applied as experimental treatments were made either; preplant using a continuous belt applicator for granules or an over the row, nitrogen pressured, sprayer at 80 lbs pressure with nozzles suspended 18" above the crop to approximate application by air.

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[3]Standard commercial fertilizer recommendations were made by Dave Williams, Wilbur-Ellis Co, Umatilla, Oregon.

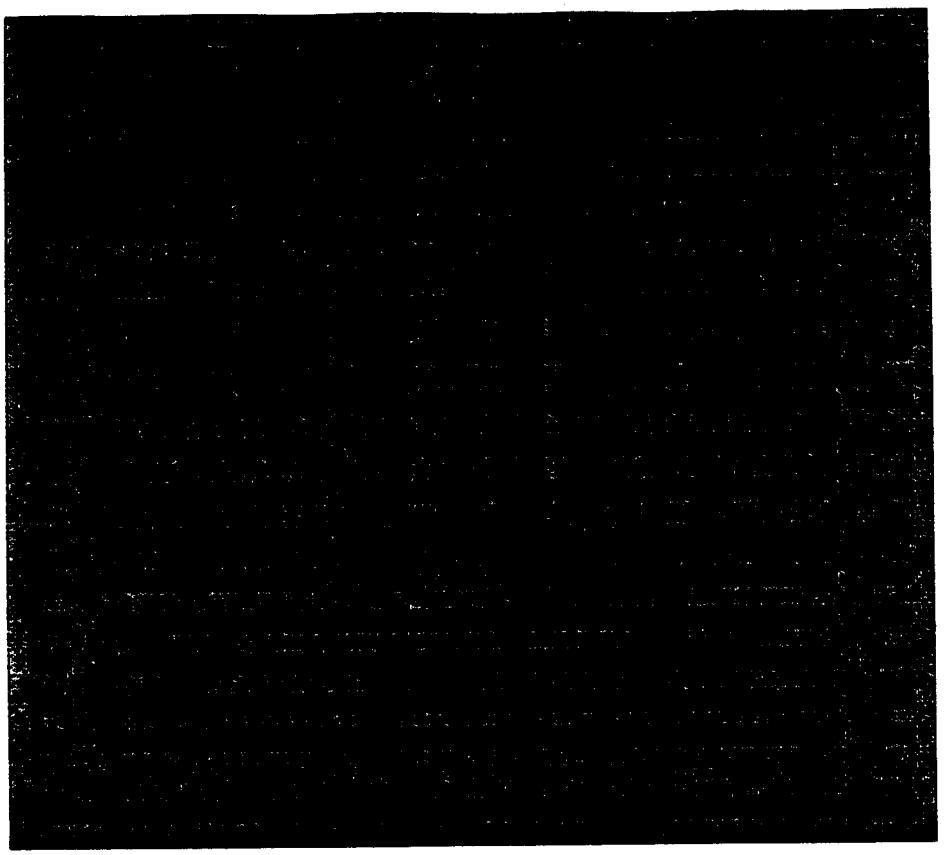

Figure 1. Integrated pest management experimental unit, Hermiston, Oregon 1993-1994. Arthropod data taken between blue and red lines, yield taken within red line, entry to yield area restricted season-long, purple circle site of pitfall trap, light green circles are plants included in treatment, dark green circles are untreated buffer plants.

The experimental unit consisted of a "treated" block of potato 46'x46' bordered on all sides by an outer walkway and by untreated normal "Russet Burbank" potato plants (Fig 1). The entire block including walkways and unprotected normal plants was 54.5'x54.5'. Inside the treatment block, the inner 16'x16' was restricted for entry during the season to prevent yield effects. The center 10' of the inner 4 rows (see red square in Fig 1) were harvested for yield. The 16'x16' yield block was surrounded by an inner walkway consisting of an unplanted row on the sides and 36" of unplanted row at the end of each 16' row to allow for collection of data.

Arthropods inhabiting the foliage were sampled twice weekly using a 28"x28" beating cloth. Beating cloth samples were collected along the outer edge of the inner walkway. The sampling procedure consisted of gently lifting the vegetation bordering the inner walkway, slipping the cloth under the tissue till it reached the main stem then folding stems from the other side of the row back over the cloth. The plant tissue over the cloth is then struck firmly 8 times using a 36"x3/4" wooden dowel . A team of 3 individuals counted all

arthropods falling to the beating cloth. Data forms were used for regularly occurring species or groups and notes made of occasional species. Consecutive samples were taken in a clockwise pattern around the inner walkway, rotating to the next side for each sample. An attempt was made not to sample the same position on a side the next time it was sampled, allowing no area to be sampled more than once in 4 weeks.

Arthropods inhabiting the soil surface were sampled twice weekly using pitfall traps. Pitfall traps were placed 17" from the southeast end of row 2 and from the northwest end of row 5 in the yield block (purple circles Fig 1). The pitfall trap consisted of a 16 oz plastic drinking cup (Solo P-16) with an inserted funnel (made by cutting the bottom out of a Solo TP9 cup). A solution containing small amounts of a wetting agent and copper sulfate was used to trap the arthropods. The pitfall traps were checked on the same days that the beating cloth samples were taken. Pitfall traps were capped and brought into the laboratory where the contents were poured onto a 12" diameter sieve upon which the insects were visually identified.

RESULTS

Collembola were the most common insect in the potato ecosystem in both 1993 and 1994. The collembola in the plots included at least four species from three families: hypogastruridae, entomobryidae, and sminthuridae. Of these the hypogastruridae and entomobryidae were common while the sminthuridae was rare. Treatment effect on collembola population in both years with both sampling techniques were very similar (Fig 2). The following observations were drawn from an average of both years and both sampling techniques (Fig 3). In all cases, collembola populations were highest where plants were treated with permethrin insecticide (Perm). The *B.t.t.* insect control protein in the genetically modified CPB resistant plants (Tg) and on plants treated with M-Trak (Mtrak) had the next highest number of observed individuals. The unprotected (None) treatment had intermediate numbers of collembola while plots treated with systemic insecticide (Sys, Tg&Sys) had the fewest collembola.

DISCUSSION

The unprotected check would in most crop systems provide the most natural treatment for comparing impact of an insect control mechanisms on non-target species such as collembola. However, that is not the case in potato where Colorado potato beetle is a pest. In our plots, CPB totally defoliates the unprotected plots at some time between the middle of July and the end of August. As potato plants become defoliated, most insect species decline in numbers, often migrating to other plots, thus numbers in the unprotected (None) plots are lower than would be expected. Numbers of collembola in plots with genetically modified CPB resistant plants and the M-Trak treatment more closely approximate the normal number of Collembola in an ecosystem where insecticides are absent. The elevated population of

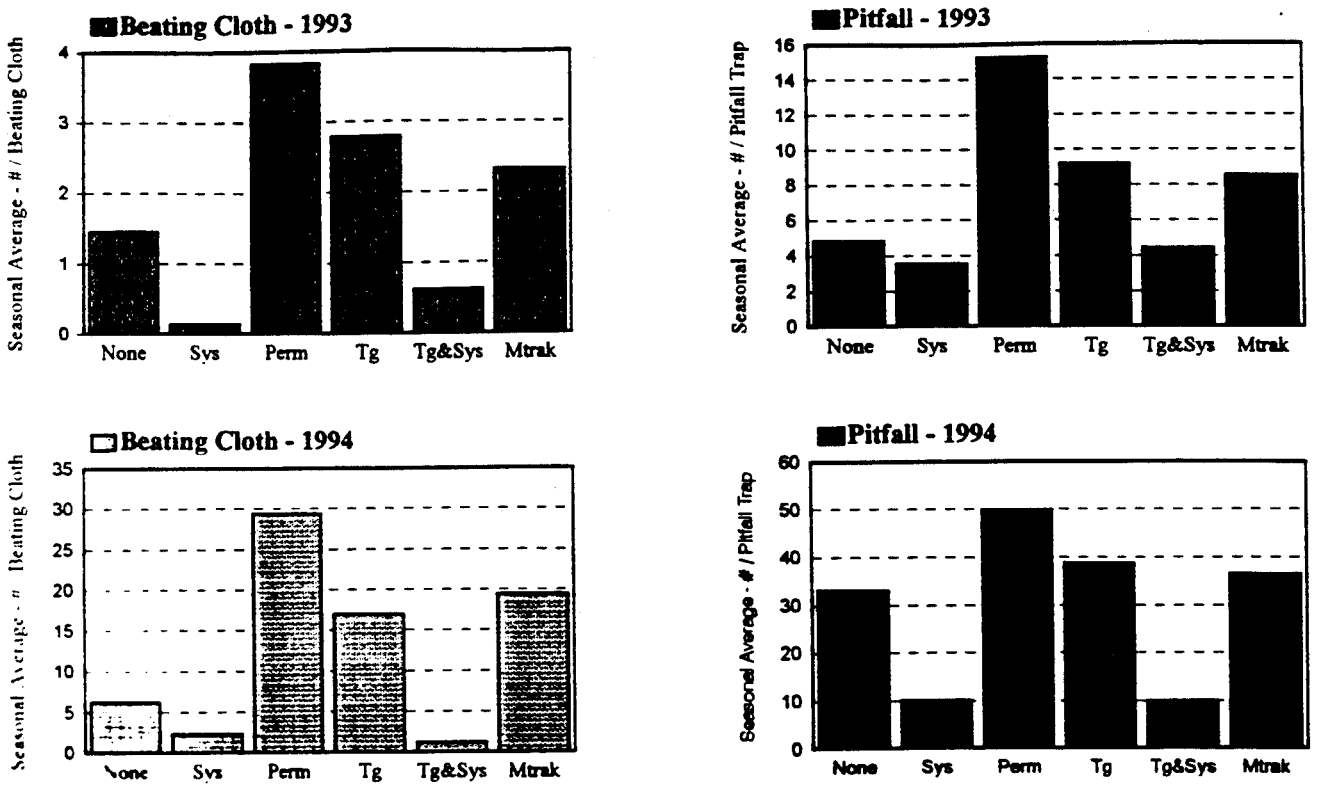

Figure 2.. Collembola counts from beating cloth samples and pitfall traps, Hermiston, Oregon, 1993 and 1994.

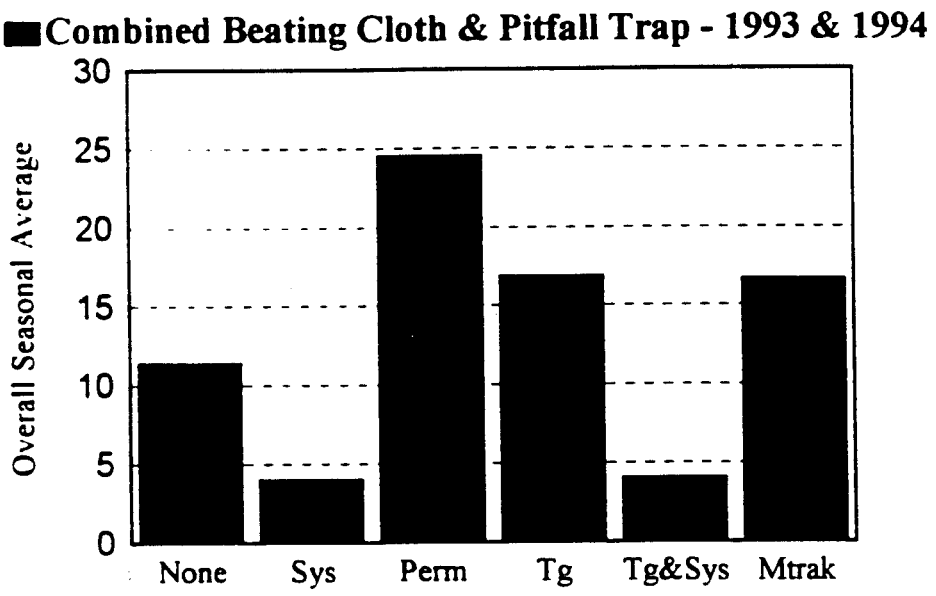

Figure 3. Average counts of collembola counted in beating cloth samples and caught in pitfall traps, Hermiston, Oregon, 1993 and 1994.

collembola in the permethrin treated plots (Perm) occurred as a direct response to reduced numbers of predators including: dwarf and crab spiders, *Geocoris pallens* Stal, nabidae, and carabidae (data not shown). A similar response was observed in aphid populations in permethrin plots both years (data not shown).

It is very important as we develop new insect control technologies for use in Integrated Pest Management strategies, that we have tools that are not disruptive of the ecosystem. This is particularly true of that segment of the ecosystem that modifies dead plant tissue and recycles it for renewable plant production. Collembola are a major component of the detritivors that decompose potato plant tissue at the end of each season. Treatments using the *B.t.t.* insect control protein including the genetically modified Colorado potato beetle resistant potato plants were the least disruptive of collembola populations of all CPB control measures evaluated. The introduction of genetically modified CPB resistance has the potential to become a major tool in allowing realistic integrated pest management in the potato industry. Collembola population conclusions merge well with data which indicated that the highest level of predators and other detritivors in these studies (Reed et.al., 1993) also occurred in the *B.t.t.* protein treatments.

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APPENDIX 11

MALE STERILITY OF RUSSET BURBANK POTATOES

United States Department of Agriculture
Agricultural Research Service
Pacific West Area
P.O. Box 307
Aberdeen, Idaho 83210
(208) 397-4162

March 30, 1989

Dr. William Belknap:
USDA,ARS
Western Regional Research Center
800 Buchanan Street
Albany, CA 94710

Dear Dr. Belknap:

Dennis Corsini asked me to write to you regarding the question of isolation of transgenic plants of cultivated potato because of the possibility of pollen escaping from these plants and contaminating other potatoes or related species. In my opinion, there is no practical possibility of this happening. Cultivated potatoes are all asexually (i.e. vegetatively) propagated. As a result, pollen has no part in the growing and reproduction of the cultivated potato. Many of the cultivated varieties grown in the U.S. and Canada have little or no pollen; this later case is true for Russet Burbank, the most popular U.S. variety.

Wild relatives of the cultivated potato are insect (bee) pollinated, not wind pollinated. Flowers of the cultivated potato are not attractive to bees, primarily because they lack nectar. There are no wild relatives of potato in any of cultivated areas of the U.S. or Canada, consequently the possibility genes escaping into a wild population is non-existent.

One other possibility to consider is the possible production of fruit from self pollination of a transformed potato plant that does have fertile pollen. This could occur with a variety such as Lemhi Russet if the growing season were cool and the humidity favorable. At maturity the fruit (berry) would fall to the ground and, under the most favorable conditions, seedlings might become established the next year. With thousands of acres grown with Lemhi Russet and similar varieties, there has been no report of a weed-like, seedling population of potatoes being established after a cultivated potato crop. And in more than 20 years of potato breeding I have grown many very fertile potato plants, but I have not seen even one seedling potato plant growing from berries that remained in the field from the previous year. In any case the possibility for survival in a cultivated field is practically nil. Fumigation, which I don't believe is needed, would further reduce the possibility for survival.

Therefore, from the standpoint of possible gene escape, I know of no need to isolate transgenic potato plants beyond the normal separation used in experimental plot practice. The 20 ft. isolation required between certified and non-certified potatoes in the production of certified potato seed would only be needed if you were saving tubers for seed.

I hope that this information will be useful. Please phone or write if you have any questions.

Sincerely,

Joseph J. Pavek
Research Geneticist

JJP:pr

OPTIONAL FORM 99 (7-90)

FAX TRANSMITTAL # of pages ▶ 2

To	From
Terry Stone	Sally VW
Dept./Agency	Phone #
Monsanto	301-436-4886
Fax #	Fax #
314-537-7085	

NSN 7540-01-317-7368 5099-101 GENERAL SERVICES ADMINISTRATION

TO: BP Staff

FROM: Sally Van Wert

SUBJECT: Fertility of several potato cultivars in the U.S.A.

In an effort to nail down the male fertility in the United States of several potato cultivars in the field I contacted three potato breeders: Dr. Joe Pavek, University of Idaho; Dr. Bob Johansen, North Dakota State University; and Dr. Bob Plaisted, Cornell University. I asked Dr. Johansen only about the "Nor" lines since he originated these. The information I obtained from them should apply to the section of the country they live in. I inquired about the cultivars I have seen as transgenics in applications for release. The results of this inquiry are as follows:

Cultivar	North West	North East	North Dakota
Atlantic	MS	MF	
Katahdin	UN	MF	
Kennebec	MS	MF*	
Lemhi Russet	MS*	MF♯	
Norchip	MS	MF♯	MF*
Norgold Russet	MS♯	UN	MF*
Norland	MS	UN	MF*
Russet Burbank	MS	MS	
Shepody	MS	MF*	
Superior	MF	MF	

Key:

MS = male sterile
MF = male fertile
UN = not grown much in area, so not known
MS* = male sterile in Idaho when temperatures are above 85F, yields berries when temperatures are moderate.
MF* = berries are rare in New York or North Dakota
MS♯ = does not flower in Idaho when night temperatures are below 60F.
MF♯ = as seen in greenhouses, no field data available

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continued on next page

Notes:

Dr. Pavek stated that when temperatures are above 85F he sees no fertility at all in the field.

Dr. Plaisted stated that at "lower elevations, with more drought" there is less seed set. Seed set is usually dependent upon night temperature, i.e., more seed set with cooler temperatures.

Dr. Plaisted stated that seed set is higher in Maine where the overall daily temperatures are lower.

Dr. Plaisted said that he has only seen the current seasons berries produce seedlings. He sees these seedlings in mid to late August the last time he walks the field prior to vine kill. When he sees these seedlings they are in spaced rows (this is an empty row between two planted rows). Such seedlings are never seen in the shade, as occurs when there are no empty rows between planted rows.

Dr. Plaisted said that in surveys of seeds obtained in the field in New York up to about 20% of the seeds were formed through cross-pollination, the remainder were formed through self-pollination.

APPENDIX 12

ECOLOGICAL RISK OF GROWING TRANSGENIC POTATOES IN THE UNITED STATES AND CANADA: POTENTIAL FOR VEGETATIVE ESCAPE OR GENE INTROGRESSION INTO INDIGENOUS SPECIES

Ecological risk of growing transgenic potatoes in the United States and Canada: potential for vegetative escape or gene introgression into indigenous species[1]

Stephen L. Love and, Joseph J. Pavek[2]

Abstract:

Numerous concerns must be addressed before regulatory policies can be relaxed sufficiently to allow large-scale field production of genetically engineered potatoes. Among the most prominent is escape and proliferation of transgenic cultivated potatoes and the introgression of transgenes into native wild species with subsequent potential for ecological disruption. Using available literature, a risk assessment was made of transgene escape and subsequent introgression into wild species growing within the geographical borders of the United States and Canada.

Escape and proliferation of domestic transgenic varieties is not of concern because potatoes are not competitive outside of cultivated areas. Potatoes will not hybridize with the

[1] Paper No. _____ of the Idaho Agricultural Experiment Station. This paper was made possible by a grant from the Monsanto Agricultural Company.

[2] Associate Research Professor, University of Idaho and Research Geneticist, USDA-ARS, respectively, Aberdeen R&E Center, Aberdeen, ID 83210.

Accepted for publication: _____.

Additional key words: ecology, genetic engineering, gene transfer, Solanum

non-tuberous Solanum weed species common to potato production sites. The presence of three tuber-bearing Solanum species (S. fendleri, S. jamesii, and S. pinnatisectum) in the southwestern United States suggests a conceivable avenue for transgene escape. A number of barriers exist to prevent natural hybridization and introgression, including geographical isolation, endosperm imbalances, multiple ploidy levels, and incompatibility. The number and magnitude of these barriers makes natural hybridization highly unlikely and transgene introgression impossible or at least highly improbable.

Commercialization and large-scale production of genetically modified potatoes is rapidly approaching realization in the United States and Canada. Due to a lack of information about important environmental and safety risk elements, all genetically engineered potato products are still heavily regulated. Before the regulation process can be relaxed and commercialization proceed, concerns about all potential risk elements must be addressed in a methodical and scientific manner. Currently,the concerns about genetically modified potatoes fall into two main categories: 1) food and product safety, and 2) environmental impact (Table 1) (8,14,28). Food safety and many environmental issues will have as a basis the potential adverse effects of genes or gene products. This is especially true when potatoes are engineered to produce substances with pesticidal properties. Policy decisions involving such potatoes will depend on the transgenes (an engineered gene expressed by a living organism) involved. Gene specific regulatory issues will always have to be resolved gene by gene and policy determined as each case requires. However, some policy decisions can appropriately and universally be made using risk management for all transgenes within a species. Situational regulation is difficult to establish and expensive to enforce. Where possible, a more global approach to identifying risk should be found.

Many environmental concerns, which are based on potential ecological disruption due to gene escape, can be addressed in a comprehensive fashion. The applicable axiom is that detrimental ecological impact is directly dependent on the potential for unmonitored escape of a transgene into the environment. It follows that minimal escape potential is accompanied by minimal environmental concern. However, if escape can and does occur, the impact then

becomes proportional to the competitive advantage of the transgenic organism in the surrounding ecosystem.

Gene escape from transgenic potatoes can be precipitated by any one of several events: 1) transfer of modified genes into microorganism or pathogen populations, 2) horizontal movement of genes into other plant species due to latant infection with a bacterial gene vector, 3) escape and proliferation of transgenic potatoes in the wild, or 4) potato hybridization with and transgene introgression into native wild species. No evidence exists for gene movement into microorganisms or for horizontal gene transfer and these issues will likely dissipate, although not before more research is completed (8). Concerns about the other two avenues, physical escape and hybridization, can be addressed without resorting to a tedious case-by-case inspection. A global approach is valid only if all potential mechanisms of escape or hybridization can be accounted for and eliminated. This paper will use currently published information as evidence to evaluate the potential for escape and proliferation of transgenic potatoes and introgression of transgenes into wild species that are indigenous to the United States and Canada. Discussion on the potential for hybridization and introgression will include both tuber- and nontuber-bearing recipient species.

Escape of Transgenic Cultivated Potatoes

The concern with the escape of cultivated transgenic potatoes is that they become a competing weed species with an accompanying change in local plant ecology. The initial

spread of transgenic potatoes can be started by vegetative propagules (tubers) or sexually produced seed.

Vegetative escape: Evenhuis and Zadoks (8) concluded that crop species, including potato, are at a competitive disadvantage and will not survive in the wild. Long distance transport and mass handling of tubers ensure that many opportunities for escape have occurred and will continue to occur. In spite of many opportunities, vegetative volunteers (plants growing from tubers left from a previous crop) have failed to survive outside of cultivated areas, where they occasionally become a short-term weed problem. Even then, weed control efforts and unfavorable environmental conditions usually limit weediness problems in fields to one or a few years (18). There is specific concern about situations resulting from the addition of herbicide resistance to potatoes which will make control of volunteers more difficult. Insertion of a such a gene could exacerbate the weediness problem in cultivated fields where weather conditions alone do not provide consistent control. The benefits of such genes will need to be carefully evaluated and balanced against potential problems. However, herbicide resistance will have no impact outside of cultivated areas are where a general lack of adaptability is the factor limiting competitiveness.

Seed production and dissemination: Brown et al. (3) found that transformation of potatoes did not necessarily detract from their ability to produce pollen and set seed. Many potato varieties produce abundant seed under typical production conditions. Potato seeds can survive and germinate for periods of time in excess of seven years (15). As a result, potato seedlings have the potential to be a more persistent problem than vegetative escapes. In spite

of greater persistence seedling vounteers, like vegetative volunteers, are limited to cultivated areas for reasons of competition and adaptation.

To conclude the discussion on escape of cultivated transgenic potatoes, current evidence indicates they have no potential as ecologically damaging weed species. This could change with the introduction of genes that cause an increase in fitness. But the increase would need to be of an unlikely magnitude. None of the genes introduced into potatoes to date, including disease and pest resistance, have had a documented effect on fitness and additionally, plants with large changes in competitiveness and fitness can be easily recognized and eliminated (8). It appears that escape and proliferation of cultivated potatoes can be ignored as a risk factor. Of much greater concern is the introgression of transgenes into adapted species where small changes in fitness can produce a large competitive advantage.

Transgene Escape Through Pollen Dispersal, Hybridization, and Introgression

Given the volume of published research, a careful examination of existing information should be sufficient to determine the potential of gene movement from cultivated to indigenous, adapted species. Evenhuis and Zadoks (8) determined that engineered genes are sexually transferred to native species only when the following conditions are met: 1) there is dispersal of transgenic pollen, 2) there is successful hybridization, 3) there is introgression into the species of concern, and 4) there is stabilization of the gene within the population of the wild species. Evidence that any one of these conditions cannot be met reduces the concern of gene movement into related wild species through hybridization.

Studies by Dale et al. (5) and Tynan et al. (29) have shown that dissemination of transgenes by movement of pollen in potatoes is very localized and cross-pollination occurs infrequently. However, even infrequent dissemination makes occasional hybridization a likelihood. Consequently, if we are to elininate hybridization as a risk factor, inability to meet one of the other three conditions must negate the chance for transgene escape. The remainder of this document examines each of the remaining three conditions and weighs their contribution to the risks for transgene movement from cultivated potatoes to related wild species.

As part of risk assessments, Evenhuis and Zadoks (8) and Kapteijns (14) concluded there was no risk in the Netherlands for transgenes to flow from cultivated potato to wild species. The major criterion for this conclusion was the lack of indigenous crossable species. The opposite situation is found in several regions of South America, the center of origin for the genus Solanum, where free exchange of genes occurs between wild and cultivated potato species. The situation in Canada is similar to that found in the Netherlands, but the situation in the United States is more complex. Closer proximity to the center of origin for the genus Solanum results in the presence of species with some potential to hybridize with potatoes. A cursory examination reveals that the potential for hybridization with nontransgenic cultivated varieties as well as with non tuber-bearing and tuber-bearing indigenous Solanum species cannot be simply dismissed.

Hybridization with nontransgenic cultivated varieties: Fertile potato varieties grown in close proximity, will likely hybridize, with a resultant flow of transgenes into new genotypes (3,5,29). However, cultivated potatoes in the United States and Canada are produced entirely as clonally (asexually) propagated varieties. This alone makes hybridization between varieties inconsequential. As described previously, seedlings from chance hybridizations may rarely become temporary weeds in cultivated fields, but uncontrolled movement of transgenes via sexual hybridization into new varieties is not possible.

Hybridization with nontuber-bearing Solanum species: Several nontuber-bearing Solanum species are widespread in North America. These include S. sarrachoides (hairy nightshade), S. triflorum (cutleaf nightshade), S. nigrum (black nightshade), S. ptycanthum (eastern black nightshade), S. dulcamara (bitter nightshade), S. elaeagnifolium (silverleaf nightshade), S. rostratum (buffalobur), S. carolinense (horsenettle), and S. torvum (turkeyberry). Most occur as weed species in cultivated areas, including potato fields and nearby waste areas (19,30). None of these species will hybridize naturally with S. tuberosum. S. nigrum will produce sterile hybrids when crossed with S. tuberosum, but only under artificial conditions and therefore is not considered to be crossable with cultivated potatoes (5,8,14). It can safely be concluded that there is no potential for transgene introgression into nontuber-bearing Solanum species. Because only nontuber-bearing species are indigenous to Canada, this information effectively eliminates concern for transgene escape in Canada through hybridization with wild species.

Hybridization with native wild tuber-bearing Solanum species: Correll (4) listed five tuber-bearing Solanum species as being indigenous to the southwestern United States. None of these species are found in Canada. The five include S. bulbocastanum, S. fendleri, S. jamesii, S. leptosepalum, and S. pinnatisectum. The presence of S. bulbocastanum in the United States was later refuted by Hawkes (9), who attributed the listing to a mix-up in labeling. The original collection has never been reproduced.

S. leptosepalum was reportedly collected in southern Texas by Hinckley in 1946 (4). No additional collections of this species have been made and there still remain some question as to its true identity. If it is a separate specie, its sparsity and isolation (one rocky canyon in a mountainous region near the southern border of Texas) makes its presence of little or no -- -- consequence.

The presence in the United States of three other species is well documented. S. fendleri has been found in Arizona, Colorado, New Mexico, and Texas (4). S. jamesii has been collected in Arizona, Colorado, Nebraska, New Mexico, Texas, and Utah (4). S. pinnatisectum has been found in an isolated valley in Arizona (4). Each of these three species represents a remote, but feasible, avenue of transgene flow into native Solanum populations. Only geographical isolation and/or genetic barriers to hybridization or introgression will eliminate the risk represented by the presence of these species.

Geographical isolation: S. pinnatisectum is limited to a small geographical area in Arizona that is isolated by hundreds of miles from potato producing regions (4). All three

species of concern are native to dry, forested areas above 1600 m in elevation. S. fendleri has been observed in areas where potato production occurs (4). S. jamesii has been found growing in and around cultivated fields, probably as a result of early attempts to domesticate this particular specie (4). Geographical isolation severely limits opportunity, but cannot be considered as a complete barrier to hybridization with respect to S. fendleri and S. jamesii.

Genetic barriers: S. fendleri, S. jamesii, and S. pinnatisectum belong to a group informally known as Mexican wild species (see Table 2 for genetic characterization). In the experience of the authors, when using normal or natural hybridization techniques, it is not possible to obtain hybrids between any of these three species and S. tuberosum. Other researchers have reported the same finding (16,20,21,27). This was confirmed by Dr. John Bamberg (personal communication) of the United States Department of Agriculture Potato Introduction Station who recently revisited the areas inhabited by S. fendleri, S. jamesii, and S. pinnatisectum. He found no evidence that hybridization occurs between these species much less between any of the three and cultivated potatoes. Dr. Bamberg reaffirms that no one has ever found hybrids between these three indigenous species and S. tuberosum cultivars. From this evidence, it would be easy to conclude that no avenue exists for transgene escape through hybridization. But several researchers have used unorthodox hybridization techniques to transfer genes of S. fendleri and S. pinnatisectum into S. tuberosum germplasm. The methods included use of bridge species such as S. demissum and S. verrucosum, crossing with partially fertile triploid hybrids, and the use of artificial polyploids (1,2,9,25,26,27,personal communication with John Bamberg). These exceptions make it difficult to universally rule out the possibility of hybridization and gene introgression without further investigation of the

genetic barriers that may prevent such an event. Known barriers to hybridization of the three indigenous species and domesticated potatoes include endosperm imbalances, multiple ploidy levels, and incompatibility.

Early attempts to elucidate mechanisms preventing hybridization among tuber-bearing Solanum species revealed that following pollination, fertilization and cell division often occurred, but abnormal endosperm development caused the embryos to abort (10,13,22). Efforts to explain this phenomenon led to the concept of endosperm balance number (EBN) (13). The genetic and physiological reasons behind EBN remain unknown, but the concept has proven valid. Any two species with identical EBN's are usually crossable, while those with different EBN's are not. The EBN's of S. fendleri, S. jamesii, and S. pinnatisectum are 2, 1, and 1, respectively (Table 2). The EBN of S. tuberosum is 4. This fact alone may eliminate any potential that natural hybrids will occur between S. tuberosum and any of the three indigenous wild species. However, Hawkes and Jackson (9) outlined exceptions that make differences in EBN a breachable barrier. In order to be confident that hybridization and/or gene introgression will not occur the exceptions must be explored.

A factorial change in ploidy level results in an equivalent change in EBN (10,21). Adiwilaga and Brown (2) capitalized on this principle by using S. tuberosum diploids (EBN=2) to make successfu crosses with S. fendleri. *In vivo* rescue-pollination of the subsequent triploids allowed hybridation with cultivated potatoes. The final result was fertile pentaploids that made possible the transfer of S. fendleri genes into S. tuberosum germplasm. The use of S. tuberosum diploids, a form not used commercially, and the artificial nature of

the hybridization procedure provided conditions that cannot be reproduced in nature. However, it demonstrates the genetic flexibility of Solanum species and the potential for defeating hybridization barriers.

The natural mechanism that could defeat the EBN barrier is an increase in ploidy level through the production of unreduced gametes, a common occurrence among Solanum species (10,13,21). The presence of unreduced gametes in S. fendleri would create an EBN of four, and theoretically create a genotype crossable with S. tuberosum. However numerous efforts to increase ploidy levels for the purpose of equalizing EBN's have failed to produce hybrids between S. tuberosum and S. fendleri, S. jamesii, or S. pinnatisectum (6, 16, 17, 21). This provides strong evidence that hybridization between cultivated potatoes and indigenous tuber-bearing wild species will not occur in nature. The effect of EBN goes beyond hybridization -- potential and allows extension of logic to transgene introgression. In the unlikely event that a fertile hybrid does occur, it will happen only under conditions of both parents having an EBN of four. In that case, the hybrid would have an EBN of four or more and not be crossable back to the native species from which the wild parent originated. The result is that the process of introgression would cease.

It is difficult to discuss the influence of differing ploidy levels without considering their indirect influence on EBN. S. jamesii and S. pinnatisectum are diploids (2x=24), while S. fendleri is a tetraploid (2x=48). EBN has a distinct influence on the result of crosses between species of different ploidy levels (10). Hybridizations involving identical EBN's and differing ploidy levels usually result in triploids, which are largely sterile (2, 22). The opposing situation, different EBN's and identical ploidy levels, results in what is known as a triploid

block (embryo abortion and subsequent death of all triploid embryoes) ultimately preventing hybridization except through the doubling of EBN that is a by-product of unreduced gametes. For all three indigenous species, there is not an EBN, ploidy level combination which could be hybridized with tetraploid, cultivated S. tuberosum and still make possible transgene introgression back into the original wild species. This effectively eliminates the possibility of meeting the conditions set out by Evenhuis and Zadoks (8) of introgression and stabilization of a transgene in the wild species of concern.

The last barrier to hybridization and gene introgression is incompatibility. Incompatibility refers to the presence or absence of genes that allow normal pollen tube formation and fertilization to occur (12). Incompatibility is present when two species do not express reciprocal genes that allow fertilization to proceed. In diploid potatoes, this is due to a genetic system similar to the S-allele system proposed for other crops (24). Pandey (23) determined that South American species have a one-gene incompatibility system, but two Mexican species, including S. pinnatisectum, have a two-gene system. If this system is prevalent among Mexican wild species, it is likely that S. tuberosum, with its South American ancestry does not possess the genes needed to be compatible with S. fendleri, S. jamesii, and S. pinnatisectum. This idea is strictly theoretical and is an area for future research. However, some evidence exists to support the idea. Dionne (6) as well as Doll and Hanneman (7) documented that incompatibility, not due to EBN, is present between S. fendleri and S. tuberosum when crosses failed because pollen tubes failed to reach the ovary. The complete lack of hybrids between all three wild species and S. tuberosum, even when other crossing barriers were addressed provides evidence that some form of incompatibility does exist (7).

Conclusion:

The available evidence overwhelmingly supports the conclusion that within the borders of the United States and Canada, there is no avenue available for escape of potato transgenes into the environment. A lack of adaptibility and competitiveness prevents potatoes from competing with native species and limits weediness to being a temporary problem and then only in cultivated fields. Transgene escape through hybridication also appears to be of little or no concern. Given the experience of a number of researchers, prudence dictates caution in concluding that natural hybrids will never occur between cultivated potatoes and indigenous tuber-bearing Solanum species, even though none have been documented. As Hermsen (11) said, "In practice, it is difficult to prove unequivocally that two related species are uncrossable. The statement 'these related species cannot be intercrossed' has to be qualified by adding: with the available genotypes, under prevailing environmental conditions, with or without special devices in vivo or in vitro." However, given the number and potency of barriers to hybridization, and more especially to introgression, and stabilization, the only sound conclusion is that transgene introgression into wild Solanum species will not occur under natural conditions. This information will have important implications for how we view transgenic potatoes in the future. It should allow us to remove one major concern from the list of regulatory criteria. Future documentation of the environmental impact of transgenes in potatoes, within the borders of the United States and Canada, need not account for transgene escape through vegetative means or hybridization with wild, indigenous Solanum species.

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Table 1. Listing of concerns that result in regulation of genetically engineered potatoes.

Concern	Potential Deleterious Impact	Methods used to Evaluate Risk
1. Food Safety:		
a. Unsafe gene products	Toxicity to humans or other mammals	Food safety evaluation
b. Changes in nutritional value	Reduced nutritional quality	Nutritional quantification
2. Environmental Issues:		
a. Adverse effects of gene products produced by transgenic potatoes	Ecological disruption by injury to non-target organisms	Controlled ecological evaluation
b. Escape and proliferation of transgenic potatoes	Ecological disruption through a change in plant community structure	Controlled ecological evaluation
c. Transfer of transgenes to wild species	Ecological disruption through a change in competitive balance within plant communities	Assessment of hybridization potential and/or controlled ecological evaluation

Table 2. Genetic characteristics of S. tuberosum and three tuber-bearing Solanum species that are native to the southwestern United States.

Species	Ploidy Level	EBN
S. tuberosum	4x (48)	4
S. fendleri	4x (48)	2
S. jamesii	2x (24)	1
S. pinnatisectum	2x (24)	1

APPENDIX 13

OVERWINTERING ABILITY OF COLORADO POTATO BEETLE RESISTANT POTATOES IN COMPARISON TO STANDARD RUSSET BURBANK

OVERWINTERING ABILITY OF COLORADO POTATO BEETLE RESISTANT POTATOES IN COMPARISON TO STANDARD RUSSET BURBANK

Introduction

When potatoes are harvested with conventional harvesting equipment, small tubers may fall through the chains and return to the soil. In areas with mild winters, these tubers may survive in the soil and reemerge the following year. Such "volunteer" potatoes can persist as weeds in rotational crops that are planted after potatoes. Volunteer potatoes are found only in cultivated fields, and are usually controlled with herbicides and cultivation. While the introduction of a gene for resistance to the Colorado potato beetle is not expected to alter the ecological success of potatoes, it is important to evaluate the potential for survival of genetically modified plants in all situations.

In the winter of 1994, research was initiated in three locations to determine the overwintering survival of CPB resistant Russet Burbank potatoes in relation to an unmodified Russet Burbank control. The information from this study will contribute to an understanding of the potential for CPB resistant potatoes to become weeds in agricultural cropping systems.

Materials and Methods

Identical field experiments were established at three sites representing geographically diverse potato production areas. Research locations were the Oregon State University-Hermiston Agricultural Research and Extension Center (HAREC), Hermiston, OR; University of Wisconsin-Hancock Research Station, Hancock, WI; and the Cornell University H.C. Thompson Vegetable Research Farm, Freeville, NY. The CPB resistant potatoes used in the study consisted of a mixture of lines BT6, BT10, BT12, BT16, BT17, BT18 and BT23 in Oregon and Wisconsin and line BT23 only, in New York. The field site at all locations was not previously planted to potatoes. The plots of CPB resistant and Russet Burbank potatoes in all locations consisted of single 20' rows, replicated three times and arranged in a randomized complete block design. Whole potato tubers were buried at a depth of 3-6 inches, 20 per plot, to simulate the random placement of tubers following harvest and fall tillage. Potato plots were established in 1993 on November 19, in Wisconsin, November 29, in Oregon and December 8, in New York. All plots were evaluated over a 6-10 week period in the spring of 1994. Emerging volunteer potatoes were counted and removed from the study area.

Results

No volunteer potatoes were found in either Wisconsin or New York, where sub-zero temperatures were reached and snow cover was minimal (Tables 1 and 2). Approximately 65% of the planted tubers emerged in Oregon, where temperatures were milder throughout the winter (Table 3). Both plant types emerged at the same time, and

no significant differences in overwintering survival were detected between Russet Burbank and CPB resistant potatoes. These results confirm that CPB resistant potatoes do not have an increased ability to overwinter and become weeds in cultivated potato producing areas.

Table 1. Mean number of emerged volunteer potato plants at Hancock, WI, 1994.

Potato type	14-Apr	9-May	24-May	14-Jun
CPB Resistant	0	0	0	0
Russet Burbank	0	0	0	0

No significant differences, Fisher's Protected LSD, .05 level.

Table 2. Mean number of emerged volunteer potato plants at Freeville, NY, 1994.

Potato type	13-May	29-Jun	Aug	Oct
CPB Resistant	0	0	–	–
Russet Burbank	0	0	–	–

No significant differences, Fisher's Protected LSD, .05 level.

Table 3. Mean number of overwintered volunteer potato plants, Hermiston, Oregon 1994.

Potato type	20-Mar	9-Apr	24-Apr	9-May	16-May	24-May	31-May	6-Jun
CPB Resistant	0	0	0	8	12	13	14	14
Russet Burbank	0	0	0	7	10	12	13	13

No significant differences, Fisher's Protected LSD, .05 level.

APPENDIX 14

LETTERS OF SUPPORT

ASHTON SEED·CO

4054 E 1300 N
ASHTON, ID 83420

CLEN ATCHLEY
SCOTT KANDLER
EMMA ATCHLEY
GREENHOUSE MGR.

April 5, 1994

Mr. Terry Stone/BB4D
Senior Regulatory Specialist
Monsanto Co.
700 Chesterfield Village Parkway
St. Louis, MO 63198

Dear Terry,

I would like to answer some of your questions about the transgenic Russet Burbank potatoes that we have grown for seed potato production for HybriTech Seed International. We have had two years experience in the green house and one year in the field. From these crops we have made the following observations:

1. The RBBt potatoes are not different from Russet Burbanks in plant or tuber appearance in the field or greenhouse. The foliage and tubers of the Bt potatoes are undistinguishable from non-transgenic Burbanks in both environments.

2. At this point there is no indication that the transgenic potatoes will set true seed or that they are any more likely to survive winters and become volunteers the following season than non-transgenic Burbanks.

3. The Bt Russet Burbanks do not appear to be any more susceptible to disease or insect damage than regular Russet Burbanks. This is evident in the virus readings in the post-harvest tests, which were similar to those of our other Russets.

I hope this information is helpful to you. Thank you.

Sincerely,

Clen P. Atchley
President

Years of Experience and Modern Technology

THE OHIO STATE UNIVERSITY
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Ohio Agricultural Research
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10 January 1994

Public Response and Program Resources Branch
Field Operations Division (7506C)
Office of Pesticide Programs
Environmental Protection Agency
401 M St. SW
Washington D.C. 20460

Comments regarding document control number OPP-30355, File symbol 524-UTU:

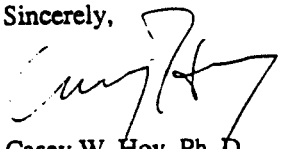
I am writing to offer my opinion in support of the use of transgenic potato plants that produce the *Bacillus thuringiensis* (Bt) d-endotoxin in agricultural production, as proposed by the Monsanto Company. In my opinion, plants containing this protein pose no more risk to producers, consumers, and the environment than do foliar sprays of Bt suspensions, which have been used in many different crops for more than 20 years. In fact, if the plant itself produces the endotoxin, then farmers can avoid damage to their soil through compaction, high foliar application costs (labor, equipment, and fossil fuels), worker exposure to sprays, and non-target contamination through drift and runoff.

Few pests are controlled exclusively with Bt foliar sprays, because they are highly selective, difficult to time properly, and have a short period of residual activity. This is particularly true for control of Colorado potato beetle with foliar Bt. We have attempted to optimize the use of foliar Bt's in Ohio potato integrated pest management programs, but have never been successful in controlling this pest entirely with Bt. The alternatives are broad spectrum insecticides that carry more environmental contamination, health and safety risks than do Bt sprays. Because the endotoxin in transgenic plants is always present in the foliage, timing and residual activity problems are avoided completely. In research conducted at the Ohio Agricultural Research and Development Center during 1993, Colorado potato beetle control was very thorough in plots containing transgenic plants. Furthermore, because no broad spectrum insecticides were used in these plots after a single early application for potato leafhopper control, aphids were successfully controlled by natural enemies. Biological control of aphids did not occur in plots where broad spectrum insecticides were used for Colorado potato beetle control. Based on last summers research results and spray records of Ohio potato growers, I estimate that use of the transgenic potato plants could reduce the number of insecticide applications, most of them broad spectrum insecticide applications, from an average of approximately 6-7 to an average of 1 application per year in our state. I believe that society as a whole would approve of that result.

The sole concern that I have with respect to the use of transgenic potatoes is the development of resistance to the endotoxin in Colorado potato beetle populations. I have the same concern, however, with respect to foliar Bt use or the use of any other insecticide. Colorado potato beetle is notoriously good at developing resistance to anything with which we try to kill it. Foliar Bt sprays have already resulted in resistance to the endotoxin in diamondback moth populations in the field, and in a Colorado potato beetle population in the laboratory. Monsanto Company has demonstrated ample concern

for this issue and has actively enlisted the assistance of professional entomologists to help devise the best strategy for avoiding resistance. In my opinion, the use of transgenic potato plants does not result in greater risk of resistance than regular use of foliar Bt sprays. The risk of resistance should not prevent the use of transgenic plants in potato production. The best way to avoid resistance is to have a large arsenal of different control measures that are used strategically in combination. Transgenic potato plants should be allowed to be part of that arsenal.

Sincerely,



Casey W. Hoy, Ph. D.
Associate Professor
Research Entomologist

Mr. Ned Berce
President-Berce Potato Co.
R.F.D. #1 Box 201
St. Agatha, ME. 04772
April 4, 1994

Mr. Terry Stone/BB4D
Senior Regulatory Specialist
Monsanto Co.
700 Chestnut Village Parkway
St. Louis, MO. 63198

Dear Terry:

I am replying to your recent questions about the transgenic Russet Burbanks that I have grown for seed potato production for HybriTech Seed International. I grew the transgenic potatoes in 1993 both from plantlets and from seed that was produced in 1992 by the Maine Seed Potato Board.

The RBBt potatoes are not different from Russet Burbank in plant and tuber type in the field, either from transplanted plantlets or seed. The plants and tubers are so similar that it would by impossible to separate plant populations if they had been intentionally mixed.

At this point I see no reason or evidence that the transgenic Russet Burbank potatoes will set seed or that they can survive through winter and grow as volunteers the following season better than nontransgenic Russet Burbanks.

If you need more information feel free to call me at (207)543-7539.

Sincerely,

Ned Berce

Guenthner Potato Company
INCORPORATED

Seed Potatoes

1818 N. EDISON STREET
POST OFFICE BOX G
ANTIGO, WI 54409-0389

OFFICE PHONE 715-623-7877
FARM PHONE 715-623-2827
HOME PHONE 715-623-4748

April 6,1994

Mr. Terry Stone
700 Chesterfiedl Parkway North GGGA
St. Louis, MO 63198

Dear Mr. Stone

Our family farm has grown Russet Burbank potatoes with the Bt gene in them for the past two seasons. We have done so in cooperation with Hybritech Seed International and under permits issued by USDA and APHIS.

As required by Hybritech we have watched these plants and tubers very closely. We can't tell any difference between the Bt plants and our regular Russet Burbanks. They emerge and blossom at the same time and are ready for vine dessication at the same time. The tubers look and feel the same.

We are certified seed producers. All of our potato plants are tested and observed for diseases. In the past two seasons there has been no difference in disease incidence.

We utilize IPM techniques to scout for and treat for pests if they reach economic thresholds. We have seen that Colorado potato beetle do not affect Bt plants. All other pests equally affect both the regular Russet Burbanks and the Bt's. These pests include; leafhoppers, verigated cutworms, potato aphids, and green peach aphids to name a few.

XXXX

★★★★

Guenthner Potato Company
INCORPORATED

Seed Potatoes

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FARM PHONE 715-623-2827
HOME PHONE 715-623-4748

We further use IPM techniques to scout areas that we had in potatoes the previous year. We do this to look for volunteer potato plants that may have overwintered. We look for them because they are potential sources to harbor insects and diseases. Fortunately , potatoes do not overwinter very easily in Northern Wisconsin. We can see no difference between regular Burbanks and Bt Russet Burbanks.

There was one growth characteristic we noticed this past season that was different than usual. We had a cold spring with very slow emergence. After the plants were 8" tall some of the leaves looked somewhat abnormal. The leaves looked like they had been compressed. This condition was in both the BT and regular Burbanks. It also appeared in other potato varieties last year.

Finally, this Bt Burbank really worked. It controlled the Colorado potato beetle 100%. We had to spray all our other potatoes two times for beetle control. The Bt Burbanks we didn't spray at all for beetle control.

Very truly yours,

Robert Guenthner

Robert Guenthner
Guenthner Potato Co.,Inc.

XXXX

★★★★

SIDDOWAY, INC.

Famous Idaho Potatoes
134 E. Main #205
Rexburg, Idaho 83440

Watts 800-234-9399
In Idaho 208-356-9399

August 19, 1993

Terry Stone
Senior Regulatory Specialist
Monsanto Agricultural Group
700 Chesterfield Parkway North
St. Louis, MO 63198

Dear Terry,

I am a Broker/Dealer of potato seed in Idaho with the bulk of my sales in the Northwest. I have been following your research on the seed which is resistant to the Colorado Potato Beetle. I have talked with many of my customers concerning the potential of this new product and have found a great deal of interest in the future availability of the seed. The CPB has been a very time consuming and expensive pest considering conventional insecticides, applications and the damage the beetle does. A product of this nature which may add to the bottom line of the farmer and reduce his time and expense in handling the current chemicals, naturally draws his immediate attention.

I recently inspected a potato field in the Rexburg area of Southeastern Idaho. The field was bordered on two sides by a grain field that was full of volunteer potatoes. As you may know volunteer potatoes in grain are the perfect host for the CPB. With an abundance of food and nothing to stop them, thousands are hatched uninhibited. Several days ago these beetles started moving into the potato field. By the time the farmer hired an airplane and had them sprayed they had totally defoliated 15 feet into the field. Luckily his timing was good and his losses were minimal but the potential for loss was explosive. This is only one situation in many where a CPB resistant potato would have never allowed the propagation of the beetles in either the volunteer potatoes or those planted this spring. It would have eliminated the need to handle the insecticide and the expense of the chemical both at pre-emergence and again this summer.

For these reasons and many others the potato industry needs this product. For the benefit of my customers and my own business I would encourage any effort toward making this seed available. If there is anything I can do to help promote or market the seed to the commercial growers please contact me. Thank you.

Thane Siddoway

Sunspiced, Inc.

SALES L.D. (208) 785-7150
ALL OTHER (208) 785-3200

P.O. BOX 592
BLACKFOOT, IDAHO 83221

August 31, 1993

Terry Stone
Senior Regulatory Specialist
Monsanto Agricultural Group
700 Chesterfield Parkway North
St. Louis, Missouri 63198

Dear Terry:

Sunspiced, Inc., and Basic American Foods wholeheartedly endorse your efforts to register Colorado Potato Beetle-resistant Russet Burbank potatoes. Sunspiced, Inc., is the largest shipper of fresh potatoes in the world, and Basic American Foods is one of the largest potato dehydrators. We have plants in Blackfoot, Idaho; Rexburg, Idaho; Moses Lake, Washington; Klamath Falls, Oregon; and Plover, Wisconsin. Colorado Potato Beetles are probably our worst potato pest.

Russet Burbanks with a Bt gene to protect them from beetles would be a welcome addition to the potato industry. Colorado Potato Beetles resistant to organophosphate and pyrethroid insecticides have already been found in many of our growing areas. A resistant Russet Burbank would certainly fit into a good Integrated Pest Management program. It would save the growers money on insecticides, limit the use of insecticides, and reduce the incidence of insects with insecticide resistance.

We as a company, and as an industry, look forward to using this resistant Russet Burbank as an integral part of our overall management plan. It is highly effective, efficient, and above all safe to the consumer and to the environment. We have no reservations at all about recommending this resistant potato to our growers and using it in our production plants.

Sincerely,

Thomas R. Owings, Ph.D.
Director, Raw Material Research
Field Department Manager

sc

NATIONAL POTATO COUNCIL
9085 E. Mineral Circle, Suite 155
Englewood, CO 80112
Phone: (303) 790-1141
Fax: (303) 790-1142

August 23, 1993

Mr. Terry Stone
Senior Regulatory Specialist
Monsanto Agricultural Group
700 Chesterfield Parkway North
St. Louis, MO 63198

Dear Mr. Stone:

I am writing this letter in support of Monsanto's submission to the Environmental Protection Agency for registration of a Colorado potato beetle (CPB)-resistant potato seed.

I speak on behalf of the National Potato Council as vice president of the NPC's Environmental Affairs Committee. The NPC is the only trade association representing 10,500 commercial potato growers in 50 states. Our growers produce both seed potatoes and potatoes for consumption in a variety of forms. Approximately 132 pounds of potatoes are consumed per person per year. Annual production in 1991 was 417,762,000 cwt with a farm value of $2,045 billion.

The Environmental Affairs Committee, as well as the entire potato industry, recognizes the CPB as one of the most damaging potato pests throughout the world. Its resistance to many insecticides is well documented.

Although devastation caused by the CPB is not apparent in every growing area, several states are severely attacked by this pest. A 1991 study showed that in Michigan alone potato growers suffered average estimated crop losses of 12.2 percent from CPB representing $4.3 million in lost revenue on 42.3 percent of the potato acreage. Average cost of CPB control on surveyed acreage in 1991 was $124.55 per acre; this was up 51.4 percent from 1989 costs of control. Michigan growers spent approximately $2.475 million on insecticides for CPB control in 1991 on the 42.3 percent of Michigan potato acreage surveyed. I am confident that these same kinds of losses and control costs occur in many northeastern potato-growing states.

I grow potatoes in the Columbia Basin in Washington State. I spray at least once and in some fields twice to protect against CPB. For the last two years I have seen a resistance to the insecticide I have been using. The cost of using and changing insecticides in my operation alone amounts to several thousand dollars annually. It would be of great commercial and productional benefit to be able to plant a potato seed that is resistant to CPB.

I appreciate the opportunity to comment on your submission to the EPA for registration of a Colorado potato beetle-resistant potato seed, Mr. Stone. Please let me know if the NPC can provide additional information.

Sincerely,

Lynn J. Olsen
Vice President

Legislative • Regulatory • Environmental Issues

Mecox Road
Bridgehampton,N.Y. 11932
April 14, 1993

Ms. Jennifer Feldman
HybriTech Seed International, Inc.
1503 Tyrell Lane
Boise, Idaho 83706

Dear Jennifer:

The presentation on transgenic potatoes which you gave at the L.I. Agricultural Forum in Riverhead, N.Y. this spring was of great interest to me. I operate a 200 acre farm here in Bridgehampton, along with my brother and father. Our farm has been in continuous operation by our family for five generations. I have been farming for 21 years since graduating from college. The farm currently supports three families, plus one worker and his family. Our primary crop is potatoes, but we also sell some grain, rye and oats, which we use for crop rotation.

The Colorado potato beetle is, without a doubt, the most expensive pest we have to contend with. Over half of our total pesticide bill is due to this single insect. At present, the best method we have of control for CPB is application of two materials, Kryocide and Novodor.

I heartily support your company's efforts in the development of transgenic CPB control for the following reasons:

1) It would likely eliminate most, if not all, CPB sprays

2) It would reduce overwintering adult populations of CPB, resulting in better crop emergence the following spring

3) It would likely break the cycle of pesticide resistance

4) It would reduce worker exposure to pesticides

5) It may reduce scouting costs

All of these things would be a benefit to our farming operation from both an economic and safety point of view. I look forward to the successful development of transgenic CPB control.

Good luck!

Sincerely yours,

Harry S. Ludlow

HSL/bal

April 27, 1993

Stephen R. Diercks
Coloma Farms Inc.
136 S. Scott St.
Coloma, WI 54930

Mr. John Cudnohufsky
HybriTech Seed International
1503 Tyrell Ln.
Boise, ID 8706

Dear Mr. Cudnohufsky,

I am a third generation potato grower in the state of Wisconsin. Our family has been growing potatoes since the 1930's, first in the Antigo area and since the mid 60's, we have been farming in Coloma, the Central Sands Area of Wisconsin. I am a 1970 graduate from the University of Wisconsin with a major in Agricultural Economics and this year I have a son graduating from the same institution with an Agricultural Engineering Degree, who will be joining my father and I on the family farm.

Our farm consists of 2200 acres of irrigated land. We grow approximately 750 acres of potatoes each year and we grow field corn, soybeans, sweet corn, peas and alfalfa in rotation with our potato crops. Our potatoes are all on a three year rotation and the crops grown in rotation are based on which crop will best suit our needs for that year. We employ 5 full time people and up to 20 people during harvest season. We purchase the majority of our inputs, approximately $500,000, from local suppliers within a 30 mile radius of the farm.

Our farm has participated in numerous research projects with the University of Wisconsin. We participated in the early development of the PCM, Potato Crop Management, and WISP, Wisconsin Irrigation Scheduling Program. We have used IPM for many years and hire scouting services to regularly scout our crops.

Even with the use of all the new Best Management Practices that we are using, we are still having problems. The publics concern over the use of pesticides, the environmental problems with some crop protectants and the probable loss of many pesticides. has led us to believe that we must find better way to control pests in our potato crop. The use of transgenic material is the next logical step.

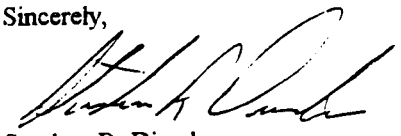
Colorado Potato Beetles (CBP) control has become one the biggest pest problems we have on the farm. The pressure in the area is very high and if we do not have control it is possible to lose the crop. With the excellent recommendations from Dr. Jeff Wyman, we have been able to maintain good control and little resistance build up. We rotate chemical classes and use PCM to determine when the correct time to make an application is. This is quite a change from 10 years ago when we applied Temik one time and had control for most of the season. Temik and other systemics have gone by the wayside because of ground water contamination and toxicity problems. Today we are still controlling CPB but we are using more sprays, introducing more pesticides into the environment and exposing workers to more pesticides because of more applications.

The use of transgenic material to control CPB is an exciting step forward. By integrating the use of this new material into our existing IPM program we should be able to better control

CPB and have less of an impact on the environment and be safer for my employees. While the transgenics are an exciting step forward, they cannot be expected to solve all our problems. We must learn how and when to use these materials. The plot work which we are doing is a beginning to see how to use these materials will be used and we are sure the data from this kind of plots will help us all.

I am looking forward to working with your people this coming growing season and am eagerly looking forward to the release of this new material in the coming years.

Sincerely,



Stephen R. Diercks
V.P. Coloma Farms Inc.

June 23, 1993

Mr. John Cudnohufsky
Manager, Customer Relations
HybriTech Seed International
300 E. Mallard Drive, Suite 220
Boise, ID 83706

Dear John:

I am writing in support of your company's research and development efforts with genetically improved potatoes. I have been a potato grower for several decades, concentrating on seed and table potatoes with production ranging from 150-200 acres/year. During the last 20 years I have observed that some pests (Colorado Potato Beetle, aphids) and diseases (Early Blight, scab) have impacted my potato crops more, while I have had less pesticides and techniques available to control the insects and diseases. In the case of Colorado Potato Beetle insecticides have lost effectiveness in a very short time and little has become available to replace them. Pesticide costs on my farm are in the $200-300/acre range, resulting in a significant expenditure each year. I find the reduced number of effective pesticides a reason for concern as we look at the future of potato production in North America. The approach of your company to develop potatoes that defend themselves without frequent and repeated pesticide applications may provide an attractive and effective alternative.

During my term as Chairman of the Potato Promotion Board I had opportunities to visit many agricultural areas in the U.S. as well as some as distant and distinct as China. Those visits reinforced my conviction that American agriculture is highly productive and successful because individual segments or areas are quick to recognize improvements and adopt new technology to be more efficient. I have also noticed that farmers are becoming more protective of their resources and environment, knowing full well that failing to do so will have serious consequences, both in the long and short term.

I see the genetically improved potatoes that your company is developing and planning to market as a major step forward in allowing a potato grower to produce a crop that continues to be safe to eat, causes less stress on the environment and require fewer inputs in terms of energy, labor and pesticides. These potatoes will also allow growers to be at the forefront of technology, a situation that will help them continue to provide high quality, inexpensive food to a rapidly expanding population.

I plan to closely follow your progress in Maine in both research and seed potato production. If your potatoes will allow me to farm smarter and better, then I will want to use them. Good luck.

Sincerely,

G. Arnold Roach

G. Arnold Roach
Forest Home Farms
P O Box 179
Smyrna Mills, Maine 04780

APPENDIX 15

SUMMARY OF EXPERIMENTAL METHODS

APPENDIX 15. SUMMARY OF EXPERIMENTAL METHODS

Determination of *B.t.t.* and NPTII Protein Expression Levels:

The following is a summary of the methods utilized in the determination of the *B.t.t.* and NPTII proteins expression levels reported in Tables V.2 to V.7 (pages 40 - 45) of this submission. A detailed description of the methods are contained in EPA MRID NO. 42932202 (Rogan, J.G., Anderson, J.S., McCreary, J.A. and Lavrik, P.B. 1993. Determination of the expression levels of *B.t.t.* and NPTII proteins in potato tissues derived from field grown plants. Study No. 92-10-37-02).

Tissue Collection

Tissues were collected during the 1992 season at seven field locations: Hancock, WI; Hermiston, OR; Riverhead, NY; Othello, WA; Aberdeen, ID; Ashland, ME; and Antigo, WI. Two field sites (Othello, WA and Aberdeen, ID) employed a six-replicate randomized complete block design for each line; the other five field sites employed a non-replicated arrangement. The tissue sampling regimen was designed to obtain tissues of similar physiological stages.

Leaf tissue. Leaf tissue was collected at approximately nine, twelve and fifteen weeks post planting. One of the youngest leafs (1/2 to 1 inch in diameter) were collected from each of four randomly chosen plants from each of the plots. The leafs from each of the four plants per plot were combined in an appropriately labelled plastic sample bag, immediately frozen on dry ice and shipped on dry ice to Monsanto where the tissue was stored frozen at approximately -80°C before being processed for ELISA analysis.

Whole plant tissue. Whole plant tissue consisted of leafs, stems and roots, excluding tubers. Approximately one week before onset of tuberization, young whole plant samples were removed from the field (one whole plant/plot for replicated sites; three whole plants/plot for non-replicated sites), immediately frozen on dry ice and shipped to Monsanto where they were stored at approximately -20°C prior to processing for ELISA analysis. Leaf sampling ensued after this timepoint at approximately three week intervals. Before the onset of senescence (or before a vine defoliant was applied) whole plant samples were once again removed from the field, immediately frozen on dry ice and shipped to Monsanto where they were stored at approximately -20°C prior to processing for ELISA analysis.

Tuber tissue. Tubers were harvested from the field, placed in temporary storage at each site and ten tuber samples non-systematically selected from each plot for ELISA analysis. Tuber samples were shipped at ambient temperatures to Monsanto. At Monsanto, tubers were stored at approximately 15°C, 60-70% relative humidity prior to processing for ELISA.

Tissue Extraction

Leaves. Frozen leaf tissue samples were crushed to a fine powder in the collection plastic sample bags. *B.t.t.* and NPTII proteins were extracted from tissues using an

aqueous extraction buffer (8.1 mM Na_2HPO_4, 1.5 mM NaH_2PO_4, 0.14 M NaCl, 1.5 mM KH_2PO_4, 2.7 mM KCl, 0.2 % Tween 20®, pH 7.4) which had been optimized to provide for maximum extraction of both proteins. Approximately one gram of each sample was extracted in 15 ml of aqueous extraction buffer.

Whole Plants. From the replicated sites, one frozen whole plant from each plot was ground to a fine powder using a food processor. At sites where a non-replicated design was used, three plants/plot were ground together. A subsample was taken from the ground whole plant sample by filling one appropriately labeled, 50-ml polystyrene tube completely full of the powder. This tube was then stored at approximately -80°C until extracts were made. Approximately one gram of each whole plant sample was extracted in 15 ml of extraction buffer (8.1 mM Na_2HPO_4, 1.5 mM NaH_2PO_4, 0.14 M NaCl, 1.5 mM KH_2PO_4, 2.7 mM KCl, 0.2 % Tween 20®, pH 7.4).

Tubers. The *B.t.t.* and NPTII protein expression levels in tubers were determined in lyophilized tuber powders. For preparation of lyophilized tuber samples, five tubers/plot/site were non-systematically selected from the ten tubers that were collected from the field. These tubers were diced into approximately 20 g pieces. One 20 g piece from each of the five tubers was sliced into thin pieces, and placed inside of an appropriately labeled paper sack and approximately 100 g of dry ice added to freeze the tuber pieces. The tuber pieces were then lyophilized at approximately -4°C for approximately seven days, and then ground into a powder in a Waring blender. The fresh weight equivalent of approximately one gram of each sample was extracted in 15 ml of extraction buffer (8.1 mM Na_2HPO_4, 1.5 mM NaH_2PO_4, 0.14 M NaCl, 1.5 mM KH_2PO_4, 2.7 mM KCl, 0.2 % Tween 20®, pH 7.4). The fresh weight of the tissue was obtained by determining the amount of water removed from the tissues during the lyophilization process.

Expression Level Assays

ELISA Assays. The expression levels in extracts of the *B.t.t.* and NPTII proteins were determined using validated ELISA's (EPA MRID NO. 42932202). Descriptive features of the *B.t.t.* protein and NPTII ELISAs are included below (pages 371 and 372, respectively). All extracts were analyzed in triplicate. Samples were repeated if the % coefficient of variation was greater than 10.5% (based on absorbance) or if any of the assays did not pass the established accept/reject criteria as described in the validation reports. Samples were also repeated if *B.t.t.* protein or NPTII were not detected in samples derived from CPB resistant plants or if NPTII or *B.t.t.* protein were detected in wild type plant extracts. This was done in order to determine if a field sampling or laboratory processing error had occurred. The expression level of each protein was converted to the amount of protein expressed in each tissue on the basis of the fresh weight of the tissue used.

ELISA Data Reduction. All data reductions were done using Microplate Manager™ from BioRad (Richmond, CA). Microsoft Excel™ was used to transform ELISA data for the statistical evaluation of the expression levels.

B.t.t. Protein ELISA Validation Data Summary

Precision:		
	Intraplate Variability:	6.5 % C.V.
	Interplate Variability:	8.3% C.V.
	Inter-assay Variability:	13.8 % C.V. (Leaf) 14.2 % C.V. (Tuber)
	Extraction Variability:	11.7 % C.V. (Leaf) 2.1 % C.V. (Tuber) 2.6 % C.V. (Whole Plant)
Range:		
	Least Detectable Dose:	32 pg/well
	Standard Curve Range:	32 to 2000 pg/well
Accuracy:		
	Extraction Efficiency:	85 % (Leaf) 100 % (Tuber) 91 % (Whole Plant)
	Spike and Recovery:	124 % (Young Whole Plant) 119 % (Old Whole Plant) 130 % (Leaf) 108 % (Tuber)
Stability:		
	Tuber Extract:	No degradation after 3 months storage at -80°C
	Leaf Extract:	No degradation after 3 months storage at -80°C
Accept/Reject Criteria:		
	Blank:	≤ 0.100 O.D. at 450 nm
	Standard curve:	Correlation Coefficient (R^2) ≥ 0.95
	Variability in replicates:	≤ 10.5 % C.V.
	Interassay Control:	< 2 standard deviations from established mean

NPTII Protein ELISA Validation Summary

Precision:

Intraplate Variability:	10.43 % C.V.
Interplate Variability:	11.3 % C.V.
Inter-assay Variability:	14.8 % C.V.
Tissue Variability:	27 % C.V. (Leaf) 36 % C.V. (Whole Plant) 26 % C.V. (Tuber)
Extraction Variability:	0.61 % CV (Leaf) 0.53 % CV (Whole Plant) 0.67 % CV (Tuber)

Range:

Least Detectable Dose:	6 pg/mg (Leaf) 10 pg/mg (Whole Plant) 40 pg/mg (Tuber)
Standard Curve Range:	0.1 - 6 ng/well

Accuracy:

Extraction Efficiency:	89.6 % (Leaf) 90.0 % (Whole Plant) 89.1 % (Tuber)
Spike and Recovery:	102 % (Leaf) 93 % (Whole Plants) 104 % (Tuber)

Stability:

Leaf Extract:	No degradation after 7 months at -80°C
Whole Plant:	No degradation after 4 months at -80°C
Tuber Extract:	No degradation after 3 months at -80°C

Accept/Reject Criteria:

Blank:	< 0.300 O.D. at 450 nm
Absorbance in 3 ng/well standard:	> 0.500 absorbance units
Standard Curve:	Standard error < 0.100
Interassay Control:	< 2 standard deviations from established mean

Compositional Analyses:

The following is a summary of the methods utilized in the determination of the potato tuber composition and quality reported in Tables V.8 and V.11 (pages 46 to 49) of this submission. A detailed description of the methods are contained in Study No. 92-01-37-19 (Lavrik, P.B. and Love, S.L. 1994. Composition and quality analysis of potato tubers derived from field grown Colorado potato beetle resistant plants).

Tuber Sample Preparation. Total solids, dextrose, sucrose, vitamin C and glycoalkaloid analyses were carried out on freeze-dried potato tuber powder. Tubers were obtained from plants grown in field trials carried out at Hancock, WI; Hermiston, OR; Othello, WA; Aberdeen, ID; Ashland, ME; Antigo, WI; during the summer of 1992. The field trials at Aberdeen, ID, and Othello, WA, consisted of six replicated plots per line. The field trials at the remaining four locations (Hancock, WI; Hermiston, OR; Ashland, ME; and Antigo, WI) consisted of a single plot per line. At harvest, tubers from each location were shipped, under ambient conditions to the test location and freeze-dried potato tuber powder samples were prepared by lyophilization of ten tubers per sample. Prior to lyophilization, tubers were washed with water, leaving the skin intact and diced by hand or with a Hobart dicer into approximately 0.6 cm cubes. After dicing, tubers were frozen in liquid nitrogen and stored frozen at -20°C until lyophilization. The frozen potato tissue was placed in the freeze drier in open containers. A temperature probe was placed into one sample on each shelf. Samples were considered completely dry when samples reached ambient temperature. After lyophilization, the freeze-dried tuber material was ground into a powder, sieved through a 40 mesh screen and stored at approximately -25°C.

Proximate (total protein, fat, carbohydrate, total dietary fiber and ash), minor nutrients (thiamine, pyridoxine, folic acid, riboflavin and niacin) and minerals (calcium, copper, iodine, iron, magnesium, phosphorous, potassium, sodium and zinc) analyses were carried out on composite subsamples of freeze-dried potato tubers processed as described above. Composite subsamples for each of the seven CPB resistant lines and a Russet Burbank control line were prepared by mixing 20 g of freeze-dried and ground potato tuber powder from each of the six-replicated plots from the field trials at Aberdeen, ID and Othello, WA. Freeze-dried composite subsamples were stored under dry refrigerated (approximately 4°C) conditions until analyzed.

Total Solids. Subsamples (20-40 g) of diced potato tuber samples, prepared from ten tubers as described above, were weighed to the nearest 0.01 g. Subsamples were dried for approximately 2 days at 60°C in a forced-air dryer to a constant weight. Percent total solids were computed from the dry weight and fresh weight (dry weight ÷ fresh weight x 100%) and reported as the mean of two analyses.

Sugar Level Determination. Dextrose and sucrose measurement was carried out on freeze-dried tuber tissue by an electrochemical method. The method employed a Yellow Spring Instrument Co. analyzer which uses an enzyme electrode technology which provides measurements that are specific for the sugar being analyzed. The procedure followed was that recommended by the manufacturer ("Dextrose and Sucrose Measurement in Potatoes." Application Note No. 102, Scientific Division, Yellow Springs Instrument Co., Yellow Springs, Ohio 45387). Two analyses were carried out

per samples. The reported values are the mean of two analyses per sample which were converted to percent dextrose and percent sucrose of fresh tissue.

Determination of Glycoalkaloid Level. Glycoalkaloid determination was carried out on freeze-dried and ground tuber tissue. The procedure is based on methods described by Bergers (Bergers, W.W. 1980. "A rapid quantitative assay for solanidine glycoalkaloids in potatoes and industrial potato protein." *Potato Research* **23**:105-110.) and measures total amount of solanines and chaconines. A single analysis was done per sample. Glycoalkaloid level is reported as total milligrams of solanines and chaconines per 100g fresh tuber weight.

Determination of Vitamin C Level. Total ascorbic acid (vitamin C) analysis was carried out on freeze-dried potato tuber following AOAC procedure (Association of Official Analytical Chemists, 1984. Official Methods of Analysis. S. Williams, Ed. Fourteenth edition. Arlington, VA.). A single analysis was performed per sample. Vitamin C level is reported as milligrams of ascorbic acid per 100 g fresh tuber weight.

Quality Analyses.

Internal defects determination – The determination of internal tuber defects was performed using ten tubers. Each tuber was cut in half along the longitudinal axis and visually inspected for hollow heart, brown center, internal brown spot (heat necrosis) and vascular discoloration (numerous causes). Only the worst defect in each tuber was recorded. The percentage of tubers expressing each defect and the percentage of total tubers showing internal defects was calculated.

Blackspot bruise susceptibility – Determination of blackspot bruise susceptibility was performed by the abrasive peel method according to SOP, which is essentially the procedure described by Pavek *et al.* (Pavek, J. Corsini, D., Nissley, F. 1985. "A rapid Method for Determining Blackspot Susceptibility of Potato Clones." *Amer. Pot. J.* **62**:511-517). Evaluation was carried on ten tubers of uniform size (6-8 0z). Each sample was placed into an abrasive peeler for 30 seconds then set aside at room temperature for approximately 18 to 24 hrs. Tubers were then rated on a scale of 1 to 5, with 5 being the blackest, the most susceptible to blackspot bruise.

Fry color characteristics – French fry color characteristic was based on tubers stored for at least 48 hrs at 40°F or 45°F. For each storage condition, three tubers from each plot were cut longitudinally into approximately 3/8 inch strips. Nine center strips were removed and fried at 375°F in vegetable oil for 3.5 minutes according to SOP. Color of fried strips were rated according to USDA fry color chart in the range of 0 to 4 with higher number equating with darker color. The reported sample rating is the average of 27 strips. The fried strips from tubers stored at 45°F were also rated for sugar-ends using the USDA fry color chart, as indicated by strips with one end, at least 1/2 inch long, that is 1 or more rating unit darker than the middle of the strip. Sugar-ends were reported as a percentage of total strips. The severity of sugar-ends was evaluated by averaging the difference in color between the dark and the light end of each sugar ended fry. The severity of sugar-ends is reported in fry chart units. For potato tubers obtained from Aberdeen, ID, five tubers from each plot were cut longitudinally into approximately 3/8 inch strips and four center strips from each tuber were removed, fried and color rated as described above. The reported sample rating is the average of 20 strips.

Statistical Analysis. Total solids, dextrose, sucrose, vitamin C and glycoalkaloid data summary reported in Table V.8 (page xx) are a result of a combined statistical analyses combined for all locations using the mixed linear model procedure (PROC MIXED) of the SAS system (Version 6.07, SAS Institute Inc., Cary, NC). For the combined analyses, the location, replication within location, location x line interaction and the residual error were all treated as random effects.

Proximate Analysis. The proximate analyses were carried according to AOAC procedures given in the references below:

Protein

Total protein analysis was carried out by Kjeldahl method using the nitrogen conversion factor of (N X 6.25).

Official Methods of Analyses (1990), 15th Edition, Method 955.04C, 979.09, AOAC, Arlington, Virginia, (Modified).

The Kjeldahl method for Organic Nitrogen, R.B. Bradstreet, Academic Press, New York, New York (1965)

Quantitative Inorganic Analysis, Kelthoff and Aandell (1948), Revised Edition.

Fat:

Official Methods of Analysis (1990), 15th Edition, Method 922.06, AOAC, Arlington, Virginia, (Modified).

Ash:

Official Methods of Analysis (1990), 15th Edition, Method 923.03, AOAC, Arlington, Virginia, (Modified).

Moisture:

70ºC Vacuum Oven.

Official Methods of Analysis (1990), 15th Edition, Method 934,06, AOAC, Arlington, Virginia, (Modified).

Carbohydrates:

The total carbohydrate level is determined by difference after the percentages of protein, moisture, ash and fat are known.

Minor Nutrient Analyses.

Thiamin Hydrochloride:

Official Methods of Analysis (1990), 15th Edition, Method 942.23, AOAC, Arlington, Virginia, (Modified).

Pyridoxine Hydrochloride:

Official Methods of Analysis (1990), 15th Edition, Method 961.15, AOAC, Arlington, Virginia, (Modified).

Atkins, et al (1943), Industrial and Engineering Chemistry, Analytical Edition, Volume 15, p. 143.

Folic Acid:

American Journal of Clinical Nutrition, 1968, 21:1202, (Modified).

Methods of Analysis for Infant Formulas, Infant Formula Council, 1985, (Modified).

Riboflavin:

Official Methods of Analysis (1990), 15th Edition, Method 960.46 and 940.33, (Turbidimetric Method), AOAC, Arlington, Virginia, (Modified).

The United States Pharmacopeia, 1990, Vol. XXII, p. 1544, (Modified).

Niacin:

Official Methods of Analysis (1990), 15th Edition, Method 960.46 and 944.13,

(Turbidimetric Method), AOAC, Arlington, Virginia, (Modified).
The United States Pharmacopeia, 1990, Vol. XXII, pp. 1539-1541, (Modified).
Methods of Analysis for Infant Formulas, Infant Formula Council, 1985, (Modified).

Minerals. All minerals, with the exception of iodine, were analyzed by ICP emission spectrometry according to the references below. Iodine was analyzed by a colorimetric method according to the reference below.

Calcium, copper, iron, magnesium, phosphorous, potassium, sodium and zinc:

Inductively Coupled Plasma-Atomic Emission Spectrometry Analysis of Biological Materials and Soils for Major Trace and Ultra-Trace Elements, Applied Spectroscopy, 1978, 22:1-29 (Modified).
Official Methods of Analysis (1985), 1st Supplement, 14th Edition, Method 3.A01 3.A04, AOAC, Arlington, Virginia, (Modified).

Iodine:

Official Methods of Analysis (1990), 15th Edition, Method 932.21, AOAC, Arlington, Virginia, (Modified).
Binnerts, W. T., Analytical Chimica Acta, 1954, 10:78 (Modified).

Insect Assays

The following is a summary of the insect assay methods utilized in the assessment of the sensitivity of selected insect species to *B.t.t.* protein which is reported in Tables VI.1 and VI.2 (pages 93 and 94) of this submission.

Leptinotarsa decemlineata (Colorado potato beetle)

The activity of *B.t.t.* protein against Colorado potato beetle was assessed by incorporation of the test material in an artificial diet. First instar larvae, reared at Monsanto Co., were individually allowed to feed on the artificial diet for a period of 5 to 7 days, after which time, the number of living larvae were counted. The *B.t.t.* protein was incorporated in the diet at five concentrations around the pre-established lethal concentration of *B.t.t.* protein. For each replicate, approximately 16 to 24 larvae were utililized per *B.t.t.* protein concentration. The LC_{50}, concentration lethal to 50% of the larvae, reported in this study was obtained from a LOGIT analysis (SAS Institute Inc., Cary, NC) of the concentration-response curve. A detailed methods description is included in EPA MRID NO. 42932204 (Lavrik, P.B. 1993. Characterization of Colorado potato beetle active *Bacillus thuringiensis* subsp. *tenebrionis* protein produced in *Escherichia coli* . Study Number 92-01-37-10, an unpublished study conducted by Monsanto Company).

Hippodamia convergens (ladybird beetle)

Assays were carried out with adult ladybird beetles obtained from a commercial supplier. The beetles were placed into test chambers which were one pint rolled paper containers covered at both ends with a disposable plastic petri dish. Twenty five beetles were placed into each test chamber. There were two test chambers used for the negative control group and 6 replicates or chambers were used for *B.t.t.* protein. Test diets were prepared on study day 0 by adding the test material to a honey/water mixture to achieve

a nominal concentration of 100 ppm *B.t.t.* protein in the diet. Diets were refrigerated and presented fresh to the beetles at 3 day intervals. Negative control diets, were prepared in the same manner with the exception that no *B.t.t.* protein was added. Ladybird beetles were observed twice on the day of test initiation for mortality and signs of toxicity and once each day thereafter. The study was terminated when mortality in the negative control group exceeded 20%. A detailed methods description is included in EPA MRID NO. 42932212 (Hoxter, K.A., and Smith, G.J., 1992. *B.t.t.* protein: a dietary toxicity study with Ladybird beetles (*Hippodamia convergens*). Project Number 139-348, an unpublished study conducted by Wildlife International, Ltd. under contract to Monsanto).

Nasonia vitripennis (parasitic wasp)

Adult parasitic Hymenoptera were obtained as pupae from a commercial supplier and appropriately incubated to allow the pupae to hatch. Test chambers were one pint rolled containers covered at both ends with a disposable petri dish. Each test chamber constituted a replicate. Each treatment and control group had two replicates. On the day of test initiation, wasps were immobilized with nitrogen and 25 wasps were assigned to each test chamber by indiscriminate draw. Test chambers were placed inside an incubator set to maintain a temperature of approximately 20-30° C throughout the test period. Test diets were prepared on study day 0 by adding the test material to a 50:50 (vol:vol) honey:water mixture. Control diet was administered in the same manner as the test diet. After preparation, diets were stored at -9 to 3°C and presented fresh to the wasps at 3 day intervals. Average estimated diet consumption was determined for each group at each change of diet. Parasitic Hymenoptera were observed twice on the day of test initiation for mortality and signs of toxicity and once each day thereafter. The study was terminated when mortality in the negative control group exceeded 20%. A detailed methods description is included in EPA MRID NO. 42932211 (Hoxter, K.A., and Smith, G.J. 1992. *B.t.t.* protein: a dietary toxicity study with parasitic Hymenoptera (*Nasonia vitripennis*). Project Number 139-349B, an unpublished study conducted by Wildlife International, Ltd. under contract to Monsanto).

Apis mellifera (honey bee larvae)

Honey bee colonies were obtained from a commercial supplier. An "even age" cohort of bee larvae was obtained by allowing queen bee oviposition on brood frames for 1 - 3 days followed by transfer of the frames to a queen - excluded hive box (super). The test was conducted on 1 - 3 day old larvae within the larval cells on these brood frames. Individual brood frames, containing at least 50 larval bees, constituted replicates. Each treatment and control group had four replicates. Two replicates of each treatment were initiated on each of two separate days. Dosing was accomplished by placing 5 µl of the test substance in distilled water or distilled water alone into the wells with the larvae. After dosing, treated frames were returned to the super for completion of larval development. Test diet was prepared on study day 0 by adding the test material to distilled water to achieve a nominal concentration of 100 ppm *B.t.t.* protein. The negative control was distilled water without *B.t.t.* protein. Once emergence from the capped cells began, all emerged adult bees were counted and placed into adult holding cages on a daily basis. Forty-eight hours after the last adult bees had emerged from the control treatment, emergence counts on the remainder of the treated frames were

stopped. Percent larval survival (from dosing to adult emergence) was determined for all treatments. A detailed methods description is included in EPA MRID NO. 42932209 (Maggi, V.M. 1992. Evaluation of the dietary effect(s) of purified *B.t.t.* protein on honey bee larvae. Study Number CAR 188-92, an unpublished study conducted by California Agricultural Research, Inc. under contract to Monsanto).

Apis mellifera (honey bee adult)

Honey bee colonies were obtained from a commercial supplier. An "even age" cohort of adult bees was obtained by allowing adults to emerge from brood frames for approximately three days under controlled environmental conditions. Test containers were one quart rolled paper cartons covered at the top end with mesh hardware cloth; the test material was introduced through the cage bottom using a glass shell vial and a cotton wick. Each test container, containing approximately 40 bees, constituted a replicate. Treatment and control group had three replicates. On the day of test initiation, bees were hand picked and assigned to each test container by indiscriminate draw. Test containers were placed in a control environment throughout the test. Test diet was prepared on study day 0 by adding the test material to a 50:50 (vol:vol) honey:water mixture to achieve a nominal concentration of 100 ppm *B.t.t.* protein in the diet. The negative control group was 50:50 (vol:vol) honey:water mixture without *B.t.t.* protein. Adult bees were observed twice on the day of test initiation for mortality and signs of toxicity and once each day thereafter. The study was terminated when mortality in the negative control group exceeded 20%. A detailed methods description is included in EPA MRID NO. 42932210 (Maggi, V.M. 1992. Evaluation of the dietary effect(s) of purified *B.t.t.* protein on honey bee adults. Study Number CAR 189-92, an unpublished study conducted by California Agricultural Research, Inc. under contract to Monsanto).

Chrysopa carnea (green lacewing larvae)

Green lacewing larvae were purchased from a commercial supplier and upon receipt, were placed in plastic cups, one larva to a cup. There were 30 larvae in each experimental group. Test diets were prepared by adding a calculated amount of test substance and reverse osmosis water to moth eggs (*Sitotroga cerealella*). The mixture was gently stirred by hand to coat the test material on the moth eggs. Test diets were made fresh daily. The negative control group was fed moth eggs mixed with reverse osmosis water only according to procedures already discussed. Larvae were observed twice on the day of test initiation for mortality and signs of toxicity and once each day thereafter. The study was terminated when mortality in the negative control group exceeded 20%. A detailed methods description is included in EPA MRID NO. 42932213 (Hoxter, K.A., and Smith, G.J. 1992. *B.t.t.* protein: a dietary toxicity study with green lacewing larvae (*Chrysopa carnea*). Project Number 139-347, an unpublished study conducted by Wildlife International, Ltd. under contract to Monsanto).

Anthonomus grandis (boll weevil), *Diabrotica undecimpunctata* (Southern corn rootworm), *Ostrinia nubialis* (European corn borer), *Manduca sexta* (tobacco hornworm), *Helicoverpa zea* (corn earworm), and *Heliothis virescens* tobacco budworm)

The sensitivity of the insects to *B.t.t.* protein was evaluated using artificial diet

incorporation assays. A single high dose of *B.t.t.* protein (50 µg of *B.t.t.* protein per ml of diet) or distilled water control were incorporated into insect diet. Treated diet was poured into 96 well insect assay trays to cool and harden and neonate larvae placed on the diet (one neonate larva per well). The boll weevil assay was conducted by adding approximately 2 - 6 boll weevil ova per well. Insect assay trays were covered and incubated under appropriate conditions. Survival of the test larvae was determined 5 to 7 days after test initiation. A total of 24 larvae were tested per treatment replicate. There were 3 replicates per treatment. The boll weevil bioassay was evaluated by determining the number of wells, in each replicate, containing at least one living boll weevil larva. A detailed methods description is included in EPA MRID NO. 42932207 (Sims, S.R. 1993. Sensitivity of selected insect species to the Colorado potato beetle active protein from *Bacillus thuringiensis* subsp. *tenebrionis* . Study Number 92-01-37-05, an unpublished study conducted by Monsanto Company).

Aedes aegypti (yellow fever mosquito)

Insect bioassay evaluation of the yellow fever mosquito was conducted by uniformly incorporating *B.t.t.* protein into artificial diet. Treated diet was poured into 96-well insect assay trays and allowed to cool and harden. Control diet contained the same ingredients and was prepared in the same manner as the treated diet, but had no *B.t.t.* protein. A sample of either treated or control diet (0.200 g) was added to 20 ml of water in a 50 ml centrifuge tube. Ten (10) 4th instar mosquito larvae were added to each treatment and control tube and the tubes incubated under approriate conditions. There were 5 replicate tubes per treatment and control. Survival of test larvae was determined approximately 24 hours after test initiation. A detailed methods description is included in EPA MRID NO. 42932207 (Sims, S.R. 1993. Sensitivity of selected insect species to the Colorado potato beetle active protein from *Bacillus thuringiensis* subsp. *tenebrionis* . Study Number 92-01-37-05, an unpublished study conducted by Monsanto Company).

Blatella germanica (German cockroach)

B.t.t. protein was uniformly incorporated into an insect diet based on pinto bean. Treated diet was poured into 50-well insect assay trays and allowed to cool and harden. Control diet contained the same ingredients and was prepared in the same manner as the treated diet, but had no *B.t.t.* protein. One cockroach nymph (3rd and 4th instar) was added to each well and the covered tray was appropriately incubated. A total of 15 nymphs were tested per treatment replicate. There were 2 replicates per treatment. The roach survivorship was determined after 5 days. A detailed methods description is included in EPA MRID NO. 42932207 (Sims, S.R. 1993. Sensitivity of selected insect species to the Colorado potato beetle active protein from *Bacillus thuringiensis* subsp. *tenebrionis* . Study Number 92-01-37-05, an unpublished study conducted by Monsanto Company).

Myzus persicae (green peach aphid)

The green peach aphid assay employed an artificial diet into which the *B.t.t.* protein was mixed. Control diet contained the same ingredients as the treated diet but no *B.t.t.* protein. Aphid diet were added to wells of 24-well culture plates and covered with

Parafilm® membrane. Aphids were added to another matching 24-well plate at a rate of approximately 12 per well. The plate with treated diet was inverted over the plate containing aphids. The wells of the opposing plates were aligned and the plates were joined using tape. The aphids fed through the overhead membrane on the treated diet above. Individual wells of the culture plates, each containing approximately 12 aphids, were replicates. There were 15 or 16 replicates (wells) per treatment and the entire test was repeated on two separate days. The assay trays were appropriately incubated for 4 days and the aphid survivorship measured. A detailed methods description is included in EPA MRID NO. 42932207 (Sims, S.R. 1993. Sensitivity of selected insect species to the Colorado potato beetle active protein from *Bacillus thuringiensis* subsp. *tenebrionis* . Study Number 92-01-37-05, an unpublished study conducted by Monsanto Company).

Statistical Analysis

The number of surviving insects, within treatment and control groups of each species, was compared using analysis of variance (SAS Institute Inc., Cary, NC).

APPENDIX 16

LIST OF SUPPORTING STUDIES

Studies Submitted to the EPA to Support the Registration and Exemption From the Requirement of a Tolerance of the *B.t.t.* Protein as a Plant Pesticide.

Keck, P.J. 1993. Molecular characterization of CPB Resistant Russet Burbank potatoes. Study Number 92-01-37-14, an unpublished study conducted by Monsanto Company. EPA MRID NO. 42932201.

Rogan, J.G., Anderson, J.S., McCreary, J.A. and Lavrik, P.B. 1993. Determination of the expression levels of *B.t.t.* and NPTII proteins in potato tissues derived from field grown plants. Study Number 92-01-37-02, an unpublished study conducted by Monsanto Company. EPA MRID NO. 42932202.

Bartnicki, D.E., Lavrik, P.B., Leimgruber, R.M., Smith, C.E., and Sims, S.R. 1993. Equivalence of microbially-produced and plant-produced *B.t.t.* protein also called Colorado potato beetle active protein from *Bacillus thuringiensis* subsp. *tenebrionis*. Study Number 92-01-37-07, an unpublished study conducted by Monsanto Company. EPA MRID NO. 42932203.

Lavrik, P.B. 1993. Characterization of Colorado potato beetle active *Bacillus thuringiensis* subsp. *tenebrionis* protein produced in *Escherichia coli* . Study Number 92-01-37-10, an unpublished study conducted by Monsanto Company. EPA MRID NO. 42932204.

Bartnicki, D.E., Lavrik, P.B., Leimgruber, R.M., Smith, C.E., and Sims, S.R. 1993. Characterization of the major tryptic fragment from Colorado potato beetle active protein from *Bacillus thuringiensis* subsp. *tenebrionis (B.t.t.)*. Study Number 92-01-37-15, an unpublished study conducted by Monsanto Company. EPA MRID NO. 42932205.

Rogan, G.J. and Lavrik, P. B. 1993. Compositional comparison of Colorado potato beetle (CPB) active *Bacillus thuringiensis* subsp. *tenebrionis* (*B.t.t.*) proteins produced in CPB resistant potato plants and commercial microbial products. Study Number 92-01-37-17, an unpublished study conducted by Monsanto Company. EPA MRID NO. 42932206.

Sims, S.R. 1993. Sensitivity of selected insect species to the Colorado potato beetle active protein from *Bacillus thuringiensis* subsp. *tenebrionis* . Study Number 92-01-37-05, an unpublished study conducted by Monsanto Company. EPA MRID NO. 42932207.

Sims, S.R. 1992. Stability of Colorado potato beetle active *Bacillus thuringiensis* subsp. *tenebrionis* protein in sucrose and honey solutions under non-refrigerated temperature conditions. Study Number 92-01-37-04, an unpublished study conducted by Monsanto Company. EPA MRID NO. 42932208.

Maggi, V.M. 1992. Evaluation of the dietary effect(s) of purified *B.t.t.* protein on honey bee larvae. Study Number CAR 188-92, an unpublished study conducted by California Agricultural Research, Inc. under contract to Monsanto. EPA MRID NO. 42932209

Maggi, V.M. 1992. Evaluation of the dietary effect(s) of purified *B.t.t.* protein on honey bee adults. Study Number CAR 189-92, an unpublished study conducted by California Agricultural Research, Inc. under contract to Monsanto. EPA MRID NO. 42932210.

Hoxter, K.A., and Smith, G.J. 1992. *B.t.t.* protein: a dietary toxicity study with parasitic Hymenoptera (*Nasonia vitripennis*). Project Number 139-349B, an unpublished study conducted by Wildlife International, Ltd. under contract to Monsanto. EPA MRID NO. 42932211.

Hoxter, K.A., and Smith, G.J., 1992. *B.t.t.* protein: a dietary toxicity study with Ladybird beetles (*Hippodamia convergens*). Project Number 139-348, an unpublished study conducted by Wildlife International, Ltd. under contract to Monsanto. EPA MRID NO. 42932212.

Hoxter, K.A., and Smith, G.J. 1992. *B.t.t.* protein: a dietary toxicity study with green lacewing larvae (*Chrysopa carnea*). Project Number 139-347, an unpublished study conducted by Wildlife International, Ltd. under contract to Monsanto. EPA MRID NO. 42932213.

Campbell, S.M., Beavers, J.B., and Jaber, M. 1993. A dietary toxicity study with Russet Burbank potatoes in the Northern Bobwhite. Project Number 139-356, an unpublished study conducted by Wildlife International, Ltd. under contract to Monsanto. EPA MRID NO. 42932214.

Campbell, S.M., Beavers, J.B., and Jaber, M. 1993. A dietary toxicity study with Russet Burbank potatoes in the Northern Bobwhite. Project Number 139-357, an unpublished study conducted by Wildlife International, Ltd. under contract to Monsanto. EPA MRID NO. 42932215.

Lavrik, P.B., Bartnicki, D.E., and Sims, S.R. 1993. Colorado potato beetle active *Bacillus thuringiensis* subsp. *tenebrionis* protein dose formulation, dose confirmation, and dose characterization for an albino mice acute toxicity study. Study Number 92-01-37-11, an unpublished study conducted by Monsanto Company. EPA MRID NO. 42932216.

Naylor, M.W. 1993. Acute oral toxicity study of *B.t.t.* protein in albino mice. Study Number ML-92-407, an unpublished study conducted by Monsanto Company. EPA MRID NO. 42932217.

Keck, P.J., Sims, S.R., and Bartnicki, D.E. 1993. Assessment of the metabolic degradation of Colorado potato beetle active protein in simulated mammalian digestive models. Study Number 92-01-37-16, an unpublished study conducted by Monsanto Company. EPA MRID NO. 42932218.

Keck, P.J. and Sims, S.R. 1993. Aerobic soil degradation of Colorado potato beetle active protein from *Bacillus thuringiensis* subsp. *tenebrionis*. Study Number 92-01-37-09, an unpublished study conducted by Monsanto Company. EPA MRID NO. 42932219.

Studies Submitted to the EPA to Support the Exemption From the Requirement of a Tolerance of the NPTII Protein as an Inert Ingredient:

Bartnicki, D.E., Leimgruber, R.M., Lavrik, P.B., Smith, C.E., Rogan, G. J. 1993. Equivalence of microbially-produced (*Escherichia coli*) and plant-produced (Colorado potato beetle resistant potato) neomycin phosphotransferase II (NPTII). Study Number 92-01-37-08, an unpublished study conducted by Monsanto Company. EPA MRID NO. 43093301.

Naylor, M.W. 1992. Acute oral toxicity study of neomycin phosphotransferase (NPTII) in albino mice. Study Number ML-91-409, an unpublished study conducted by Monsanto Company. EPA MRID NO. 43054701.

Ream, J.E. 1993. Assessment of degradation of neomycin phosphotransferase II in *In vitro* mammalian digestion models. Study Number IRC-91-ANA-06, an unpublished study conducted by Monsanto Company. EPA MRID NO. 43093302.

Studies Supporting the Safety and Wholesomeness of Colorado Potato Beetle Resistant Potatoes.

Lavrik, P.B. and Love, S. L. 1994. Composition and quality analysis of potato tubers derived from field-grown Colorado potato beetle resistant potato plants. Study No. 92-01-37-19, an unpublished study conducted by Monsanto Company.

Naylor, M.E. 1993. One month feeding study with CPB (Colorado Potato Beetle) control potatoes in sprague dawley rats. Study No: ML-92-528, an unpublished study conducted by Monsanto Company.