97-339-01p
Revised

MONSANTO
*Food · Health · Hope*

MONSANTO COMPANY
700 CHESTERFIELD PARKWAY NORTH
ST. LOUIS, MISSOURI 63198
PHONE (314) 694-1000
http://www.monsanto.com

October 13, 1998

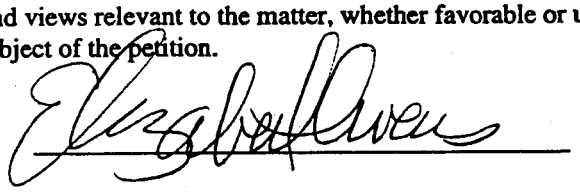
Coordination, and Technical Assistance,
Biotechnology, and Scientific Services,
Plant, Protection, and Quarantine,
Animal and Plant Health Inspection Service,
U.S. Department of Agriculture
4700 River Road
Riverdale, MD 20737

**Subject: PETITION FOR DETERMINATION OF REGULATORY STATUS FOR NEWLEAF® Y POTATOES.**

Enclosed is a revised copy of USDA petition 97-339-01P for determination of the regulatory status of *Solanum tuberosum* cultivar Russet Burbank and Shepody lines RBMT15-101, SEMT15-02, and SEMT15-15.

Based on the data and information contained in the enclosed petition, we believe that there is no longer "reason to believe" that the modified potato plants should be deemed to be regulated articles. The modified potato plants do not present a plant pest risk and are not otherwise deleterious to human health or the environment. The enclosed petition does not contain confidential business information.

The undersigned certifies that, to the best of his/her knowledge and belief, this petition includes all data, information, and views relevant to the matter, whether favorable or unfavorable to the position of the undersigned, which is the subject of the petition.

Signature 

Elizabeth D. Owens, Ph.D.
Regulatory Affairs Manager
Monsanto Company
700 Chesterfield Parkway North
BB1K
St. Louis, MO 63198
Phone: 314-737-5721
FAX: 314-737-7085
Email: Elizabeth.D.Owens@monsanto.com

# PETITION FOR DETERMINATION OF REGULATORY STATUS FOR NEWLEAF® Y POTATO
# Lines RBMT15-101, SEMT15-02, and SEMT15-15

**Revised Submission**

**October 13, 1998**

**Submitted by**

**Elizabeth D. Owens, Ph.D.**
**Monsanto Company**

**97-357U3**

**Contains No Confidential Business Information**

# Table of Contents

# LIST OF FIGURES



# LIST OF TABLES

# LIST OF APPENDICES

## ABBREVIATIONS

| | |
|---|---|
| *aad* | Gene encoding aminoglycoside adenylyltransferase |
| bp | Basepairs |
| *B.t.t.* | *Bacillus thuringiensis* subsp. ***tenebrionis*** bacterium |
| ca. | About |
| cm | Centimeters |
| CCMV | Cowpea chlorotic mottle virus |
| CP | Coat Protein |
| CPB | Colorado Potato beetle |
| *cry3A* | Gene encoding Cry3A δ-endotoxin protein |
| cvs. | Cultivars |
| cwt | Centiweight |
| EBN | Endosperm Balance Number |
| ELISA | Enzyme-linked immunosorbent assay |
| EPA | Environmental Protection Agency |
| FDA | Food and Drug Administration |
| FIFRA | Federal Insecticide, Fungicide and Rodenticide Act |
| FMV | Figwort mosaic virus |
| g, ng, µg, mg, pg | Grams, nanograms, micrograms, milligrams, picograms |
| Hl | HiLite |
| Kb | Kilobases |
| kD | Kilodaltons |
| lb | Pound |
| ml | Milliliter |
| mRNA | Messenger RNA |
| *nptII* | Gene encoding neomycin phosphotransferase II |
| nt | Nucleotide(s) |
| OD | Optical Density |
| ORF | Open Reading Frame |
| PCR | Polymerase chain reaction |
| PLRV | Potato LeafRoll Virus |
| *PVYcp* | PVY coat protein gene |
| PV-STMT15 | Plant Vector |
| $PVY^{O}$ | PVY strain O |
| $PVY^{NTN}$ | PVY strain necrotic tuber necrosis |
| PVA | Potato A potyvirus |
| PVX | Potato X potexvirus |
| PVY | Potato Y potyvirus |
| PVM | Potato M carlavirus |
| PVS | Potato S carlavirus |
| Rb | Russet Burbank |
| RBMT15-15, *etc.* | Potato Lines |
| RLaSV | Red Lakota virus |
| RNA | Ribonucleic acid |
| Se | Shepody potato |
| sp. | Species |
| T-DNA | Transfer DNA |
| TRV | Tobacco Rattle Virus |
| USDA | United States Department of Agriculture |
| UTR | Untranslated Region |
| vRNA | Viral RNA |

# I. RATIONALE FOR THE DEVELOPMENT OF NEWLEAF® Y POTATOES

Potato is the world's fifth most abundant crop with 265,436,000 metric tons produced world-wide in 1994 (National Potato Council, 1996). Monsanto Company has developed NewLeaf® Y potatoes which are highly resistant to the Colorado Potato Beetle (CPB) and to infection by potato virus Y (PVY), two of the most damaging potato pests in North America (Perlak *et al.*, 1993). Fourteen NewLeaf® potato lines expressing the *cry3A* gene for CPB resistance have already been granted a Determination of Nonregulated Status by the USDA, Animal and Plant Health Inspection Service (USDA Determinations for 95-338-01p and 94-257-01p). NewLeaf Y potato lines were developed using new transformation events to have the additional trait of PVY resistance.

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). To date, no traditionally bred cultivars have been produced that are resistant to the CPB. For traditional potato varieties, approximately one-third of the 2.8 million pounds of chemical insecticides annually applied to potatoes are targeted for its control (USDA, 1997). 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% (Harcourt, 1971). If poorly managed, the CPB is capable of completely defoliating some potato plants, resulting in yield reductions of as much as 85%, which is sufficient to prevent potato production in some areas (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 and trap plots (Roush and Tingey, 1993). These options are not often practical, effective, economical nor easily implemented throughout the season.

Potato virus Y causes rugose mosaic. Symptoms vary widely with virus strain and potato cultivar, ranging in severity from weak symptoms to severe foliage necrosis to death of infected plants. Infection by PVY is associated with reduced yield which may be as high as 80% in susceptible cultivars (Banttari *et al.*, 1993). The virus can be transmitted mechanically, via the transfer of sap from an infected plant, or biologically by over 30 species of aphids. The main control of PVY is to remove from the field infected potatoes which serve as an inoculum source for non-infected potatoes. This is usually accomplished by rogueing, although it is often difficult to visually identify infected potatoes. In some cultivars, detection of PVY is difficult because visible symptoms may be transient or absent (Banttari *et al.*, 1993). Additionally, insecticides are applied to control aphids which transmit PVY and result in the spread of PVY infection throughout the potato field. Certification programs assay to identify virus-infected plants which helps to reduce the amount of virus-infected seed potatoes used for commercial production. However, even with these procedures, it is difficult to produce certified seed for some potato varieties, such as Shepody, because of their susceptibility to PVY. A survey of potato tubers grown in Colorado, Idaho, Washington, and Wisconsin, and available in grocery stores in Missouri, USA, found that between 19% and 38% of the potatoes were infected by PVY (Naumovich and Kaniewski, 1994).

NewLeaf Y potato lines provide many advantages to potato farmers, especially to seed potato producers. In 1995, growers applied insecticides to 88% of the potato acreage in the U.S., amounting to 2,553,000 total pounds. Available statistics on insecticide usage for control of CPB, and/or aphids which transmit viruses, are listed in Table I.1. Methamidophos, phorate, carbofuran, disulfoton, and endosulfan are all very widely used for CPB and aphids. Phorate, carbofuran, and disulfoton are soil-applied systemic insecticides, while methamidophos and endosulfan are applied as foliar insecticides. The use of NewLeaf Y potato lines could reduce the need for these

synthetic insecticides in potato farming by providing insecticide-free control of the Colorado potato beetle and potato virus Y.

**Table I.1. Insecticide use on potato for 1995 (USDA, 1997).**

| Insecticide | Area Applied (%) | Number of Applications | Rate per application (Pounds per acre) | Rate per crop year (Pounds per acre) | Total (1000 lbs) | Insect Targets |
|---|---|---|---|---|---|---|
| Azinphos-methyl | 8 | 1.4 | 0.45 | 0.64 | 59 | CPB, APHID |
| Carbaryl | 3 | 1.3 | 0.83 | 1.08 | 34 | CPB |
| Carbofuran | 24 | 1.7 | 0.84 | 1.40 | 386 | CPB, APHID |
| Cryolite | 0 | 1.0 | 11.19 | 11.19 | 49 | CPB |
| Dimethoate | 4 | 1.2 | 0.42 | 0.53 | 23 | CPB, APHID |
| Disulfoton | 4 | 1.4 | 2.38 | 3.36 | 158 | CPB, APHID |
| Endosulfan | 14 | 1.4 | 0.74 | 1.02 | 160 | CPB, APHID |
| Esfenvalerate | 18 | 1.3 | 0.03 | 0.05 | 9 | CPB, APHID |
| Ethoprop | 7 | 1.1 | 4.38 | 4.61 | 358 | WIREWORM, NEMATODE |
| Fonofos | 3 | 1.0 | 2.64 | 2.69 | 94 | WIREWORM, NEMATODE |
| Imidacloprid | 15 | 1.2 | 0.14 | 0.17 | 29 | CPB, APHID |
| Methamidophos | 23 | 1.7 | 0.87 | 1.50 | 393 | APHID |
| Methylparathion | 1 | 1.7 | 0.70 | 1.18 | 15 | CPB, APHID |
| Oxamyl | 2 | 1.3 | 0.83 | 1.08 | 22 | CPB, APHID |
| Permethrin | 16 | 1.3 | 0.13 | 0.16 | 31 | CPB |
| Phorate | 21 | 1.0 | 2.67 | 2.67 | 647 | CPB, APHID |
| Phosmet | 1 | 1.4 | 0.85 | 1.15 | 17 | CPB, APHID |
| Piperonylbutoxide | 2 | 1.1 | 0.28 | 0.30 | 6 | CPB |
| Propargite | 3 | 1.2 | 1.74 | 2.07 | 62 | MITES |

This revised petition includes three NewLeaf Y plant lines that are under consideration for commercial introduction. These include one line of NewLeaf Y Russet Burbank (RBMT15-101) and two lines of NewLeaf Y Shepody (SEMT15-02 and SEMT15-15). All NewLeaf Y potato lines were transformed with the plant vector PV-STMT15, which contains the *cry3A* gene for resistance to the CPB, the PVY coat protein gene for resistance to PVY, and the *nptII* gene for resistance to kanamycin as a selectable marker. Field experiments were conducted from 1993 to 1997 under notifications from the USDA (93-357-03n, 94-067-12n, 94-298-03n, 95-023-05n, 95-041-06n, 95-041-08n, 95-041-10n, 95-061-01n, 95-061-02n, 95-121-01n, 96-038-02n, 96-040-06n, 96-071-17n, 96-072-03n, 96-079-11n, 96-079-12n, 96-086-05n, 96-086-06n, 96-260-01n, 96-278-01n, 97-020-04n, 97-020-06n, 97-049-06n, 97-049-07n, 97-049-08n, 97-049-11n, 97-080-05n, 97-111-09n, 97-114-04n, 97-114-05n, 97-114-06n, 97-114-08n); final Data Reports are submitted under separate cover. Data and information presented in this petition demonstrate that the modified potato plants do not present a plant pest risk and are not otherwise deleterious to human health or the environment.

# II. THE POTATO FAMILY

A Consensus Document on the Biology of *Solanum tuberosum* subsp. *tuberosum* (Potato) in the OECD Series on the Harmonization of Regulatory Oversight in Biotechnology No. 8 (1997) has been published. This document, which was prepared by the Netherlands as lead country in collaboration with the United Kingdom, addresses the biology of the crop plant *Solanum tuberosum* subsp. *tuberosum* and contains information for use during the regulatory assessment of potato products.

## *Properties of the Non-transformed Potato Cultivars*

The information in the following section on potato cultivars was provided by Dr. Steve Love (1997), a potato expert at the University of Idaho.

### Russet Burbank

Russet Burbank is the dominant potato cultivar produced in the U.S. It is estimated by the USDA to represent 50.9% of total fall potato production of 1.14 million planted acres (National Potato Council, 1997). 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, 1997).

- Uses: Multiple end uses make Russet Burbank unique among the major cultivars. It has good consumer quality for boiling, and it is 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 dehydrated products, such as potato flakes, and cattle feed.

- 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).

- 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.

- Characteristics: Russet Burbank is a male sterile, tetraploid (2n=48) potato variety. It is a high yielding, high specific gravity potato with a very late maturity. It is resistant to common scab (caused by *Erwinia carotovora*) and is highly resistant to blackleg (caused by *Streptomyces scabies*). It is highly susceptible to potato virus Y and potato leafroll virus. 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).

### Shepody

Shepody is grown primarily in the northern tier of the United States and Canada. In 1996, it was planted on approximately 130,000 acres in the U.S. and 102,000 acres in Canada in 1996.

- Uses: It is used primarily for frozen french fry processing. It is excellent when baked or fried, and it also boils very well making it a very good table or fresh product.

- **Parentage**: It was selected in New Brunswick, Canada from the progeny of a cross between Bake-King and F58050. It was released by Agriculture Canada-New Brunswick in 1980.

- **Description**: Plants are medium sized and spreading with large medium green leaves. Leaflets are broadly ovate and overlapping. Flowers are numerous, light violet with white tips. Tubers are oblong to long and have a smooth to lightly netted buff skin. The eyes are medium deep and evenly distributed. The flesh of the tuber is white.

- **Characteristics**: Shepody is a male fertile tetraploid (2n=48) potato variety (Hawkes, 1990). It is a high yielding, medium to low gravity (1.075 to 1.100) potato. It is very susceptible to common scab, PVX and PVY. It is susceptible to *Verticillium* wilt and early blight (caused by *Alternaria solani*). It is resistant to PLRV-induced net necrosis and tolerant to heat stress. It is highly resistant to PVA. Plants are normally spaced at 8 to 10 inches when planted for seed and 10 to 12 inches when planted for processing and tablestock. It sets tubers late; however, they size quickly. Tubers are uniform in size and the percentage over 10 oz. is high.

# III. DESCRIPTION OF TRANSFORMATION SYSTEM

The NewLeaf Y potato lines RBMT15-101, SEMT15-02, and SEMT15-15 were developed by transforming the parental lines (Russet Burbank, Shepody) with PV-STMT15 using the *Agrobacterium tumefaciens* transformation system. The *A. tumefaciens* transformation method used in the generation of the commercial NewLeaf lines 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 transgenes into plant cells. The vectors were assembled in *E. coli* MV1190, a derivative of the common laboratory *E. coli* K-12 strain (Bachmann, 1987) and mated into the *Agrobacterium* strain ABI. 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 *Agrobacterium* strain ABI containing the plant expression vector PV-STMT15 was added to potato stem sections (Newell *et al.*, 1991) in tissue culture dishes. The transgene expression cassette which includes the *cry3A*, *PVYcp* and *nptII* genes was transferred into the genome of individual potato cells thereby allowing selection in medium containing 200 µg kanamycin. After a few days, the remaining viable *Agrobacterium* cells were killed using carbenicillin and cefotaxime. Prior to planting any new transformed line, the plants were grown on medium without antibiotics for two weeks, then a sample of tissue from each plant was placed in Luria broth and shaken at 25°C. The plant was rejected if bacteria were found in either of these assays. Subsequently, the potato tissues were treated to stimulate regeneration of transgenic cells into shoots. Ultimately plantlets were grown in soil.

# IV. THE DONOR GENES AND REGULATORY SEQUENCES

Genetic elements contained in the vector PV-STMT15 (Plasmid map Fig. V.1) are summarized in Table IV.2.

## *A. Construction of Plant Vectors*

PV-STMT15 was used in the generation of the transgenic NewLeaf Y potato lines. It is a double border plant transformation vector. It contains well-characterized DNA segments required for selection and replication of the plasmid in bacteria, as well as right and left T-DNA borders for delineating the region of DNA to be transferred into the plant genomic DNA. The host for DNA cloning and vector construction was *Escherichia coli* MV1190, a derivative of the common laboratory *E. coli* K-12 strain (Bachmann, 1987). These vectors are composed of the following genetic elements. The first segment is the 0.45 Kb fragment from the octopine Ti plasmid, pTi15955 (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 RK2 plasmid (Stalker *et al.*, 1981). The next segment (*ori-322/rop*) is a 1.8 Kb segment of pBR322 plasmid which provides the origin of replication for maintenance in *E. coli* and the *bom* site for the conjugational transfer into the *Agrobacterium tumefaciens* cells (Bolivar *et al.*, 1977; Sutcliffe, 1978). This is fused to the 0.93 Kb fragment isolated from

transposon Tn7 that 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 *PvuI* to *BclI* restriction fragment from the pTiT37 plasmid containing the nopaline-type T-DNA right border region (Depicker *et al.*, 1982).

Three chimeric constructs were introduced between the right and left border regions of the plant transformation vectors. In lines SEMT15-02 and SEMT15-15 elements outside the borders were transferred to the potato plants. Each line is described in detail in Section V.

- The chimeric *nptII* expression cassette (*P-NOS/nptII/NOS* 3') consists of the promoter region of the nopaline synthase gene from Ti plasmid of *A. tumefaciens* (Fraley *et al.*, 1983), the neomycin phosphotransferase type II (*nptII*) gene (Beck *et al.*, 1982) and the nontranslated 3' region of the nopaline synthase gene referred to as nos 3' (Depicker *et al.*, 1982; Bevan *et al.*, 1983).
- The chimeric *cry3A* expression cassette (*AraSSU1Ac/cry3A/NOS* 3') consists of the *Arabidopsis thaliana* ribulose-1,5-bisphosphate carboxylase small subunit ats1A promoter (Almeida *et al.*, 1989; Wong *et al.*, 1992), the *cry3A* gene that encodes the Cry3A protein (McPherson *et al.*, 1988; Perlak *et al.*, 1993) and the nontranslated 3' region of the nopaline synthase gene referred to as nos 3' (Depicker *et al.*, 1982; Bevan *et al.*, 1983).
- The chimeric *PVYcp* expression cassette (*FMV/PVYcp/E9* 3') consists of the 35S promoter region of the figwort mosaic virus (Richins *et al.*, 1987), the Hsp 17.9 leader sequence from *Glycine max*, the full length *PVYcp* gene and 3' untranslated region from a naturally occurring PVY isolate (Lawson *et al.*, 1990) and the nontranslated 3' region of the pea small subunit of ribulose-1,5-bisphosphate carboxylase referred to as E9 3' (Coruzzi *et al.*, 1984).

## *B. Description of the Inserted Genes*

### *1. cry3A* gene and its encoded Cry3A protein

The *cry3A* gene was isolated from the DNA of *Bacillus thurigiensis, sp. tenebrionus (B.t.t.)* strain BI 256-82 (Krieg *et al.*, 1983). This gene encodes the protein responsible for the control of CPB. A full-length clone and the complete nucleotide sequence has been reported by McPherson *et al.* (1988) and Perlak *et al.* (1993). The *cry3A* 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 (Sims, 1993; 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 73 kD full-length protein, the *B.t.t.* bacterium also produces a smaller form of this protein called Cry3A band 3. The Cry3A 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). The gene encoding the Cry3A band 3 protein, modified with potato preferred codons for increased plant expression, was introduced in the transformed potato plants. 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 plant-preferred codon modifications changed 399 out of 1791 nucleotides within the gene that codes for the Cry3A band 3 protein, without altering any of the encoded amino acids. This modified gene encodes the identical amino acid sequence of band 3 protein as produced by the *B.t.t.* bacterium (Perlak *et al.*, 1993).

### *2. PVYcp* gene and its encoded PVY coat protein

The PVY coat protein gene (*PVYcp*) was obtained from a PVY strain O (Murphy *et al.*, 1995; De Bokx and Huttinga, 1981) isolated in the USA by Dr. Pete Thomas, USDA-ARS from an infected potato in Washington State. The characteristics of PVY are summarized in Table IV.1. The gene sequence engineered into lines RBMT15-101, SEMT15-02 and SEMT15-15 is identical to the native viral gene, except for the ATG start codon which was synthesized using site-directed mutagensis; therefore, the protein produced is expected to be identical to the native viral coat protein with the exception of an additional methionine at the 5' terminus. Additionally, this gene contains the complete 3' untranslated region of the PVY genome directly downstream of the coat protein gene. PVY is a polyadenylated virus and the poly-A tail was used to clone the coat protein gene using a poly-A primer for the

reverse transcriptase polymerase chain reaction. At the time these transgenic lines were developed, Monsanto scientists thought that the presence of 3' UTR produced a more stable transgenic viral coat protein mRNA.

**Table IV.1. Description of PVY donor organism for coat protein gene.**

| Characteristic | Description | References |
|---|---|---|
| Taxonomic Name | Potato virus Y strain O | Murphy *et al.*, 1995; De Bokx and Huttinga, 1981 |
| Nucleic Acid Type | ssRNA | Murphy *et al.*, 1995; De Bokx and Huttinga, 1981 |
| Localization | Systemic | Murphy *et al.*, 1995; De Bokx and Huttinga, 1981 |
| Association with any satellite or helper viruses | None | not published |
| Natural Host Range | Solanaceous plants (potato, tobacco, tomato, nightshade, *etc.*) | Murphy *et al.*, 1995; De Bokx and Huttinga, 1981 |
| Means of Transmission | Mechanical and Aphid [primarily (*Myzus persicae* Sulzer) and *Aphis nasturtii*] | Murphy *et al.*, 1995; De Bokx and Huttinga, 1981 |
| Mode of Transmission | Nonpersistent | Murphy *et al.*, 1995; De Bokx and Huttinga, 1981 |
| Known Synergy | PVY/PVX synergism | De Bokx, 1996 |
| Origin of Virus | Infected potato plant in Washington State, USA | Kaniewski *et al.*, 1990 |

## 3. *nptII* gene and its encoded NPTII protein

The *nptII* gene was isolated from the prokaryotic transposon Tn5 present in *E. coli* (Beck *et al.*, 1982). The *nptII* gene encodes the NPTII protein or neomycin phosphotransferase, which 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 protein uses ATP to phosphorylate neomycin and the related kanamycin, thereby inactivating these aminoglycoside antibiotics and preventing them from killing the cells producing the NPTII protein. The sole purpose of inserting the *nptII* gene into potato cells with the *cry3A* and *PVYcp* genes is to have an effective method of selecting cells that contain the insect and virus-resistant genes. In general, the frequency of cells 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 are used. Consequently, cells selected for plant regeneration contain the *cry3A, PVYcp* and *nptII* genes.

**Table IV.2. Summary of DNA Components in PV-STMT15**

| Genetic Element | Size, Kb | Function and Source |
|---|---|---|
| *aad* | 0.79 | Aminoglycoside adenylyltransferase gene from Tn7 transposon in *E. coli* (Fling *et al.*, 1985) conferring spectinomycin or streptomycin resistance |
| RB | 0.36 | *Pvu*I to *Bcl*I restriction fragment from the pTiT37 plasmid containing the nopaline-type T-DNA right border region (Depicker *et al.*, 1982) |
| P-FMV | 0.57 | The 35S promoter region of the Figwort mosaic virus (FMV) (Richins *et al.*, 1987) |
| Hsp17.9 | 0.08 | Heatshock protein 17.9 kD, 77-nucleotide leader sequence from *Glycine max* (Raschke *et al.*, 1988) |
| *PVYcp* | 0.81 | Coat protein gene from Potato virus Y strain O (Lawson *et al.*, 1990) used to provide resistance to PVY |
| 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 *PVYcp* mRNA |
| P-Arab- SSU1A | 1.7 | The *Arabidopsis thaliana* ribulose-1,5-bisphosphate carboxylase small subunit *ats*1A promoter (Almeida *et al.*, 1989; Wong *et al.*, 1992) used to direct transcription of the *cry3A* gene. |
| *cry3A* | 1.8 | The *cry3A* delta-endotoxin gene from *Bacillus thuringiensis* subsp. *tenebrionis* encodes the 68 kD band 3 protein having an amino acid sequence identical to the *Cry3A* band 3 protein found in *B.t.t.* (Perlak *et al.*1993) |
| NOS 3' | 0.26 | A 3' nontranslated region of the nopaline synthase gene which functions to terminate transcription and direct polyadenylation of the mRNA (Depicker *et al.*, 1982; Bevan *et al.*, 1983) |
| P-NOS | 0.3 | The promoter region of the nopaline synthase gene from the Ti plasmid of *Agrobacterium tumefaciens* (Fraley *et al.*, 1983) |
| *nptII* | 0.79 | Neomycin phosphotransferase II gene isolated from the Tn5 *E. coli* transposon. The encoded protein confers kanamycin resistance and serves as a selectable marker for transformation (Fraley *et al.*, 1983) |
| LB | 0.45 | A restriction fragment from the octopine Ti plasmid, pTI15955, containing the 24 bp T-DNA left border (Barker *et al.*, 1983) |
| *oriV* | 1.3 | Origin of replication from plasmid RK2 isolated from *Agrobacterium* strain ABI (Stalker *et al.*, 1981) |
| *ori-322/rop* | 1.8 | A segment of pBR322 which provides the origin of replication for maintenance of the PV-STMT15 plasmid in *E. coli* (*rop*) and *bom* site for conjugational transfer into *Agrobacterium* (Bolivar *et al.*, 1977; Sutcliffe, 1978) |

# V. GENETIC ANALYSIS, AGRONOMIC PERFORMANCE AND COMPOSITIONAL ANALYSIS

## *A. Genetic Analysis*

Product characterization studies were conducted on the transformed NewLeaf Y potato plant lines. Data from lines RBMT15-101, SEMT15-02, and SEMT15-15 are discussed. The studies included: characterization of the DNA inserted, characterization of the mRNA produced by the *PVYcp* gene, characterization of proteins expressed, estimation of expression levels of the proteins, estimation of PVYcp mRNA levels produced in the recipient plants, and field observations of beetle feeding damage.

### 1. Characterization of inserted DNA

Genomic DNA was isolated from young leaf tissue from the transgenic NewLeaf Y lines as well as nontransformed control lines from each cultivar collected from 1996 field trials. The isolated DNA was characterized by polymerase chain reaction (PCR) and Southern blot analyses. Detailed methodology is included in Appendix I. The PCR analyses defined the genetic elements which were transferred between the left and right border region of plasmid PV-STMT15 to the genome of the potato lines. PCR was used also to establish the linkage between the genetic elements (*PVYcp*, *cry3A*, and *nptII*). Southern blots were used to detect genetic elements transferred from outside the left and right borders of PV-STMT15. These sequences include the bacterial origins of replication (*oriV* and *ori322*) and the bacterial gene for spectinomycin and streptomycin resistance (*aad*).

A summary of genetic elements for each line is presented in Table V.1. Line RBMT15-101 contains the *PVYcp*, *nptII*, and *cry3A* expression cassettes and portions of the left and right borders. For transfer of the DNA by *Agrobacterium* transformation, the plasmid DNA is nicked between the nucleotides 3 and 4 of the borders; therefore, each transformed line contains some of the border sequences (Albright *et al.*, 1987; Wang *et al.*, 1987). Lines SEMT15-02 and SEMT15-15 contain *PVYcp*, *nptII*, and *cry3A* expression cassettes plus the LB, RB, *oriV* element, *ori322* element, and the *add* gene. Until recently, it was commonly thought that during *Agrobacterium*-mediated plant transformation, the DNA sequences transferred to the plant were delimited by the left and right borders. However, in 1997, Kononov and co-workers (1997) reported that 75% of tobacco plants transformed using disarmed *Agrobacterium tumefaciens* contained sequences outside the border. Therefore, the results for lines SEMT15-02 and SEMT15-15 are consistent with published literature. Only the *PVYcp*, *nptII* and *cry3A* genes are expressed in the transgenic lines; *aad* expression is under the control of a bacterial promoter and is, therefore, not expected to be expressed in plants (Appendix V).

**Table V.1. Genetic elements present in each NewLeaf Y line.**

| | Genetic Element Present | | | | | |
|---|---|---|---|---|---|---|
| Line | *PVYcp*[1] | *nptII*[1] | *cry3A*[1] | *aad*[2] | *oriV*[2] | *ori322*[2] |
| RBMT15-101 | √ | √ | √ | | | |
| SEMT15-02 | √ | √ | √ | √ | √ | √ |
| SEMT15-15 | √ | √ | √ | √ | √ | √ |

[1] Analyzed using PCR (see section V.A.1.a.).
[2] Analyzed using Southern blots (see section V.A.1.b.).
The √ indicates the presence of the genetic element.

## a) PCR Analysis

The primers used and expected size of the product are provided in Table V.2. A schematic representation of the PV-STMT15 vector and the location of the primers is provided in Figure V.2.

*PCR reactions with primers for the PVYcp gene.* Two primers (PVY1 and PVY2) were designed to amplify the coding sequence of the *PVYcp* gene. Based on the sequence of the plant vector, PV-STMT15 (Figure V.2), a 786 bp product was predicted. The location of these primers is depicted in Figure V.2. PCR reactions with this primer set and genomic DNA isolated from nontransformed control lines were conducted. As expected, no product was observed with any of the nontransformed control cultivars. A 786 bp band was produced from each of the transformed lines (Figs. V.3 - V.5). These results demonstrate that the three commercial of NewLeaf Y potato lines contain an intact *PVYcp* gene.

*PCR reactions with primers for the cry3A gene.* Two primers (B1 and B2) were designed to amplify the coding sequence of the *cry3A* gene. Based on the sequence of the PV-STMT15, a 1793 bp product was predicted. The location of these primers is depicted in Figure V.2. PCR reactions with this primer set and genomic DNA isolated from nontransformed control lines were also carried out. As expected, no product was observed from any of the three nontransformed control cultivars. A 1793 bp band was produced from each of the transformed lines (Figs. V.3 - V.5). These results establish that the three commercial lines of NewLeaf Y potato lines contain at least one intact *cry3A* gene.

*PCR reactions with primers directed to the nptII gene.* Two primers (N1 and N2) were designed to amplify the coding sequence of the *nptII* gene. Based on the sequence of PV-STMT15, a 768 bp product was predicted. The physical location of these primers is depicted in Figure V.2. PCR reactions with this primer set and genomic DNA isolated from nontransformed control lines were also carried out. As expected, no product was observed from any of the three nontransformed control cultivars. A 768 bp band was produced from each of the transgenic lines (Figs. V.3 - V.5). These results establish that the three commercial lines of NewLeaf Y potato lines contain at least one intact *nptII* gene.

*Linkage between PVYcp* and *cry3A genes.* Two primers (PVY1 and BC1) were used to amplify a specific sequence that demonstrates that the *PVYcp* and *cry3A* coding sequences were linked in the transgenic plant lines. PVY1 and BC1 anneal near the 5' end of *PVYcp* and 3' end of *cry3A*, respectively. Based on the sequence of PV-STMT15, a 2171 bp product was predicted. A 2171 bp band was produced from each of the transgenic lines (Figs. V.6 - V.7). These results demonstrate that the *PVYcp* and *cry3A* genes are linked in all commercial NewLeaf Y potato lines.

*Linkage between cry3A and nptII genes.* Initial attempts to amplify across the entire promoter for *cry3A* (i.e., P-Arab-SSU1A) to demonstrate linkage between *cry3A* and *nptII* were unsuccessful. These results may have been due to the fact that this sequence is very A-T rich. Formation of a secondary structure may have prevented polymerization across this region. To circumvent this problem, two sets of primers were designed to amplify DNA fragments which overlap in sequence (Figure V.2). The first primer set containing PB2 and SS1 is expected to anneal at the 5' end of *cry3A* and within the *cry3A* promoter (P-Arab-SSU1A). A 1665 bp product was predicted to be generated following PCR amplification with PB2 and SS1. The second primer set (SS2 and N1) anneal within the P-Arab-SSU1A promoter and near the 5' end of *nptII*. From the sequence of PV-STMT15, a 1607 bp product was predicted to be generated following PCR amplification with this primer set. The fragments generated from the two sets contain overlapping sequence within the P-Arab-SSU1A promoter and encompass the entire region of the DNA from the 5' end of *cry3A* to the 5' end of *nptII*. PCR reactions with the two primer sets and genomic DNA from the transgenic lines generated the expected size products (Figs. V.6 - V.7). These results support the fact that the *cry3A* and *nptII* genes are linked in all NewLeaf Y potato lines.

## b) Southern Analysis

*Presence of the oriV, ori322 and aad sequences.* The presence of the *aad*, *ori322* and *oriV* genetic elements was determined by Southern analysis on isolated genomic DNA from the NewLeaf Y lines digested with the restriction

endonuclease *Bam*HI. Plasmid PV-STMT15 contains a single restriction site for this enzyme which is indicated in Figure V.1. In the case of single copy insertion events, digestion with *Bam*HI is expected to yield two fragments containing the inserted DNA linked to plant DNA. If elements outside the left and right border were inserted into the plant, only one of these two fragments would be expected to hybridize with probes for either *aad*, *ori322* or *oriV*. In the case of multicopy insertion events, more than one fragment would be expected to hybridize with probes for either *aad*, *ori322* or *oriV*. The results of the Southern analysis are shown in Figures V.8 through V.10. The Southern blot analysis for the NewLeaf Y lines probed with the *aad* gene is shown in Figure V.8. Positive hybridization of the *aad* probe to a single band was detected for line SEMT15-15. Positive hybridization to two fragments was detected for line SEMT15-02 (Figure V.8, Lane 3). No hybridization to the *aad* probe was detected with line RBMT15-101. The Southern blot analyses for the NewLeaf Y lines probed with the *oriV* gene and with the *ori322* are shown in Figures V.9 and V.10., respectively. The results with the *oriV* and *ori322* probes parallel the results obtained with the *aad* probe. Positive hybridization of the *oriV* and *ori322* probes to a single fragment was detected for lines SEMT15-02 and SEMT15-15 and positive hybridization to two fragments for line SEMT15-02. No hybridization to *oriV* or *ori322* probes was detected with line RBMT15-101. Hybridization with *oriV* and *ori322* probes to two bands was observed in the case of a control sample, DNA from Russet Burbank control plants spiked with plasmid DNA (Figures V.9 and V.10, Lane 7). The two positive hybridizing bands were attributed to incomplete digestion with *Bam*HI; the larger size band corresponds to nicked open-circular plasmid DNA and the lower molecular size fragment corresponds to linear plasmid DNA.

## c) Conclusions

The PCR analyses established that the *PVYcp*, *cry3A* and *nptII* genes were inserted in the genome of all NewLeaf Y lines. Integrity of the linkage between these genetic elements was maintained during the transfer process for all lines. Southern blot analyses indicated that no genetic elements outside the left and right borders of plasmid PV-STMT15 were transferred to the genome of line RBMT15-101. For lines SEMT15-02 and SEMT15-15, insertion of the DNA was not delimited by the right border; therefore, in addition to the elements between the left and right borders, these lines also contain the *aad*, *ori322*, and *oriV* genetic elements.

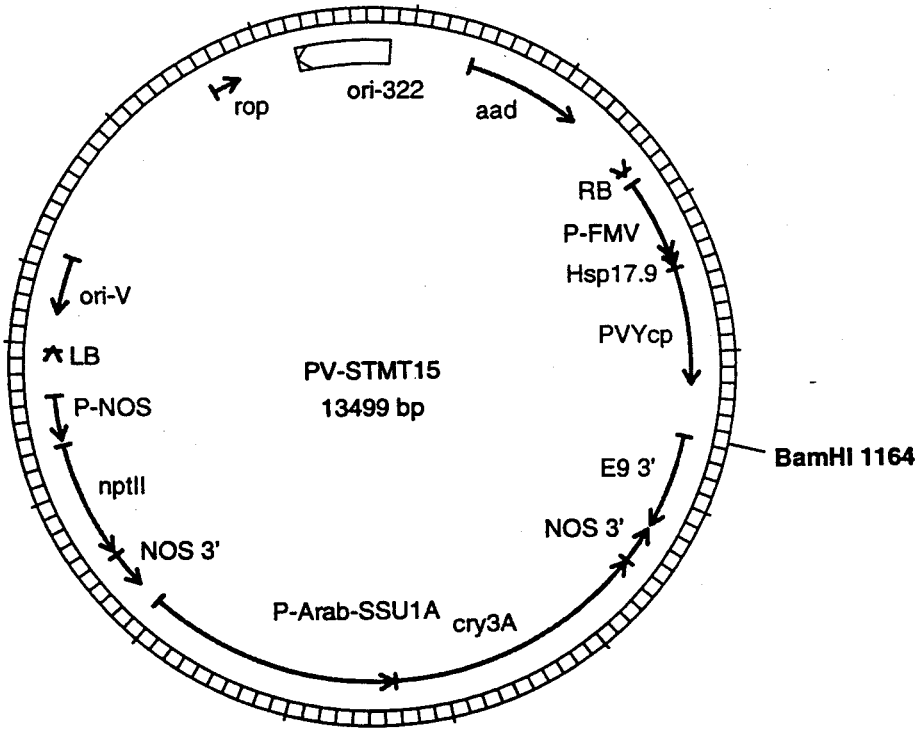



**Figure V.1. Plasmid map of PV-STMT15.**

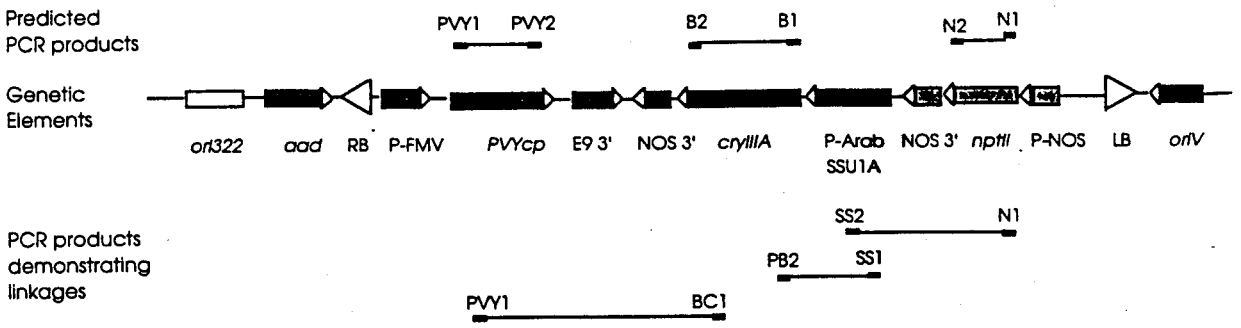



**Figure V.2. Schematic Representation of Predicted PCR Products.**

Detailed map of the portion of PV-STMT15 containing all the genetic elements expected to be integrated, as well as elements flanking the right border (*aad*) and left border (*oriV*). Directly above the map is a representation of PCR products expected using primer sets designed to amplify specific elements. Below the map is a representation of PCR products from reactions designed to assess linkages between genetic elements. Detailed information concerning the primers (annealing position and sequence) may be found in Table V.2., below, and Appendix I. (Figure not drawn to scale.)

**Table V.2. Primers Used for PCR Analysis**

| Primer 1 | Size (nt) | Primer 2 | Size (nt) | PCR Product Size, bp | Anneal. Temp[1], °C |
|---|---|---|---|---|---|
| PVY1 | 25 | PVY2 | 26 | 786 | 60 |
| B2 | 23 | B1 | 27 | 1793 | 60 |
| N2 | 24 | N1 | 23 | 768 | 60 |
| PVY1 | 25 | BC1 | 21 | 2171 | 60 |
| PB2 | 23 | SS1 | 29 | 1665 | 60 |
| SS2 | 39 | N1 | 23 | 1607 | 60 |

1 "Anneal Temp" refers to temperature(s) used in annealing step during PCR reactions.

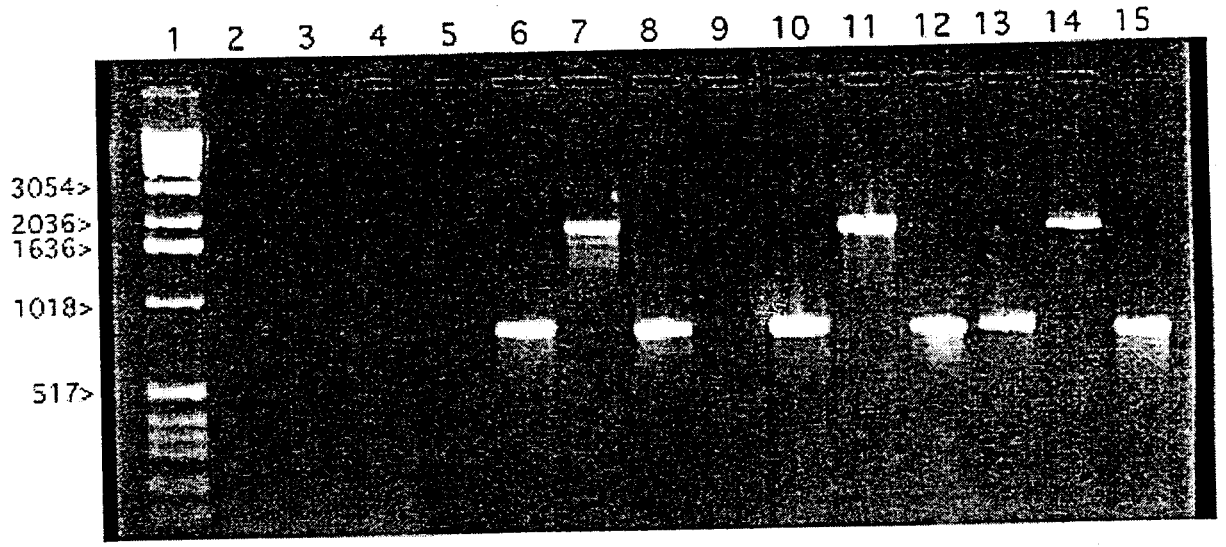



**Figure V.3. PCR Analysis of Lines SEMT15-02 and SEMT15-7 utilizing primers directed toward the *PVYcp*, *cry3A*, and *nptII* genes.**

***Note: Line SEMT15-7 has been withdrawn from this petition.***

Photograph of agarose gel electrophoresis of PCR products following amplification of genomic DNA using primers for specific genetic elements.

**Lane 1** contains molecular weight markers.

**Lanes 2-4** contain PCR products from amplification of control genomic DNA from nontransformed tissue of Shepody variety.

**Lanes 6-8** contain PCR products from amplification of control genomic DNA from nontransformed Shepody tissue plus 150 ng of PV-STMT15 plasmid DNA.

**Lanes 10-12** contain PCR products from amplification of SEMT15-02 genomic DNA.

**Lanes 13-15** contain PCR products from amplification SEMT15-7 genomic DNA.

**Lanes 2, 6, 10, and 13** contain PCR products from amplification with primers (PVY1-PVY2) directed toward the *PVYcp* gene.

**Lanes 3, 7, 11, and 14** contain PCR products from amplification with primers (B1-B2) directed toward the *cry3A* gene.

**Lanes 4, 8, 12, and 15** contain PCR products from amplification with primers (N1-N2) directed toward the *nptII* gene.

**Lanes 5 and 9** are empty.

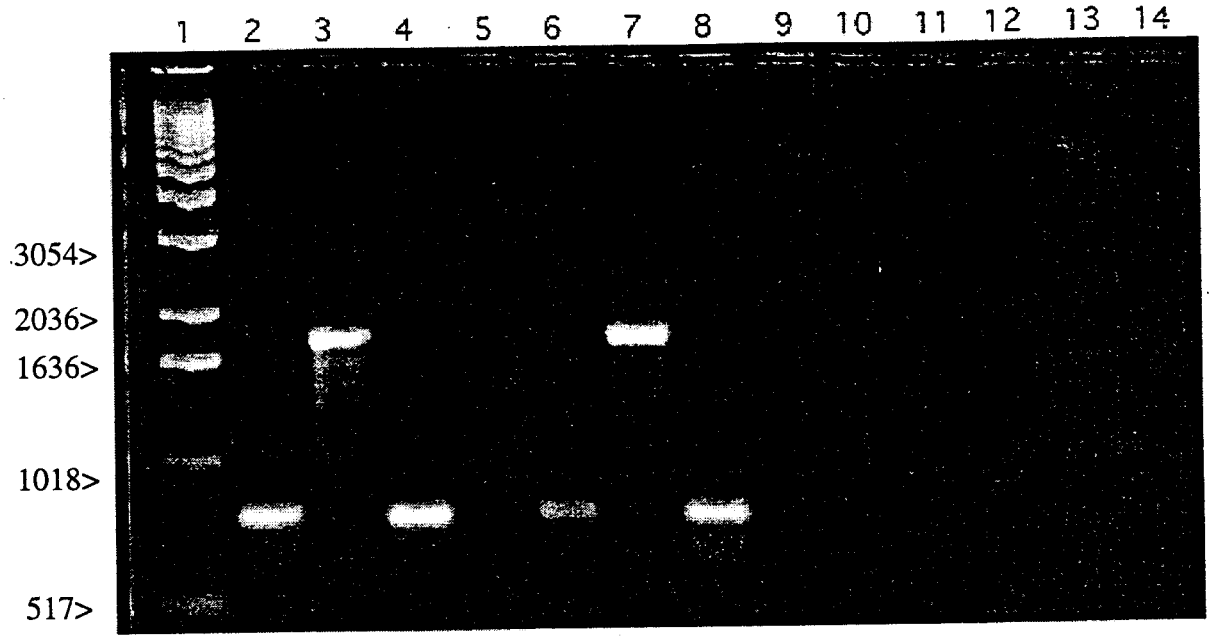



**Figure V.4. PCR Analysis of Line SEMT15-15 utilizing primers directed toward the *PVYcp*, *cry3A*, and *nptII* genes.**

Photograph of agarose gel electrophoresis of PCR products following amplification of genomic DNA using primers for specific genetic elements.

**Lane 1** contains molecular weight markers.

**Lanes 2-4** contain PCR products from amplification of control genomic DNA from nontransformed tissue from the Shepody variety plus 150 ng of PV-STMT15 plasmid DNA.

**Lanes 6-8** contain PCR products from amplification of SEMT15-15 genomic DNA.

**Lanes 2 and 6** contain PCR products from amplification with primers (PVY1-PVY2) directed toward the *PVYcp* gene.

**Lanes 3 and 7** contain PCR products from amplification with primers directed toward the *cry3A* gene (B1-B2).

**Lanes 4 and 8** contain PCR products from amplification with primers (N1-N2) directed toward the *nptII* gene.

**Lanes 5 and 9-14** are empty.

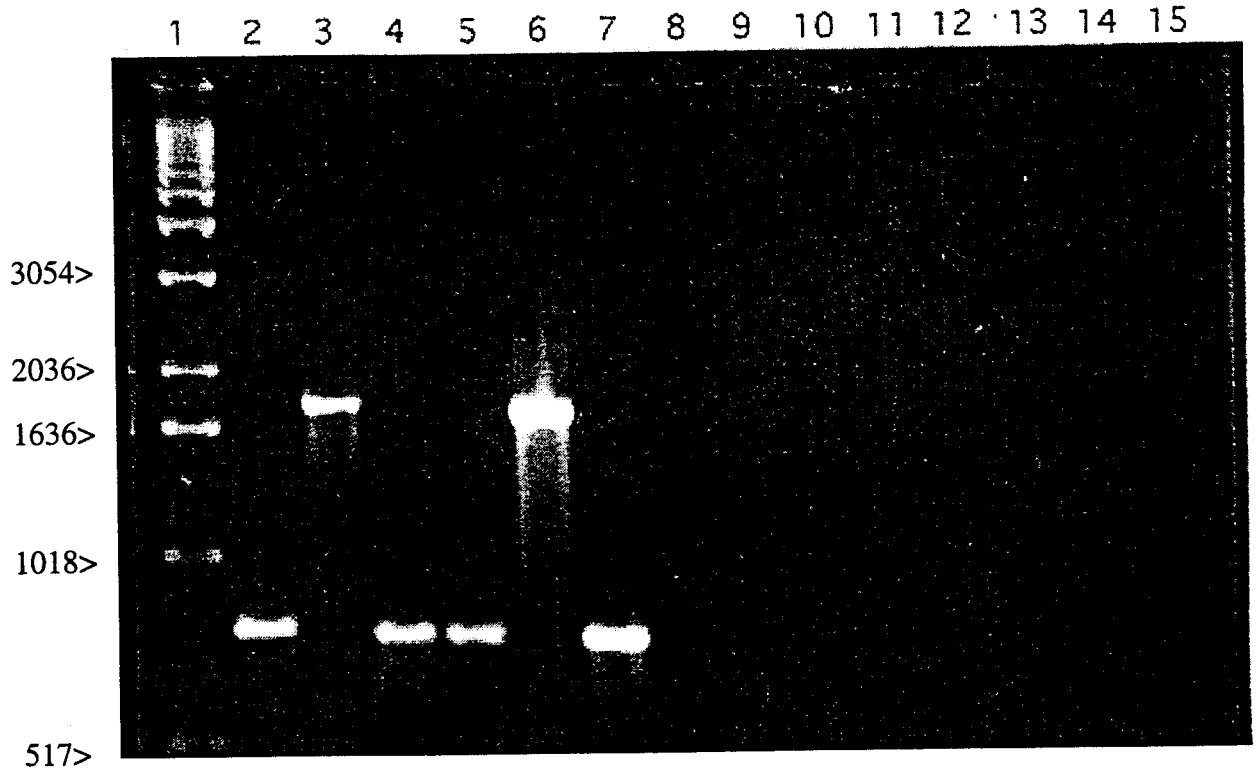



**Figure V.5. PCR Analysis of Line RBMT15-101 utilizing primers directed toward the *PVYcp*, *cry3A*, and *nptII* genes.**

Photograph of agarose gel electrophoresis of PCR products following amplification of genomic DNA using primers for specific genetic elements.

**Lane 1** contains molecular weight markers.

**Lanes 2-4** contain PCR products from amplification of control genomic DNA from nontransformed Russet Burbank tissue plus 150 ng of PV-STMT15 plasmid DNA.

**Lanes 5-7** contain PCR products from amplification of RBMT15-101 genomic DNA.

**Lanes 8-10** contain PCR products from amplification of control genomic DNA from nontransformed tissue.

**Lanes 2, 5, and 8** contain PCR products from amplification with primers (PVY1-PVY2) directed toward the *PVYcp* gene.

**Lanes 3, 6, and 9** contain PCR products from amplification with primers (B1-B2) directed toward the *cry3A* gene.

**Lanes 4, 7 and 10** contain PCR products from amplification with primers (N1-N2) directed toward the *nptII* gene.

**Lanes 11-15** are empty.

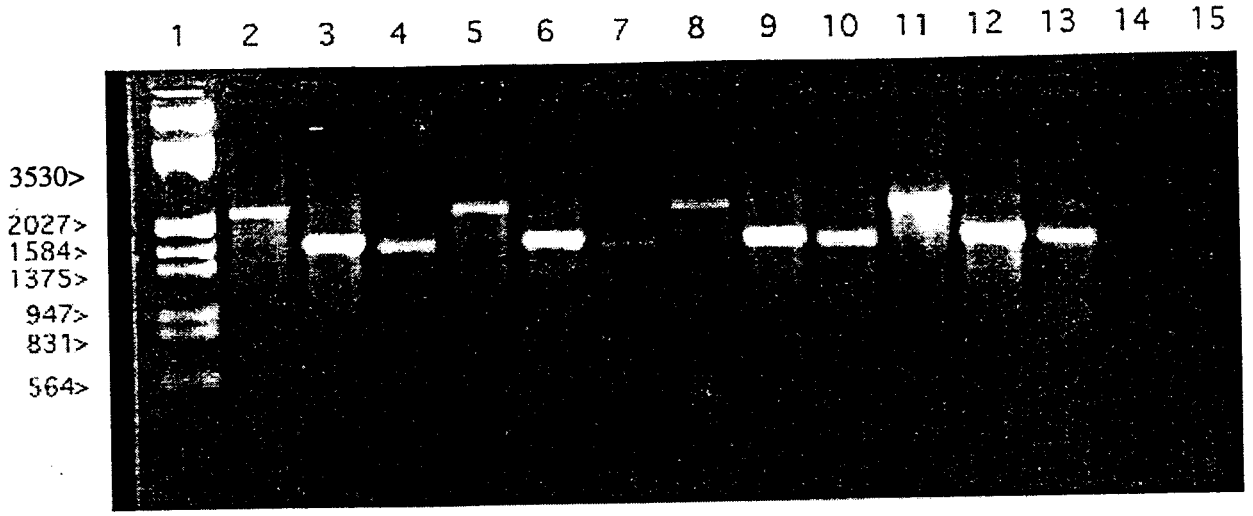



**Figure V.6. PCR Analysis of Genetic Element Linkage in Lines SEMT15-02, SEMT15-7 and SEMT15-15.**

***Note: Line SEMT15-7 has been withdrawn from this petition.***

Photograph of gel electrophoresis of PCR products following amplification of genomic DNAs using specific primers to test linkage between integrated genetic elements.
**Lane 1** contains molecular weight markers.
**Lanes 2-4** contain the PCR products from amplification of SEMT15-02 genomic DNA.
**Lanes 5-7** contain the PCR products from amplification of SEMT15-7 genomic DNA.
**Lanes 8-10** contain the PCR products from amplification of SEMT15-15 genomic DNA.
**Lanes 11-13** contain the PCR products from amplification of PV-STMT15 DNA.
**Lanes 2, 5, 8, and 11** contain PCR products from amplification with primers (PVY1-BC1) to ascertain linkage between *PVYcp* and *cry3A* genes.
**Lanes 3, 4, 6, 7 9, 10, 12, 13** contain PCR products from amplification with primers (PB2-SS1 and SS2-N1) to ascertain linkage between *cry3A* and *nptII* genes.
**Lanes 14 and 15** are empty.

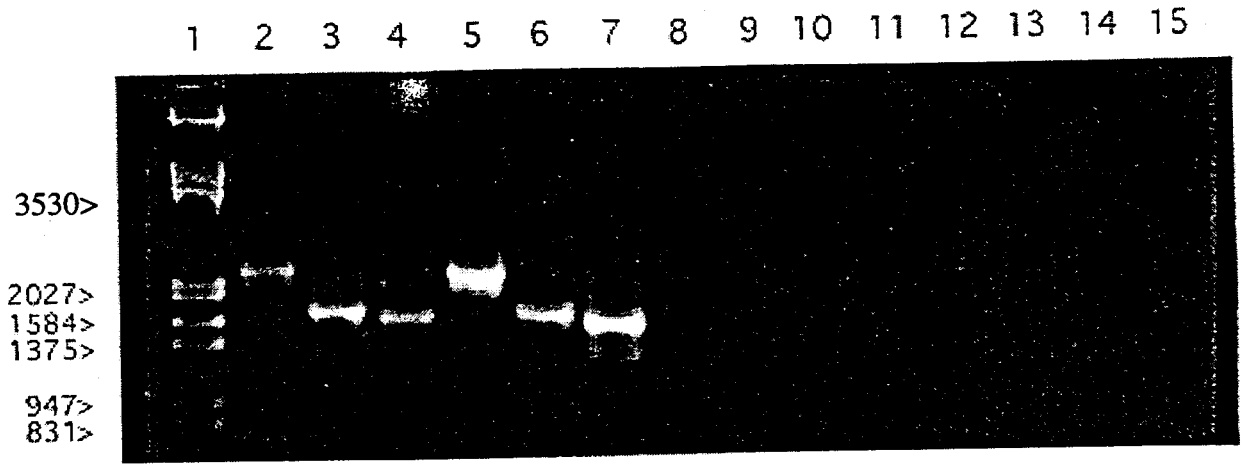



**Figure V.7. PCR Analysis of Genetic Element Linkage in Line RBMT15-101.**

Photograph of gel electrophoresis of PCR products following amplification of genomic DNAs using specific primers to test linkage between integrated genetic elements.

**Lane 1** contains molecular weight markers.

**Lanes 2-4** contain the PCR products from amplification of RBMT15-101 genomic DNA.

**Lanes 5-7** contain the PCR products from amplification of PV-STMT15 DNA.

**Lanes 2 and 5** contain PCR products from amplification with primers (PVY1-BC1) to ascertain linkage between *PVYcp* and *cry3A* genes.

**Lanes 3, 4, 6, and 7** contain PCR products from amplification with primers (PB2-SS1 and SS2-N1) to ascertain linkage between *cry3A* and *nptII* genes.

**Lanes 8-15** are empty.

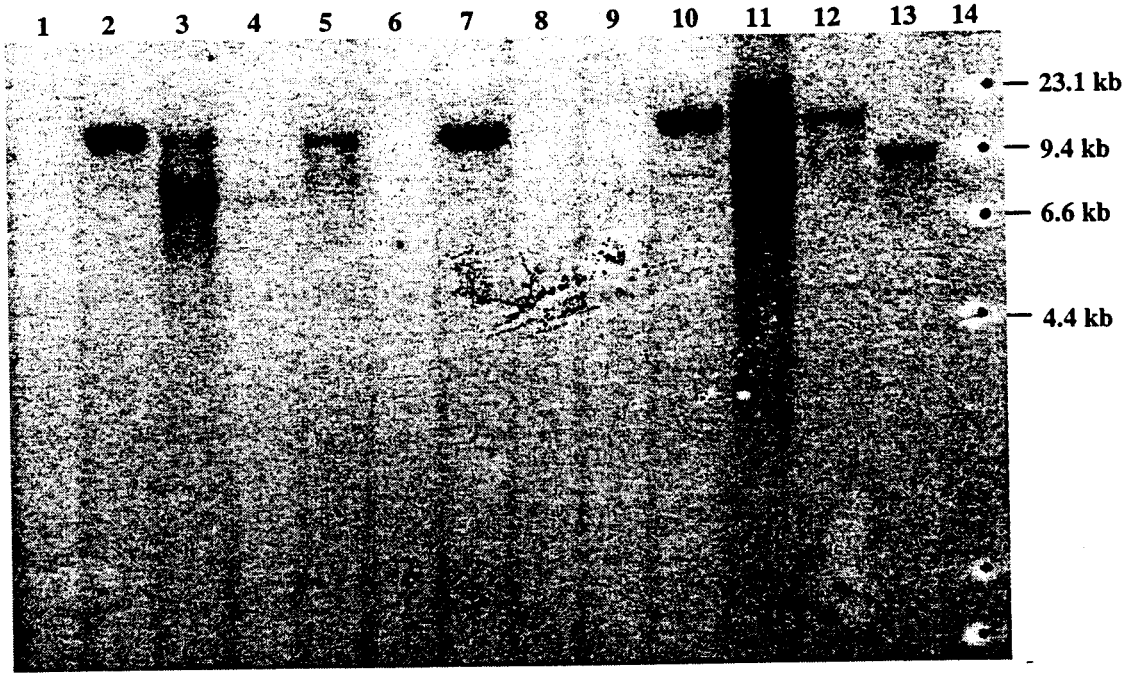



**Figure V.8. Southern blot analysis of NewLeaf Y lines probed with the *aad* gene.**

***Note: Lanes 4, 8-13 contain data from lines which have been withdrawn from this petition.***

Genomic DNA was extracted from leaf tissue of field grown plants. DNA samples were digested with *Bam*HI endonuclease, electrophoresed through a 0.8% agarose gel, and blotted onto charged nylon membrane. The nylon membrane was probed with the *aad* gene labeled with $^{32}P$ dCTP before detection on Kodak X-OMAT imaging film.
**Lane 1** contains 10 µg of genomic DNA from control Shepody plants.
**Lane 2** contains 10 µg of genomic DNA from control Shepody plants spiked with 15 pg of PV-STMT15 plasmid DNA.
**Lanes 3 through 5** contain 10 µg of genomic DNA from SEMT15-02, SEMT15-7, and SEMT15-15 plants, respectively.
**Lane 6** contains 10 µg of genomic DNA from control Russet Burbank plants.
**Lane 7** contains genomic 10 µg of DNA from control Russet Burbank plants spiked with 15 pg of PV-STMT15 plasmid DNA.
**Lanes 8** through 13 contain 10 µg of genomic DNA from HiLite plants, which are not the subject of this petition.
**Lane 14** contains the molecular weight markers.

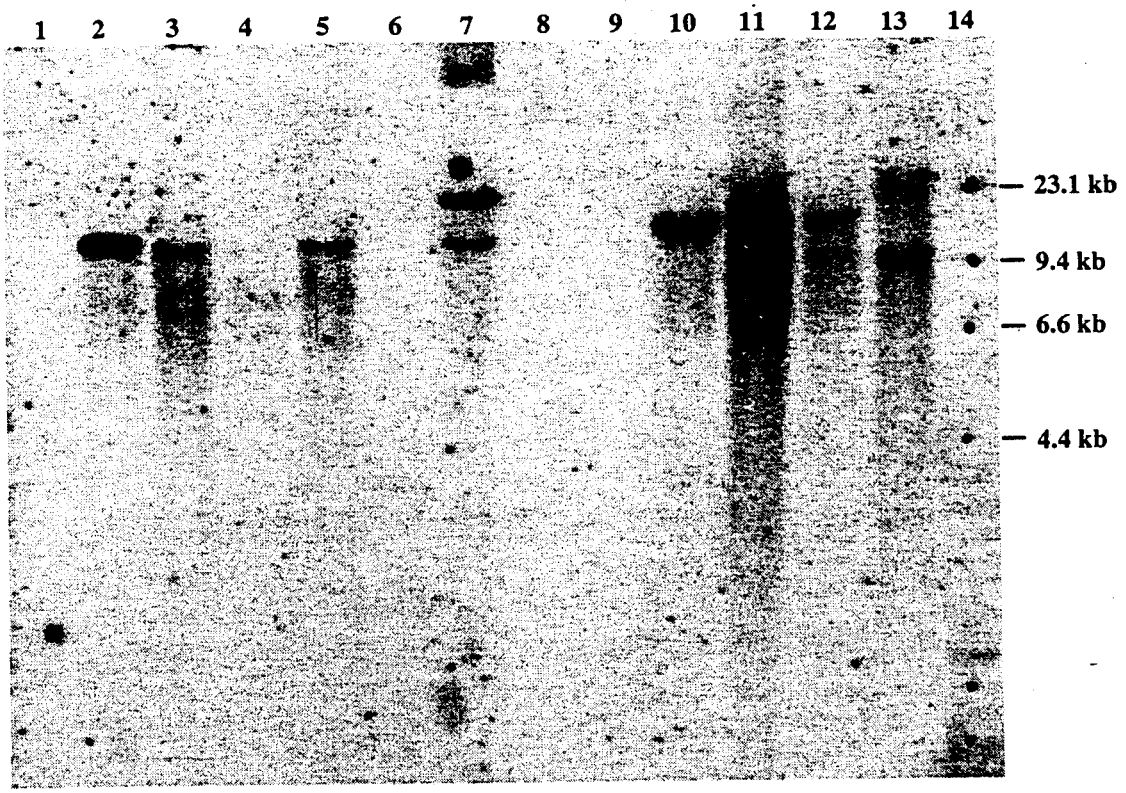



**Figure V.9. Southern blot analysis of NewLeaf Y lines probed with *oriV*.**

***Note: Lanes 4, 9-13 contain data from lines which have been withdrawn from this petition.***

Genomic DNA was extracted from leaf tissue of field grown plants. DNA samples were digested with *Bam*HI endonuclease, electrophoresed through a 0.8% agarose gel, and blotted onto charged nylon membrane. The nylon membrane was probed with the oriV genetic element labeled with $^{32}$P dCTP before detection on Kodak X-OMAT imaging film.

**Lane 1** contains 10 µg of genomic DNA from control Shepody plants.

**Lane 2** contains 10 µg of genomic DNA from control Shepody plants spiked with 15 pg of PV-STMT15 plasmid DNA.

**Lanes 3 through 5** contain 10 µg of genomic DNA from SEMT15-02, SEMT15-7, and SEMT15-15 plants, respectively.

**Lane 6** contains 10 µg of genomic DNA from control Russet Burbank plants.

**Lane 7** contains genomic 10 µg of DNA from control Russet Burbank plants spiked with 15 pg of PV-STMT15 plasmid DNA.

**Lane 8** contains 10 µg of genomic DNA from RBMT15-101 plants.

**Lanes 9** through 13 contain 10 µg of genomic DNA from HiLite plants, which are not the subject of this petition.

**Lane 14** contains the molecular weight markers.

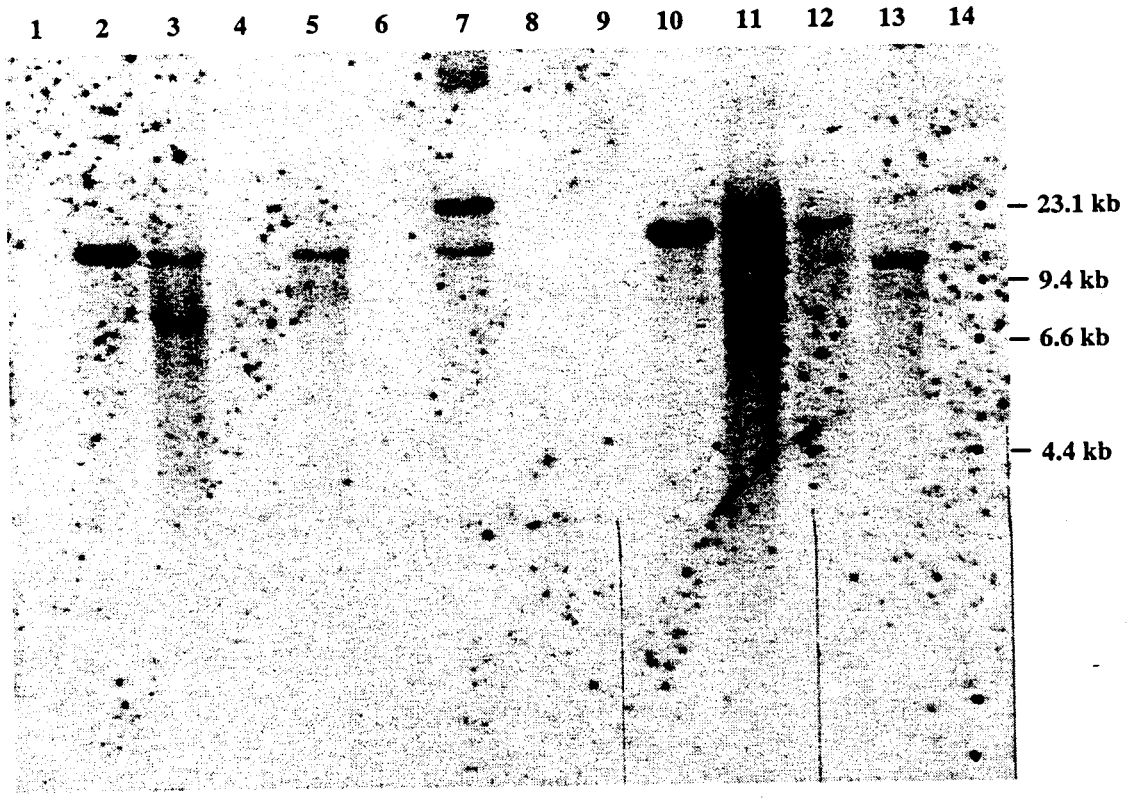



**Figure V.10. Southern blot analysis of NewLeaf Y lines probed with *ori322*.**

***Note: Lanes 4, 9-14 contain data from lines which have been withdrawn from this petition.***

Genomic DNA was extracted from leaf tissue of field grown plants. DNA samples were digested with *Bam*HI endonuclease, electrophoresed through a 0.8% agarose gel, and blotted onto charged nylon membrane. The nylon membrane was probed with the *ori322* genetic element labeled with $^{32}$P dCTP before detection on Kodak X-OMAT imaging film.

**Lane 1** contains 10 µg of genomic DNA from control Shepody plants.
**Lane 2** contains 10 µg of genomic DNA from control Shepody plants spiked with 15 pg of PV-STMT15 plasmid DNA.
**Lanes 3 through 5** contain 10 µg of genomic DNA from SEMT15-02, SEMT15-7, and SEMT15-15 plants, respectively.
**Lane 6** contains 10 µg of genomic DNA from control Russet Burbank plants.
**Lane 7** contains genomic 10 µg of DNA from control Russet Burbank plants spiked with 15 pg of PV-STMT15 plasmid DNA.
**Lane 8** contains 10 µg of genomic DNA from RBMT15-101 plants.
**Lanes 9 through 13** contain 10 µg of genomic DNA from HiLite plants, which are not the subject of this petition.
**Lane 14** contains the molecular weight markers.

## 2. Characterization of gene products; expressed proteins and PVYcp mRNA

### a) Cry3A and NPTII

A protein characterization study established that the Cry3A protein produced in the transformed lines is equivalent to the previously characterized *E. coli*-produced Cry3A reference standards and equivalent to the Cry3A protein produced in previously registered plant lines (Rogan *et al.*, 1997). Since NPTII protein could not be detected using highly sensitive immunological techniques, the NPTII protein characterization is based on the identity of the *nptII* gene, which was introduced into *E. coli* and used to produce the NPTII reference standard.

### b) PVY Coat Protein (PVYcp) and mRNA

The PVY coat protein gene (*PVYcp*) was obtained from a PVY-infected potato in Washington State, USA by Dr. Pete Thomas, USDA-ARS. The gene sequence engineered into the NewLeaf Y potato lines RBMT15-101, SEMT15-02 and SEMT15-15 is identical to the native viral gene; therefore, the PVY coat protein and mRNA produced is expected to be identical to the native viral coat protein and viral mRNA. Since PVYcp could not be detected using highly sensitive immunological techniques, the PVYcp characterization is based on the identity of the *PVYcp* gene which was introduced into these potato lines. Exemption from the requirement of a tolerance under the Federal Insecticide Fungicide and Rodenticide Act (FIFRA) has been granted by the U.S. Environmental Protection Agency (EPA) for the potato virus Y coat protein in all plants and raw agricultural commodities (EPA, 1997).

## 3. Levels of expressed proteins and gene products

The NewLeaf Y lines, control Russet Burbank and control Shepody plant lines were grown in field trials during the summer of 1995, 1996 and 1998 at several locations in the United States (Aberdeen, ID; Caldwell, ID; Coloma, WI; Echo, OR; Hermiston, OR; Homestead, FL; Island Falls, ME; Lakeview, MI) and Canada (Lethbridge, Alberta; New Denmark, New Brunswick; Sainte Foy, Quebec). The locations selected represent environments in which potatoes are grown for commercial tuber production in the U.S. and Canada. For field trials conducted in 1995 and 1996, leaf samples were collected at approximately ten weeks post-planting, tuber samples were collected at harvest. The field trials were performed using a randomized complete block design with four, six, eight or ten replicates per line. Samples were obtained from at least four plots from each site for determination of Cry3A, NPTII, and PVYcp protein expression levels. In order to evaluate season-long expression of the Cry3A protein, additional field trials were performed in 1998 at two sites in Canada (New Maryland, New Brunswick, Canada and Saint Croix de Lotbiniere, Quebec, Canada). At these sites, leaf samples were obtained from NewLeaf Y plants at 6, 10 and 14 weeks post planting. Cry3A expression levels were determined on leaf tissue obtained from each genetically modified and control line. Expression levels were determined in potato tissue extracts using validated enzyme linked immunosorbent assays (ELISAs), which are highly specific sandwich assays developed and validated to determine the concentration of the respective protein in extracts derived from potato tissues. Detailed methodology and specific results by field site and line number can be found for the Cry3A and NPTII proteins in Appendices II and III. The control lines for PVY coat protein determination were grown in the field or a growth chamber. The expression level for each protein is summarized below.

### a) Cry3A

Mean Cry3A protein expression level of line RBMT15-101 among all sites for samples obtained in 1995 and 1996 was 20.44 and 0.246 µg/g tissue fresh weight for leaf and tuber, respectively. The expression of the Cry3A protein in leaf tissue obtained from 1998 Canadian field sites was greatest early in the season and showed a decline over the growing season. Cry3A expression was 60, 34 and 24 µg/g tissue fresh weight for samples taken at 6, 10 and 14 weeks post planting, respectively. The Cry3A protein expression levels for line RBMT15-101 correspond to a range of 0.13-0.38% of foliage protein and 0.0012% of tuber protein, using total protein levels of 1.6 and 2.0% for foliage and tuber fresh weight, respectively.

Mean Cry3A protein expression levels of lines SEMT15-02 and SEMT15-15 among all sites ranged from 22.5 to 28.5 µg/g tissue fresh weight for leaf tissue and from 0.126 to 0.194 µg/g tissue fresh weight for tuber tissue.

Expression of the Cry3A protein in leaf tissue obtained from 1998 Canadian field sites in SEMT15-02 was the greatest in samples obtained 6 weeks post-planting and declined over the length of the growing season. The expression level was 51, 24 and 10 μg/g tissue fresh weight for samples taken at 6, 10 and 14 weeks post planting, respectively. Expression of the Cry3A protein in leaf tissue obtained from 1998 Canadian field sites in Line No. SEMT15-15 was also greatest at 6 weeks post planting and declined over the length of the growing season. Over-season expression in this line was 63, 37 and 9 μg/g tissue fresh weight for samples taken at 6, 10 and 14 weeks post planting, respectively. The Cry3A protein expression levels for these lines correspond to a range of 0.06 to 0.39% of total foliage protein and 0.0006 to 0.001% of total tuber protein, using total protein levels of 1.6 and 2.0% for foliage and tuber fresh weight, respectively.

Overall, the Cry3A protein expression in NewLeaf Y lines is comparable to the corresponding expression levels in tissue of previously deregulated NewLeaf Atlantic plants that utilize the same promoter (the *Arabidopsis thaliana* ribulose-1,5-bisphosphate carboxylase small subunit *ats1A* promoter) to regulate expression of the c*ry3A* gene. Since the LD99 for the Cry3A protein is approximately 0.3 ppm (Perlak *et al.*1993), the decrease in expression of the Cry3A protein over the length of the growing season has no impact on the ability of these lines to provide season long control of CPB.

Furthermore, observations taken of beetle feeding damage on the foliage of these plants confirmed that expression levels of the Cry3A protein in these lines were highly effective at controlling CPB throughout the growing season. In conjunction with determination of expression levels, plants were also rated for incidence of beetle feeding damage at four different time points throughout the 1998 Canadian growing season. The results of these observations are presented in Table V.3. No feeding damage was noted at any of the time points in the plots that contained genetically modified NewLeaf Y lines, whereas measurable feeding damage was noticed in the corresponding control plots.

**Table V.3. Degree of defoliation of genetically modified NewLeaf Y Lines: RBMT15-101, SEMT15-02, SEMT15-15 and non-modified potato varieties over the length of the 1998 growing season by Colorado potato beetle.**

| | Weeks Post Planting | | | |
|---|---|---|---|---|
| Line (Replicates) | 9 | 10 | 11 | 13 |
| RB control (1) | 1[1] | 2 | 2 | 2 |
| RBMT15-101 (1) | 0 | 0 | 0 | 0 |
| SE control (1) | 1 | 1 | 1 | 1 |
| SEMT15-02 (1) | 0 | 0 | 0 | 0 |
| SEMT15-15 (1) | 0 | 0 | 0 | 0 |
| RB control (2) | 0 | 1 | 1 | 1 |
| RBMT15-101 (2) | 0 | 0 | 0 | 0 |
| SE control (2) | 0 | 0 | 1 | 1 |
| SEMT15-02 (2) | 0 | 0 | 0 | 0 |
| SEMT15-15 (2) | 0 | 0 | 0 | 0 |
| RB control (3) | 1 | 1 | 1 | 1 |
| RBMT15-101 (3) | 0 | 0 | 0 | 0 |
| SE control (3) | 2 | 2 | 2 | 2 |
| SEMT15-02 (3) | 0 | 0 | 0 | 0 |
| SEMT15-15 (3) | 0 | 0 | 0 | 0 |
| RB control (4) | 1 | 1 | 1 | 1 |
| RBMT15-101 (4) | 0 | 0 | 0 | 0 |
| SE control (4) | 2 | 2 | 2 | 2 |
| SEMT15-02 (4) | 0 | 0 | 0 | 0 |
| SEMT15-15 (4) | 0 | 0 | 0 | 0 |

[1]Rating scale used:

0=no defoliation,
1=2-60% of plants with leaflets slightly damaged
2=2% of plants with at least 1 compound leaf with more than 50% defoliation
3=2-9% of plants with at least 1 stem with more than 50% defoliation
4=1-24% of plants at least 1 stem with more than 50% defoliation
5=25-49% of plants at least 1 stem with more than 50% defoliation
6=50-74% of plants at least 1 stem with more than 50% defoliation
7=75-90% of plants at least 1 stem with more than 50% defoliation

## b) NPTII

The levels of NPTII protein in leaf and tuber tissue of all NewLeaf Y potato lines were below the detection limits of the assay. The detection limit of the NPTII ELISA assay was determined to be 2.7 ng/g tissue fresh weight. The NPTII protein levels in leaves and tubers of the NewLeaf Y Russet Burbank and Shepody lines are much lower than previously deregulated NewLeaf® cvs. Russet Burbank, Atlantic and Superior potato plants. The difference in NPTII protein expression reflects the strength of the promoters used in these plants. Previously registered lines utilize the cauliflower mosaic virus 35S promoter to regulate the *nptII* gene expression; whereas, the NewLeaf Y transgenic lines utilize the nopaline synthase promoter (NOS).

## c) PVYcp

To determine the expression level of PVYcp in genetically modified potato plants, a western blotting procedure was developed. This procedure was used to estimate the level of PVYcp in leaf samples obtained from field grown plants. For comparison, PVYcp expression was also estimated in PVY infected non-modified plants of the same varieties using a commercially available sandwich ELISA.

PVYcp derived from purified virions produced two major bands on the western blot (Figure V.11, lanes 2 and 3). The lower band has an approximate molecular weight of 32 kD and corresponds to the theoretical molecular weight predicted for PVYcp monomer (Lawson, *et al.* 1990), the upper band is a dimer composed of two 32 kD monomer subunits and has an approximate molecular weight of 64 kD. Minor multi-meric forms of the coat protein were also observed at higher protein loading concentrations. The multiple bands observed on the western blot are due to the inability to completely reduce the coat protein in the virion to monomers. The same bands were also observed on SDS-PAGE gels stained with coomassie (data not shown). Western blot analysis of extracts prepared from potato plants infected with PVY also showed multiple bands with major bands at the expected molecular weights for the monomer and dimer (Figure V.11). Additional minor higher, lower and intermediate molecular weight bands were also observed and concluded to be non-specific bands.

On the basis of western blot analysis, PVY coat protein expression levels were estimated to be less than 2 ng/mg fresh leaf tissue (< 0.125% total soluble protein) in leaf extracts of the transgenic plant lines RBMT15-101, SEMT15-02, SEMT15-15, and a HiLite line (not the subject of this petition). In comparison, PVYcp levels in infected potato plants of the same varieties ranged from 24 to 488 µg/g tissue fresh weight, as determined by ELISA (Appendix IV). Thus, the amount of PVY coat protein in PVY-infected tissues is at least 12 to 244-fold greater than in NewLeaf Y plant lines. Details of the western blot analysis can be found in Appendix IV.

The *PVYcp* gene product was further characterized by Northern blot analysis. For this analysis, leaf samples were obtained from genetically modified NewLeaf Y potato lines.: RBMT15-101, SEMT15-02 and SEMT15-15 from NatureMark's Island Falls facility (Island Falls, ME). Control, non-modified leaf tissues were obtained from greenhouse grown plants at Monsanto's Chesterfield Village site (Chesterfield, MO). Leaves were harvested from healthy plants, immediately placed on dry ice and shipped on dry ice to Monsanto (Chesterfield, MO). Samples were stored frozen at Monsanto at -80°C until analysis. For extraction of RNA, approximately 0.5 g of powdered leaf tissue was suspended in 10 mL of TRIZOL® Reagent (Gibco BRL, Life Technologies, Gaithersburg, MD) and total RNA was isolated from the tissue using the standard TRIZOL® protocol in accordance with the manufacturer's instructions. For Northern blot analysis, approximately 15 µg of total RNA was separated on an 1.2% agarose/formaldehyde gel and transferred to Hybond N (Amersham, Arlington Heights, IL) according to the method described by Sambrook *et al.* (1989). Membranes were probed with a PCR-generated fragment of the PVY gene. In addition, membranes were also probed with a PCR-generated fragment of the ubiquitin gene which was used to assess the integrity of the RNA and the relative amount of mRNA loaded per line. PCR fragments were labeled with $\alpha^{32}$P using an $\alpha^{32}$P-dCTP random primer kit in accordance with the manufacturers instructions (Gibco BRL, Life Technologies, Gaithersburg, MD). After hybridization, the membranes were washed in several changes of SSC buffer. The final wash was done in 0.1 X SSC at 65°C. and exposed to film (48 hours for ubiquitin and 120 hours for PVY). All exposures were done at -80°C.

**Figure V.11. Western blot of NewLeaf Y and PVY infected plants extracts probed with anti-PVY serum.**

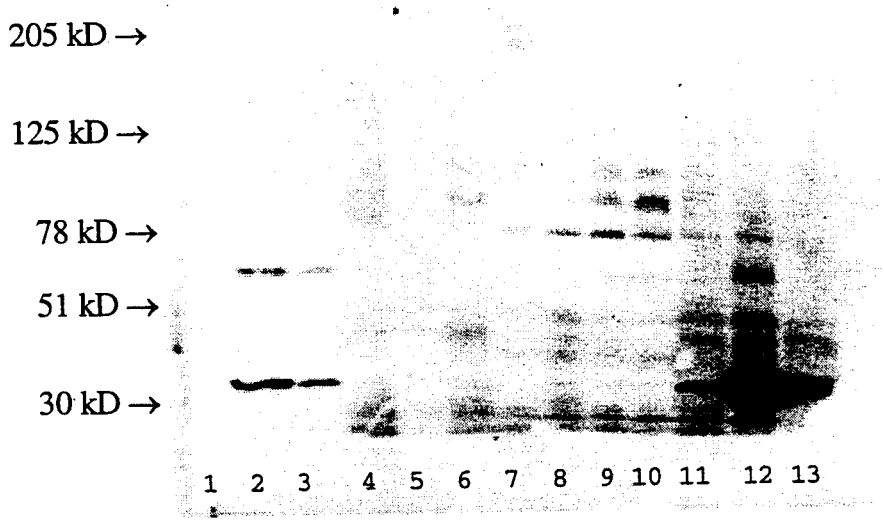



**Figure V.11.** Western blot of PVYcp derived from density gradient purified PVY virions, PVY infected and non-infected Russet Burbank, Hi-Lite, and Shepody potato varieties or genetically modified NewLeaf Plus Russet Burbank, Hi-Lite, and Shepody lines RBMT 15-101, HLMT 15-46 (not the subject of this petition), SEMT 15-2, SEMT 15-15 . Lane Nos. contain: (1) molecular weight markers, (2) 5 ng PVYcp virion, (3) 2 ng PVYcp virion, (4) non-modified Russet Burbank variety, (5) non-modified Hi-Lite variety, (6) non-modified Shepody variety, (7) genetically modified potato Line No. RBMT15-101, (8) genetically modified line No. HLMT15-46 not subject of this petition, (9) genetically modified potato Line No. SEMT15-02, (10) genetically modified potato Line No. SEMT15-15, (11) PVY-infected Russet Burbank, (12) PVY-infected Hi-Lite, and (13) PVY infected Shepody. Leaf tissue was obtained from potato plants grown in growth chambers and in 1996 field trials. Tissue extraction and western blot conditions are described in the text. Samples were extracted at a 1:10 tissue fresh weight to volume ratio, therefore the protein obtained from approximately 1 mg of tissue fresh weight was loaded per lane for all tissue extracts. The membrane was probed with PVYcp-antigen-affinity-purified antiserum produced by immunization of rabbits with density-gradient purified PVY virions. Expression levels were estimated by comparing band intensities in lanes loaded with extracts derived from genetically modified plants in comparison to lanes loaded with potato virus Y density-gradient purified whole virions. PVYcp was not detected by western blot analysis in genetically modified lines or non-modified, non-infected tissues obtained from the same varieties. The detection limit for the western blot was estimated to be approximately 2 ng/mg tissue fresh weight (2 ppm).

The results from Northern blot analysis of genetically modified NewLeaf Y lines RBMT15-101, SEMT15-02 and SEMT15-15 are presented in Figure V.12. Through the use of the ubiquitin specific probe, the RNA samples used for this analysis contained intact mRNA which was seen as a distinct band of the expected size (for each genetically modified and control line) with some products of its degradation observed as smearing in all lines and control. On the basis of band intensity observed in each lane when the membrane was probed with the ubiquitin probe, it can be concluded that comparable amounts of RNA were loaded in the control lanes in comparison to the genetically modified lines. Comparison of the banding patterns observed for the membranes probed with either the ubiquitin or the PVY probe show that the hybridization is highly specific since no PVYcp mRNA product was detected in RNA isolated from control samples. Visual analysis of the band pattern observed when the blot was hybridized with the PVY probe, leads to the conclusion that all lines produce a full length PVYcp mRNA of the expected size (approximately 0.8 Kb). The lower size bands observed when the blot was hybridized with the PVY probe are assessed to be due to minor degradation of the RNA due to sample handling and extraction.

**Figure V.12.** **Northern blot analysis of PVY coat protein (a) and ubiquitin (b) mRNA produced by NewLeaf Y genetically modified and non-modified potato varieties.**

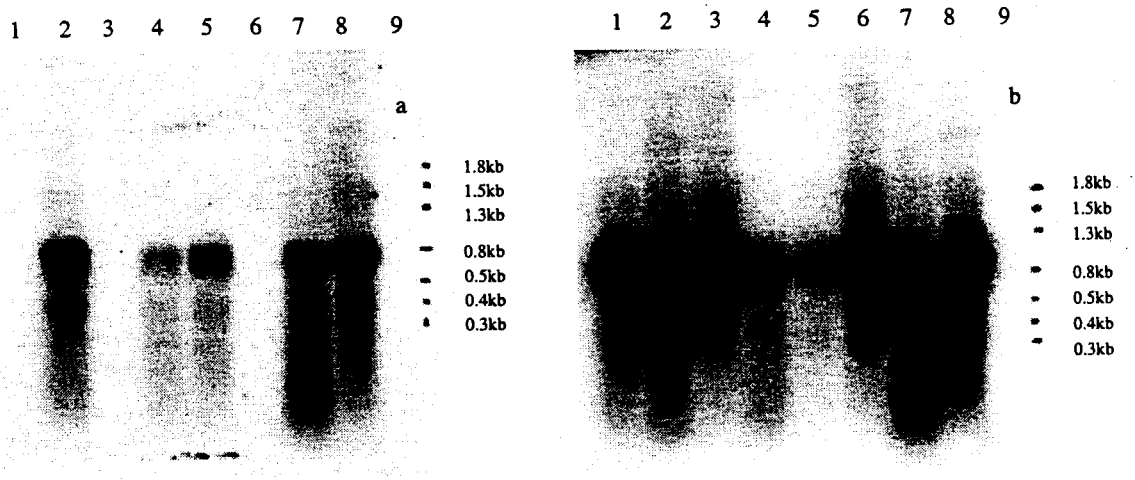



**Figure V.12: Panel a.** Northern blot analysis of NewLeaf Y genetically modified and non-modified potato varieties, probed with random primer labeled PVY coat protein probe. Lanes contain RNA (15 μg) isolated from genetically modified NewLeaf Y and non-modified potato varieties. Lane Nos.: (1) non-modified Russet Burbank; (2) genetically modified Russet Burbank NewLeaf Y Line No. RBMT15-101; (3) non-modified Hi-Lite; (4) genetically modified Hi-Lite HLMT15-46 (not the subject of this petition); (5) Hi-Lite HLMT15-298 (not the subject of this petition); (6) non-modified Shepody; (7) genetically modified Shepody NewLeaf SEMT15-02; (8) genetically modified Shepody SEMT15-15; and (9) molecular size standards (0.16 1.77 kb ladder, Gibco BRL, cat no.15623-010). On the basis of the band pattern observed when the blot was hybridized with the PVY probe, it can be concluded that all lines produce a full length mRNA of the expected size (approximately 0.8 kb).

**Panel b.** Northern blot analysis of NewLeaf Y genetically modified and non-modified potato varieties, probed with ubiquitin probe. Lanes contain RNA (15 μg) isolated from genetically modified NewLeaf Y and non-modified potato varieties. Lane Nos.: (1) non-modified Russet Burbank; (2) genetically modified Russet Burbank RBMT15-101; (3) non-modified Hi-Lite; (4) genetically modified Hi-Lite HLMT15-46 (not the subject of this petition), (5) Hi-Lite HLMT15-298 (not the subject of this petition); (6) non-modified Shepody; (7) genetically modified Shepody SEMT15-02; (8) genetically modified Shepody SEMT15-15; and (9) molecular size standards (0.16 1.77 kb ladder, Gibco BRL, cat no.15623-010). The RNA samples used for this analysis contained intact mRNA which was seen as a distinct band of the expected size and some products of its degradation which were observed as smearing in all lines and control. On the basis of band intensity observed in each lane, it can be concluded that equivalent or greater amounts of RNA were loaded in the control lanes in comparison to the genetically modified lines and that hybridization is highly specific since no PVYcp mRNA product was detected in RNA isolated from control samples.

### d) AAD

The aminoglycoside adenylyltransferase (*aad*) gene was detected in lines SEMT15-02 and SEMT15-15. The purpose of this gene is to provide a selectable marker for bacterial transformation. It does not serve any intended function in the transgenic plant. This bacterial gene is regulated by a bacterial promoter and therefore is not expected to be expressed in the transgenic potato lines. As expected, when these transgenic lines were assayed for the presence of AAD protein by ELISA, no AAD protein was detected (Appendix V).

## *B. Agronomic Performance*

Commercial NewLeaf Y potato lines RBMT15-101, SEMT15-02 and SEMT15-15 were evaluated in the field from 1995 - 1997 under USDA notifications, listed in Section I (page 7). In 1996, the transgenic lines were evaluated for agronomic performance by measuring stand, vigor, yield, hollow heart, brown center, and specific gravity. The data demonstrate that the transgenic lines are agronomically equivalent to nontransformed potatoes (Appendix VI). The

lines selected for commercialization are agronomically comparable to nonmodified commercial potato lines and are additionally highly resistant to the Colorado Potato Beetle and the Potato Virus Y. During field trial evaluation of the transgenic potato lines, the potato plants were visually monitored for any unusual susceptibility to potato insect pests or pathogens (Table V.4.). Throughout the growing season, the insect pest and diseases noted varied depending on the location. Some level of leafroll disease, early blight, early dying, and canker were observed in all trial locations throughout the U.S. Leafhoppers were seen, but mostly in the midwestern states (MN and WI). However, except for the intended CPB and PVY resistance traits in the transgenic lines, no differences in susceptibility to insect pests or diseases between the transgenic and control lines were noted.

**Table V.4. Insect and disease symptoms visually monitored during monthly scouting of transgenic potato field trials.**

| Organism or Pathogen | Disease or Symptoms |
|---|---|
| **Insect** | |
| *Emposasca fabae* (Potato leafhopper) | Leaf feeding damage |
| *Epitrix* species (Flea Beetle) | Shotholes in leaves |
| *Leptinotarsa decemlineata* (Colorado Potato Beetle) | Defoliation |
| *Limonius californicus* (Wireworm) | Bored holes in tubers and shoots |
| *Ostrinia nubilalis* (European Corn Borer) | Severe vine wilting above point of injury |
| *Paratrioza cockerelli* (Potato Psyllid) | Yellows |
| *Phthorimaea operculella* (Tuberworm) | Foliar and tuber damage |
| Various aphid species | Leaf suckling damage |
| **Virus and Virus-Like** | |
| Aster yellows MLO | Purple top disease |
| Potato leafroll virus | Rolling of leaves and net necrosis |
| Potato spindle tuber viroid | Potato spindle tuber disease |
| Potato virus A,M,X,Y | Mosaic symptoms |
| Tobacco rattle virus | Stem mottling |
| **Bacteria and Fungi** | |
| *Erwinia carotovora* | Blackleg, Aerial stem rot and Tuber soft rot |
| *Corynebactium sepedonicum* | Bacterial ring rot |
| *Alternatia solani* | Early blight |
| *Fusarium sp.* | Dry rot |
| *Phytophthora and Pythium* | Water rot |
| *Phytophthora infectans* | Late blight |
| *Rhizoctonia solani* | Canker |
| *Sclerotinia sclerotiorum* | Sclerotinia stalk rot |
| *Streptomyces scabies* | Scab |
| *Verticillium spp.* | Early dying |
| **Nematodes** | |
| *Globodera rostochiensis* | Cysts |
| *Meloidogyne sp.* | Root knot |
| *Paratrichodorus sp.* | Stubby root |
| *Pratylenchus sp.* | Root lesions |

## *C. Compositional Analysis*

Monsanto has completed the consultation with FDA on the compositional analysis of potato tubers produced from the transformed lines. The data demonstrates and FDA concurs that the potato tubers from the NewLeaf Y transgenic lines are substantially equivalent to the nontransformed parental potato cultivars with regard to

nutritional composition (total solids, sugars, glycoalkaloid, and vitamin C) and proximate composition (soluble protein, moisture, total fat, ash, crude fiber, carbohydrates, and calories). The data submitted to FDA is attached as Appendix VIII.

# VI. ENVIRONMENTAL CONSEQUENCES OF INTRODUCTION

## *A. Weediness*

NewLeaf Y potato lines (lines RBMT15-101, SEMT15-02 and SEMT15-15) have been field tested under USDA permits and/or notification since 1993. These plants have not exhibited any weedy tendencies compared to non-transgenic control plants. Potato plants, in general, do not have any inherent weediness under North American growing conditions. Although potatoes may arise as volunteers from tubers remaining in the field after harvest, these volunteers are easily managed and are not considered to be weeds. The combined traits of Colorado potato beetle resistance and PVY resistance do not change the weediness characteristics of potatoes. NewLeaf potatoes which contain only the CPB resistant trait have been grown commercially in the U.S. and Canada since 1995. There have been no reports of increased weediness in those lines. Furthermore, farmers typically apply insecticides to control damage from CPB and PVY in the same field. There are no reports that control of both pests results in plants that are more likely to become weeds.

## *B. Gene Escape (Outcrossing)*

Over nine hundred species of *Solanum* have been identified (Correll, 1962; Hawkes, 1990), most near the centers of origin in Central and South America. 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 (Hawkes, 1990). 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 potato. 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 (S. Love, USDA petition 94-257-01P).

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 hybrid 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. This results in very limited pollen dissemination. Tynan *et al.* (1990) found that dispersal of pollen from transgenic plants did not occur outside a range of five meters.

If the transgenes escape into the environment in a persistent manner, it is most likely to do so in Central or South America 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 due to susceptibility of various viral, fungal and bacterial diseases. Furthermore, the impact on biodiversity would be insignificant and would not be any different than the impact from introduction of traditionally bred potatoes.

## *C. Impact on Threatened, Endangered and Beneficial Organisms*

The Cry3A protein has been reviewed in previous submissions to the USDA and EPA (EPA, 1995). USDA, APHIS came to a Finding Of No Significant Impact to the Environment for the potato plants expressing the Cry3A protein (USDA, 1995; 1996). Similarly, the Environmental Protection Agency has granted an Exemption from the Requirement of a Tolerance for the Cry3A protein (EPA, 1995). In data evaluated by the EPA and USDA in those previous submissions, Monsanto demonstrated that Cry3A protein is non-toxic to non-target organisms including larval and adult honeybee, ladybird beetle, green lacewing, parasitic wasp, *Collembola* sp., earthworm, mice and bobwhite quail.

The PVY coat protein has been evaluated by EPA and has been granted an Exemption from the Requirement of a Tolerance in all crops and raw agricultural commodities (EPA, 1997). Plant viruses only infect certain plants and are not known to have any adverse effects on other organisms.

## *D. Benefits to the Environment*

### 1. Control of CPB and PVY

NewLeaf Y potatoes provide substantial protection against the Colorado potato beetle without the input of synthetic insecticides. In field studies reported by Perlak *et al.* (1993), potatoes expressing 0.002% Cry3A protein (appx. 0.32 ppm based on total protein level in foliage of 1.6% of fresh weight) caused 100% mortality of neonate CPB larvae. At levels of expression above 0.005% (appx. 0.8 ppm), feeding damage by adult beetles was negligible. The three NewLeaf Y potato lines express between 5.96 and 47.35 ppm of Cry3A (Appendix II). This level is 19 to 145-fold and 7 to 58-fold higher than the level required to provide complete protection against neonate and adult CPB, respectively. As expected, no damage has been observed from CPB in any NewLeaf potato fields during the two years of commercial production in the U.S. and Canada. The mean expression of Cry3A in the commercial NewLeaf® 6 (Russet Burbank line BT6) line is 16.36 ppm in leaf tissue. NewLeaf Y potatoes lines RBMT15-101, SEMT15-02, SEMT15-15 express similar levels of the Cry3A protein and have performed equally well in the field. Monsanto has not observed or received any reports of damage by CPB in any NewLeaf Y field trial.

In addition to the Colorado potato beetle resistance trait, the NewLeaf Y potato RBMT15-101, SEMT15-02 and SEMT15-15 are highly resistant to the potato virus Y. To evaluate resistance of NewLeaf Y potato lines to PVY infection, potatoes were field tested in Florida in 1995. Four replicates of each transgenic line and control nontransgenic line were planted as a seed piece adjacent to an PVY infected non-transgenic control seed potato. Between 50 and 100 aphids were placed on each infected control potato. The aphids were allowed to move onto and inoculate the control and transgenic test lines. Plants were observed for visual symptoms of PVY infection and analyzed for the presence PVY by Agdia Inc. using ELISA. All of the control lines showed visible signs of PVY infection and tested positive for PVY using ELISA. The transgenic lines RBMT15-101, SEMT15-02 and SEMT15-15 showed no signs of PVY infection and tested negative for PVY using ELISA.

Additional challenges with $PVY^{NTN}$ (Slovanian isolate) and $PVY^{NTN}$ (California) produced similar results (Table VI.1) in Canadian greenhouse challenge experiments. However, all transgenic lines, except the Shepody lines which are naturally resistant, were susceptible to PVA.

**Table VI.1. Canadian greenhouse virus challenge experiments conducted in 1996.** Greenhouse grown plants (5 per line) were mechanically inoculated with either PVA or one of three strains PVY. Two tests were completed. Plants were analyzed for virus presence using ELISA and PCR methods.

| Potato line # | PVA | PVY (common) | PVY (California) | PVY (Slovenian) |
|---|---|---|---|---|
| RBMT15-101 | Infected | Not infected | Not infected | Not infected |
| RB Control 12 | Infected | Infected | Infected | Infected |
| SEMT15-02 | Not infected | Not infected | Not infected | Not infected |
| SEMT15-15 | Not infected | Not infected | Not infected | Not infected |
| SE Control 5 | Not infected | Infected | Infected | Infected |

The NewLeaf Y transgenic lines were also tested in 1994, 1995 and 1997 in greenhouse studies conducted by Dr. John J. Cho at the University of Hawaii at Manoa (Table VI.2 and Appendix VII). The plants were directly inoculated with PVY° using infected tobacco as the source. Approximately 4 and 8 weeks after inoculation, plants

were visually observed for symptoms and tested using ELISA. In this testing, transformed potato lines RBMT15-101, SEMT15-02, and SEMT15-15 showed resistance to infection by PVY. Control lines of the same varieties were infected using the same procedure and exhibited both visual symptoms and positive ELISA results. It can be concluded that these NewLeaf Y potato lines are resistant to infection by potato virus Y and are not symptomless carriers of the virus.

**Table VI.2. Hawaiian greenhouse PVY challenge experiments.** Over the course of 3 years, each transgenic line was tested 3-4 times by mechanically inoculating, with PVY, groups of five virus-free plants grown under greenhouse conditions. Control plants, with more groups tested per line, were treated similarly. Symptoms were observed and ELISA's conducted to confirm virus infection at approximately four weeks after inoculation.

| Potato line # | Total Number of Plants Tested from 1994 - 1997 | Symptoms Observed* | Average ELISA (OD**) reading per group at first sample period |
|---|---|---|---|
| RBMT15-101 | 15 | None | 0.153 |
| RB Control 11 | 30 | M, P, VC | 1.869 |
| RB Control 12 | 55 | M, P, VC | 0.932 |
| RB Control 13 | 25 | M, P | 0.923 |
| SEMT15-02 | 20 | None | 0.134 |
| SEMT15-15 | 20 | None | 0.126 |
| SE Control 1 | 65 | M | 2.217 |
| SE Control 5 | 55 | M | 1.963 |

* Symptom - M = Leaf mottling due to mosaic; P = leaf puckering; VC = vein clearing.
** OD = Optical Density

To evaluate the virus resistance of NewLeaf Y potato lines of the varieties Russet Burbank (RBMT15-101) and Shepody (SEMT15-02, SEMT15-15), field experiments were conducted over three growing seasons on Prince Edward Island (1994-1997) ( Table VI.3 and Appendix VII). These experiments, conducted by Dr. Robert Coffin, Ph.D., P. Ag. Crop Specialist, Cavendish Farms, Prince Edward Island (PEI), Canada, were designed to simulate worst case potato virus Y (PVY) pressure under field conditions. Viral infection was determined through visual observation and ELISA's on the three NewLeaf Y potato lines and non-transformed commercial lines of the same variety. In three seasons of field testing under extreme potato virus Y exposure, the transformed potato lines showed high resistance to infection by this virus. Control lines of the two varieties, Russet Burbank and Shepody, were very infected by the end of the field testing. It can be concluded that these three transformed potato lines are resistant, with a stable and persistent trait, to infection by potato virus Y.

**Table VI.3. Prince Edward Island field challenge experiments. Challenges with PVY to transgenic lines of Russett Burbank and Shepody potatoes. ELISA results from July 1998, the final field season.** Potato tubers, saved from the plants grown in the field under high virus pressure in 1996, were planted in the field at the beginning of the 1997 growing season and subjected to natural infection pressure for potato viruses. Tubers from the resulting plants were grown out on PEI in the summer of 1998 and rated for virus using ELISA detection methods for potato Potato Virus Y. The 1998 plot was a randomized block design, with 3 replications of plots of 20 feet of row. This plot design allowed a total of 50 to 60 plants per line to be observed for virus symptoms. In July 1998, 10 leaves from 10 individual plants were collected from each line to conduct ELISA's. Results are presented as the percent of the ten sampled plants with positive infections as determined from ELISA's.

| Potato Line #[1] | % of plants with PVY Infection[2] |
|---|---|
| RBMT15-101 | 0 |
| RB CONTROL 11 | 20 |
| RB CONTROL 12 | 30 |
| SEMT15-02 | 0 |
| SEMT15-15 | 0 |
| SE CONTROL 5 | 100 |
| SE COMMERCIAL CONTROL | 100 |

[1] **Line #** - The tested line of transformed Russet Burbank (RB), Shepody (SE) and control lines of each variety.
[2] **% PVY** - Percent of plants with ELISA determined Potato Virus Y infection, as determined by leaf samples from 10 plants per line

As additional data to substantiate the efficacy against PVY, no incidence of PVY infection has been detected in the seed lots of the three transformed lines evaluated in the U.S. and Canada seed certification programs (Appendix VII).

## 2. Reduction of pesticide use

Use of NewLeaf Y potatoes for CPB control alone is expected to significantly reduce the overall amount of pesticides applied to potato fields. In commercial production of NewLeaf potatoes (CPB resistant trait only), the total number of all pesticide treatments were reduced by 40%, active ingredient was reduced by over 20% per acre, and formulation pound per acre was reduced by 10% or more compared to non-NewLeaf potatoes (Table VI.4.). A secondary benefit of potatoes expressing Cry3A to control CPB was observed. In field trials, beneficial predaceous and parasitic arthropods such as big-eyed bugs, damsel bugs, minute pirate bugs, species of Hymenoptera, and spiders were significantly more abundant in the NewLeaf potato plots than in those treated with conventional chemical insecticides to control the Colorado Potato Beetle (USDA Petition 94-257-01p). Insect resistance management continues to be a high priority for Monsanto. We continue to consult with the EPA and the Canadian Food Inspection Agency to ensure that biotechnology for insect resistance is used responsibly and will remain a viable tool for agriculture in the future.

Management of PVY in potato fields relies on removal of infected plants by roguing and treating the aphid vectors with insecticides to prevent the spread of the virus. Seed potato producers typically apply 1 - 3 applications of an insecticide per growing season to control the aphid population (Preston, 1997). The aphid population must be controlled so that the seed will meet the strict standards to qualify as certified seed to be sold for replanting in subsequent growing seasons. However, even with the measures taken to produce certified seed, many plots are rejected or downgraded because of PVY infection (Altman, 1997). As NewLeaf Y lines are completely resistant to infection by PVY, use of aphicides is not needed to control PVY infection.

**Table VI.4. Comparative analysis of total insecticide usage in commercial NewLeaf® potato fields; U.S., 1996.**

| Farms using NewLeaf program[3] | Farms | NewLeaf[1] (RB variety) | | | Russet Burbank[2] | | | Reduction | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Trts[4] | ai/ acre[5] | Form. lb/acre[6] | Trts[4] | ai/ acre[5] | Form. lb/acre[6] | Trts[4] | % Trt reduction | ai/ acre[5] | %ai/acre reduction | Form. lb/acre[6] | %Form. lb/acre reduction |
| Avg. all regions | 35 | 1.77 | 1.91 | 8.07 | 3.12 | 2.43 | 8.90 | 1.35 | 43.2 | 0.52 | 21.3 | 0.83 | 9.3 |
| ME, NB | 3 | 1.67 | 1.29 | 2.62 | 3.33 | 1.89 | 5.63 | 1.67 | 50.1 | 0.60 | 31.7 | 3.01 | 53.4 |
| MN, ND, WI | 15 | 2.58 | 1.73 | 3.46 | 3.85 | 1.60 | 4.12 | 1.27 | 32.9 | -0.12 | - | 0.66 | 16.0 |
| Idaho | 14 | 0.53 | 1.58 | 13.62 | 1.56 | 3.25 | 16.00 | 1.03 | 66.0 | 1.67 | 51.3 | 2.38 | 14.8 |
| OR, WA | 4 | 3.25 | 4.53 | 13.23 | 4.00 | 6.16 | 24.09 | 0.75 | 18.7 | 1.63 | 26.4 | 10.86 | 45.0 |

1 NewLeaf Russet Burbank variety.
2 Traditional non-transformed Russet Burbank variety.
3 Farms which grew non- transformed Russet Burbank and NewLeaf Russet Burbank using pest management practices as recommended by Monsanto/NatureMark (Appendix X).
4 The average number of treatments per field.
5 The active ingredient of insecticide used per acre.
6 The pounds of formulated insecticide used per acre.

## *E. Plant Pest Issues Related to PVY Resistance*

For virus-resistant transgenic plants, three main issues related to plant pest risk have been identified: transencapsidation, synergism and recombination (Robinson, 1996; Miller *et al.*, 1997). Issues related to viral coat protein gene-mediated resistance have been addressed in detail by OECD (1996).

### 1. Transencapsidation

Transencapsidation requires that the inserted gene encodes for a viral coat protein. Progeny genomes of the incoming virus are assembled into particles in which some or all of the protein subunits may be derived from the coat protein of another virus. This phenomenon has been detected with potyviruses (Bourdin and Lecoq, 1991; Lecoq *et al.*, 1993). Farnelli *et al.* (1992) demonstrated that the detection of CP transgene can be enhanced in transgenic plants when the transgenic plants are infected with a taxonomically related virus to which they are not resistant. It is hypothesized that the transgene CP is stabilized in the masked virus particles produced upon infection.

NewLeaf Y potato lines contain the native coat protein gene from PVY strain O; therefore, it is conceivable that the PVY strain O coat protein expressed from the transgene may transencapsidate another invading virus. In order to address this concern, data has been compiled derived from field samples obtained from NewLeaf Y potato lines that were infected with potato virus A (PVA). PVA is the only other common potyvirus prevalent in the United States known to infect potato. For assessment of the potential for transencapsidation to occur in NewLeaf Y plants, NewLeaf Y Russet Burbank potato plants, resistant to infection by potato virus Y, were grown in winter nurseries in Florida City, FL in the winters of 1996 -1998. Over the course of these seed certification field trials, visual observations of the foliage growing from some of the seed lots showed a low incidence of plants displaying "mosaic" symptoms. Samples were obtained from individual plants within each plot that displayed the mosaic symptoms and sent to the commercial plant pathogen screening company, Agdia (Elkhart, IN) for qualitative analysis (by ELISA) for PVY and PVA. To confirm that samples were obtained from genetically modified potatoes, Cry3A protein expression levels were also evaluated. All analyses were done using ELISA tests routinely used by NatureMark and the potato industry for seed certification purposes.

The results from these qualitative analyses of NewLeaf Y potato plants that displayed mosaic symptoms showed that all of the plants were infected with PVA a potyvirus known to infect potato plants and produce symptoms similar to PVY. Furthermore, PVY coat protein was not detectable by ELISA in RBMT15-101 and RBMT15-088 plants which were infected with PVA (Table VI.5). As expected, all of the NewLeaf Y lines tested expressed the Cry3A protein, thereby confirming that samples were obtained from genetically modified NewLeaf Y lines. Although NewLeaf Y Russet Burbank Line No. RBMT15-088 is not included in this petition, it displays commercial levels of resistance to PVY infection, thereby confirming the results obtained from NewLeaf Y Russet Burbank Line No. RBMT15-101 and further extending the conclusions drawn from these analyses to other PVY resistant potato lines developed using the same vector. These analyses were not performed for the other lines included in this petition (SEMT15-02, SEMT15-15) because of natural resistance to PVA in the Shepody variety.

**Table VI.5 PVY coat protein levels in PVA-infected NewLeaf Y potato lines.**

| | Optical Density | | |
|---|---|---|---|
| Plant Number[1] (line) | PVA[2] | PVY[3] | Cry3A |
| 1 (RBMT15-101) | 2.172 | 0.055 | ND[4] |
| 2 (RBMT15-101) | 0.970 | 0.021 | 0.556 |
| 3 (RBMT15-101) | 0.950 | 0.027 | 0.557 |
| 4 (RBMT15-101) | 1.188 | 0.023 | 0.610 |
| 5 (RBMT15-101) | 0.778 | 0.027 | 0.609 |
| 6 (RBMT15-101) | 1.141 | 0.024 | 0.543 |
| 7 (RBMT15-101) | 0.737 | 0.024 | 0.604 |
| 8 (RBMT15-088) | 4.000 | 0.040 | 0.650 |
| 9 (RBMT15-088) | 4.000 | 0.078 | 0.650 |
| 10 (RBMT15-088) | 2.872 | 0.040 | 0.505 |

1. Leaf samples were obtained from NewLeaf Y potato plants grown in 1996-1997 and 1997-1998 winter nurseries in Florida City, Florida. Plants were assayed for the presence of PVA, PVY and Cry3A using ELISA. All analyses were performed by AgDIA, (Elkhart, IN). Results are presented as optical density (O.D.) readings observed for each sample for the PVA, PVY and Cry3A, respectively.
2. All samples in this column were positive for PVA. Positive control value for plant number 1 was 0.912 OD units. For plant numbers 2-10, positive control value was 1.318 OD units.
3. All samples in this column were negative for PVY. Positive control value for plant number 1 was 1.463. For plant numbers 2-10, positive control value was 0.473 OD units.
4. Cry3A analyses were not done for this sample. Qualitative PVA, PVY and Cry3A ELISA analyses were performed by AgDia (Elkhart, IN).

The results from these analyses establish that PVY coat protein is not detected in NewLeaf Y potato lines infected by PVA and that mosaic symptoms observed in a limited number of NewLeaf Y plants are due to infection with a different potyvirus, PVA. Given this information, it must be concluded that expression levels of PVY coat protein remain extremely low (less than 2 µg/g tissue fresh weight) even in the presence of an invading potyvirus, thus establishing that the transgenic PVY coat protein is not incorporated into the PVA virion at a detectable level. Finally, since PVA and PVY are found within the same cells of the potato plant (Brunt, 1996), much greater concentrations of PVY and PVA coat protein accumulate during natural infections (Table VI.6), therefore the risk for transencapsidation due to the agronomic use of NewLeaf Y potato plants is likely much less than that already present in the environment due to natural co-infections with PVA and PVY that have already occurred throughout history due to cultivation of potato plants.

The hypothetical scenarios involving any environmental risk associated with genetically modified plants expressing viral coat proteins pose no long-term environmental plant pest issues (AIBS, 1995). In the event that transencapsidation were to occur, the transencapsidated virus has not acquired the genetic material required for subsequent production of coat protein subunits, therefore, the transcapsidated virus particles cannot be maintained in subsequent rounds of viral infection. Any potential impacts due to transencapsidation can only occur with the first round of infection of the masked virus upon infection of a susceptible host plant. Furthermore, the "mismatched" or heterologous viral coat produces a risk only if it provides new properties to the encapsidated virus. This is not the case for potyviruses where the primary determinant of aphid transmissibility is the helper component, not the coat protein (Murphy *et al.*, 1995). Therefore, transencapsidation with the PVY coat protein would not affect the aphid transmissibility of the new virus, and the transencapsidated virus would not likely gain any new beneficial properties.

**Table VI.6 PVA and PVY qualitative virus levels in dual infected potato plants.**

| Plant Number[1] | Optical Density | | |
|---|---|---|---|
| | PVA[1] | PVY[2] | Cry3A |
| 1 | 4.000 | 0.466 | 1.866 |
| 2 | 0.311 | 2.682 | 2.042 |
| 3 | 4.000 | 0.540 | 2.199 |
| 1 | 4.000 | 0.159 | 1.863 |

1 Leaf samples were obtained from NewLeaf potato plants grown in 1997-1998 in winter nurseries in California, USA. Plants were assayed for the presence of PVA, PVY and Cry3A using ELISA. All analyses were performed by AgDIA, (Elkhart, IN). Results are presented as optical density (O.D.) readings observed for each sample for the PVA, PVY and Cry3A, respectively.

2 All samples in this column were positive for PVA. Positive control value was 1.533 OD units.

3 All samples in this column were positive for PVY. Positive control value was 2.493 OD units.

## 2. Synergy

In synergism, the virus or gene product of a virus potentiates the effect of the incoming virus on the host plant. A well-recognized example of synergistic disease occurs between potyviruses such as PVY and potato virus X (Vance, 1991). In this synergistic interaction, the level of PVX increases 3 to 10-fold in systemically infected leaves of doubly infected plants, while the level of potyvirus in the same leaves is unchanged from that of singly infected plants. The increase in virus level is also correlated with an increase in disease severity. Shi *et al.* (1997) have mapped the potyviral synergistic component to the helper component protease (HC-Pro). Mutations within this region eliminate synergism. The coat protein gene was not shown to produce any syngeristic effects. Therefore, for the potyvirus (PVY)-potexvirus (PVX) synergism, the HC-Pro gene is responsible. NewLeaf Y potato lines contain the PVY coat protein gene and do not contain the HC-Pro gene. Therefore, no synergism would be expected. In field trials conducted in the U.S. and Canada, there has been no observed effect of the presence of the PVY coat protein gene on susceptibility of NewLeaf Y lines to other viral diseases.

## 3. Recombination

RNA recombination involves the exchange of RNA templates during virus replication and results in a product that contains the union of two previously distinct RNA templates (Allison *et al.*, 1996). Several researchers have shown that recombination is possible between transgenes and invading viruses which are closely related to the donor organism for the transgene (Greene and Allison, 1994; Schoelz and Wintermantel, 1993). The important issue for addressing risk from recombination is not whether it may happen, but whether recombination will produce a novel virus resulting in a new disease or an increase in disease severity.

Formally, there are two types of RNA recombination: homologous and nonhomologous (for a review, see Simon and Bujarski, 1994; Bujarski and Nagy, 1994). Homologous recombination may occur between related viral RNAs at corresponding sites and leads to regeneration of wild-type or close to wild type RNAs (Lai, 1992). In contrast, nonhomologous recombination may occur between unrelated RNA molecules at non-corresponding sites (Bujarski and Nagy, 1994). Using such definitions, homologous RNA recombination occurs among strains of the same virus or viruses of the same group and leads to related variants of the virus. Nonhomologous recombination occurs among different, unrelated viruses and leads to the emergence of new viral species.

Two possible mechanisms of recombination, 'breakage and re-ligation' and 'template switching or copy-choice' have been proposed. However, no evidence for the 'breakage and re-ligation' model exists. The results from several laboratories strongly suggest that template switching is responsible for both homologous and nonhomologous crossover events (Kirkegaard and Baltimore, 1986, Cascone *et al.*, 1993,

Bujarski and Nagy, 1994, Nagy *et al.*, 1995). Shoelz and Wintermantel (1993) also implicated template switching as the mechanism responsible for recombination. Together these reports have concluded that replicase-mediated template switching is the most likely mechanism responsible for recombination between plant RNA viruses. Template switching predicts that viral replicase, the enzyme which makes new viral RNA molecules, changes RNA templates during RNA synthesis. Thus, properties of both viral RNAs and viral replicases must be responsible for the efficiency of RNA crossovers. Nonhomologous recombination can occur during minus strand synthesis (Rao and Hall, 1993), Ishikawa *et al.* (1991) and Nagy and Bujarski (unpublished results). Recent data suggest that homologous recombination can occur mostly during plus strand synthesis (Nagy and Bujarski, 1997). Nagy *et al.* (1995) found mutations in certain regions of replicase proteins can affect the frequency of crossovers, the location of crossovers or the precision of crossovers. Greene and Allison (1994) showed that a viral transgene representing a small 3' portion of viral RNA that includes the viral replicase binding region could correct via recombination a defective infecting virus. When this same group (Allison *et al.*, 1996) made several different deletions of the viral 3' replicase binding region included in the transgene, none of the 479 transgenic plants challenged with the defective virus supported a recombination event. These results have been supported by Ishikawa *et al.* (1991) and Cascone *et al.* (1993) where they found that elimination of replication through alteration of the 3' replicase binding region resulted in the lack of detectable recombinants. Since template switching is the likely mechanism of recombination between plant RNA viruses, and the PVYcp transgene message does contain the 3' putative replicase binding region of the virus, the discussion regarding recombination risk of the PVYcp transgene message will focus on recombination via template switching.

Probability of Recombination with the PVYcp mRNA

PVY is perhaps the most common virus found infecting potatoes in the world, and mixed infections with other potato viruses are quite common (McDonald, 1984). Transgenic potato plants expressing the *PVYcp* gene exhibit extreme resistance to PVY infection. The level of transgene mRNA is approximately 6 to 9-fold less than the level of PVY RNA in a naturally infected potato (Appendix IX). Thus the amount of template available for template-switching based recombination is much lower in a transgenic potato as compared to one naturally infected with PVY. In a natural mixed infection, each virus serves as a template for its own replicase and as a source of genetic information for replicase production. However, in potatoes expressing only the *PVYcp* gene, the genetic information for potyviral replicase is not present and furthermore will not be supplied from an invading PVY strain because these plants do not support replication of PVY. Template switching recombination would only be predicted to occur in the transgenic potato where a related potyvirus was able to establish an infection and actually bind to and replicate the transgene mRNA. The transgene mRNA would not be a template for any non-potyviral replicase and therefore the presence of the viral 3' end on the transgene mRNA would not contribute to increased frequency of template switching where non-potyviral replicases were involved. Therefore, recombination would not occur with the viral-derived transgene in plants that are highly resistant to virus from which the transgene was derived. The only time recombination would occur with the viral derived transgene is when another potyvirus has successfully infected the plants. Acquisition of the transgene that confers resistance to the invading virus will not give an evolutionary advantage to the virus. Therefore, the probability that a new viral disease will arise due to recombination with the *PVYcp* transgene is less than the probability of such an event occurring naturally in a mixed infection.

Consequences of Recombination with Other Potyviruses

In addition to PVY, the other potyviruses naturally found in potato are PVA and PVV. Both of these viruses cause very minor disease symptoms if any at all, and typically are not responsible for economic loss. Potato serves as the only known natural host for these viruses (Rich, 1983). Symptoms of viral diseases in potato are stable and this feature of virus infection is the foundation of the seed certification system. Therefore, since mixed infections are common but viral diseases are stable, history and experience shows that recombination between different viruses in potato is not a frequent source of new diseases. If recombination between potyviruses occurs, it would appear that the consequences have been uneventful to date. In fact, recombination has not been shown to play a major role in the evolution of potyviruses, even though there is an extensive database of partial and complete sequences of potyvirus genomes published. In only one case is recombination thought to have contributed to the evolution of a potyvirus strain. Based on sequence comparison, Cervera *et al.* (1993) suggested that plum pox virus (PPV)-06 evolved from a

homologous recombination event between PPV-PS and PPV-Rankovic isolate. A severe necrotic or N strain of PVY appears to have arisen from a series of single nucleotide changes that are distributed throughout the genome (Robaglia *et al.*, 1989; Suidarsono *et al.*, 1993) and apparently did not arise from recombination of gene cassettes.

Potyviruses are long single stranded RNA viruses of approximately 10 kilobases in length. They encode a single polyprotein which is post-translationally processed into functional proteins, and thus there is only a single translational start signal on the virus and this resides at the 5' end of the genome. The coat protein gene resides at the very 3' end of the genome. The viral-derived transgene only carries the information necessary to encode the PVY coat protein, and so the only potentially new gene a recombinant virus would obtain is the CP gene of PVY. Potyviral coat proteins are not sufficient to impart insect transmission nor cell-to-cell movement (Murphy *et al.*, 1995) nor are they thought to be determinants in disease severity. Therefore it is difficult to envision a scenario where a viable incoming potyvirus would gain a competitive advantage by obtaining part or all of the *PVYcp* gene. A more likely outcome would be that the recombinant virus would have incomplete gene sequences or out of frame sequences due to the polyprotein nature of potyviruses, and would be less viable than the wild type virus. Recombinant viruses can only appear from natural mixed infections between potyviruses in potato if they gain a selective advantage relevant to the parental viruses. Again, such an event from natural mixed infections is much more likely to occur than in a transgenic potato given the abundance of templates available for recombination in mixed infections versus the level of transgene mRNA.

Consequences of Recombination with Non-Potyviruses

Non-homologous recombination has not been shown to have played a major role in the evolution of potyviruses (Cervera *et al.*, 1993), perhaps because of the polyprotein nature of the virus. Since multiple virus infections are common in potato, and the viral RNAs are abundant in such infections, one would expect that considerable opportunity exists in nature for recombination between PVY and non-potyviruses infecting potato. The fact that no such recombinant has been detected suggests that the frequency of viable recombinant viruses appearing from such a recombination event is extremely low. While the transgene mRNA does contain the 3' end of the virus, the transgenic plant does not pose an increased risk for recombination between PVY and non-potyviruses. First, the level of transgene mRNA is 6 to 9-fold lower in the transgenic plant versus the level of viral RNA in a naturally infected potato (Appendix IX). Second, homology is typically a requirement of template switching recombination (Nagy *et al.*, 1995, Allison *et al.*, 1996) and the transgene is roughly 13% of the length of the entire viral genome, so much less viral sequence is present in the transgenic potato. Therefore the total frequency of template switching mediated recombination would be lower in the transgenic plant versus a natural mixed infection due to a reduction in total template available for such an event. Last, the 3' end of the PVY genome would not be expected to serve as a replicase binding region for unrelated, non-potyviral replicases. In the absence of a potyviral replicase the transgene message is an unlikely substrate for template-switching-mediated recombination.

It is possible that a potyvirus, such as PVA, could establish an infection in potato plants expressing the *PVYcp* gene. A second non-potyvirus also could establish a mixed infection in this same plant. In this case, a potyviral replicase would be present to potentially bind to and replicate the *PVYcp* transgene and make this message available for template switching recombination with the second virus. In this situation it is much more likely that the recombination will occur between PVA and the second, non-potyvirus given that the PVA viral RNA would be more abundant and much longer (greater chance of homology) than the transgene. Therefore in this situation the transgenic plant does not create a unique or increased risk versus the situation in nature today. However, should the transgene be involved in a recombination event with a non-potyvirus, the only function transferred to the recombinant virus would be the *PVYcp* gene. Potyviral coat proteins are not sufficient for determining host range, vector transmission or viral movement, nor are they thought to be determinants of disease severity. Therefore a resultant recombinant would not gain a competitive advantage over naturally occurring viruses in nature. In fact, recombinant viruses have been shown to be typically less fit than wild type viruses.

Summary

- The probability that a new viral disease will arise due to the expression of the *PVYcp* transgene is less than the probability of such an event occurring naturally in a mixed infection. The level of transgene mRNA is 2.6 to 485-fold lower than the level of PVY coat protein RNA in a naturally infected potato plant.
- There is no evidence that any new disease has arisen due to a recombination event involving PVY in potato. Mixed infections are very common in potato, especially with PVY which is the most prevalent potato virus.
- A recombinant virus gaining a portion of the *PVYcp* gene would not gain a competitive advantage since potyviral coat proteins are not sufficient to impart insect transmission, cell-to-cell movement nor are they determinants of disease severity.
- A recombinant virus gaining the *PVYcp* gene would likely not be viable in the transgenic potato plant given the extreme resistance to PVY.

### *F. Conclusion*

NewLeaf® Y potatoes present an opportunity for farmers to grow modified potato varieties that previously have not been protected from infection by PVY without the application of insecticides. The plant pest issues related to transencapsidation, synergism, and recombination have been addressed. The arguments presented demonstrate that that there are no increased plant pest risks associated with the use of PVY coat protein-mediated resistant potatoes.

## *VII. ADVERSE CONSEQUENCES: NEW CULTIVAR INTRODUCTION*

With respect to the virus resistant potatoes that are the subject of this petition, Monsanto Company knows of no possible adverse consequences due to the introduction of these new lines of standard commercial potato varieties. These plants are exempt from the requirement of registration under EPA with respect to the genetic material and product of the virus coat protein gene. They are registered with the EPA relative to the expression of genetic material for Cry3A protein.

# VIII. REFERENCES


AIBS (American Institute of Biological Sciences). 1995. Transgenic Virus-Resistant Plants and New Plant Viruses. A Workshop Sponsored by the USDA-APHIS and the Biotechnology Industry Organization, Inc. 44 pp.

Albright, L.M., M.F. Yanofsky, B. Leroux, D. Ma, and E.W. Nester. 1987. Processing of the T-DNA of Agrobacterium tumefaciens generates border nicks and linear, single-stranded T-DNA. J. Bacteriol. 169:1046-1055.

Allison, R.F., W.L. Schneider, and A.E. Greene. 1996. Recombination in plants expressing viral transgenes. Seminar Virology. 7:417-422.

Almeida, E. R. P., V. Gossele, C.G. Muller, J. Dockx, A. Reynaerts, J. Botterman, E. Krebbers, and M.P. Timko. 1989. Transgenic Expression of Two Marker Genes Under the Control of an Arabidopsis rbcS Promoter: Sequences Encoding the Rubisco Transit Peptide Increase Expression Level. Mol. Gen. Genet. 218:78-86.

Altman, J.E. 1997. Personal Communication.

Bachmann, B. G. 1987. Derivatives and Genotypes of Some Mutant Derivatives of Escherichia coli K-12. In: F.C. Neidhardt (ed.), Escherichia coli and Salmonella pyphimurium, Vol.2. American Society for Microbiology, Washington, DC.

Banttari, E.E., P.J. Ellis, and S.M.P. Khurana. 1993. Management of diseases caused by viruses and viruslike pathogens, In: R. C. Rowe (ed.), Potato Health Management. APS Press, St. Paul, Minnesota.

Barker, R. F., Idler, K. B., Thompson, D. V. and Kemp, J. D. 1983. Nucleotide Sequence of the T-DNA Region from the Agrobacterium tumefaciens Octopine Ti Plasmid pTi15955. Plant Molecular Biology 2:335-350.

Barkley, G.M. and W.W.M. Schrage. 1993. Potato varieties in Canada, 5th Edition. New Brunswick Department of Agriculture, Fredericton, NB, Canada.

Beck, E., G. Ludwig, E.A. Auerswald, B. Reiss, and H. Schaller. 1982. Nucleotide Sequence and Exact Localization of the Neomycin Phosphotransferase Gene from Transposon Tn5. Gene 19:327-336.

Bevan, M., W.M. Barnes, M. Chilton. 1983. Structure and Transcription of the Nopaline Synthase Gene Region of T-DNA. Nucleic Acids Res. 11:369-385.

Boiteau, G., C. Misener, R. P. Singh and G. Bernard. 1992. Evaluation of a vacuum collector for insect pest control in potato. Am. Pot. J. 69:157-166.

Bolivar, F., R.L. Rodriguez, P.J. Greene, M.C. Betlach, H.L. Heyneker, and H.W. Boyer. 1977. Construction and Characterization of New Cloning Vehicles II. A Multipurpose Cloning System. Gene 2:95-113.

Bourdin, D. and H. Lecoq. 1991. Evidence that heteroencapsidation between two potyviruses is involved in aphid transmission of a nonaphid transmissible isolate from mixed infection. Phytopathology 81:1459-1464.

Bujarski, J.J. and P.D. Nagy. 1994. Genetic RNA-RNA recombination in positive-stranded RNA viruses of plants. In: J. Paszkowski (ed), Homologous recombination in plants. Kluwer Academic Publisher, Dordrecht, The Netherlands.

Casagrande, R.A. 1987. The Colorado potato beetle: 125 years of mismanagement. Bull. Entomol. Soc. 33:142-150.

Cascone, P.J., T.F. Haydar, A.E. Simon. 1993. Sequences and structures required for recombination between virus-associated RNAs. Science 260: 801-805.

Cervera, M.T., J.L. Riechmann, M.T. Martin, and J.A. Garcia. 1993. 3'-terminal sequence of the plum pox virus PS and O6 isolates: evidence for RNA recombination within the potyvirus group. J. Gen. Virol. 74:329-334.

Correll, D.S. 1962. The potato and its wild relatives. Texas Research Foundation, Renner, Texas.

Coruzzi, G., R. Broglie, C. Edwards, and N-H. Chua. 1984. Tissue-specific and Light-regulated Expression of a Pea Nuclear Gene Encoding the Small Subunit of Ribulose-1,5-bisphosphate Carboxylase. EMBO J. 3:1671-1679.

De Bokx, J.A. and H. Huttinga. Potato Virus Y, 1981, CMI/AAB Descriptions of Plant Viruses, No. 242

DeBlock, M., L. Herrera-Estrella, M. Van Montagu, J. Schell, and P. Zambryski. 1984. Expression of foreign genes in regenerated plants and in their progeny. EMBO J. 3:1681-1689.

Depicker, A., Stachel, S., Dhaese, P., Zambryski, P. and Goodman, H. M. 1982. Nopaline Synthase: Transcript Mapping and DNA Sequence. J. Molec. Appl. Genet. 1:561-573.

EPA. 1997. Coat protein of potato virus Y and the genetic material necessary for its production; exemption from the requirement of a tolerance. Federal Register . 62: 43653-43657.

Farnelli, L., P. Malnoe, and G.F. Collet. 1992. Heterologous encapsidation of potato virus Y strain O ($PVY^{O}$) with the transgenic coat protein of PVY strain N ($PVY^{N}$) in Solanum tuberosum cv. Bintje. Bio/Technology. 10:1020-1025.

Ferro, D.N. and G. Boiteau. 1992. Management of major insect pests of potato. In Plant Health Management in Potato Production (R. C. Rowe ed.) pp. 209-234, Am. Phytopath. Soc. Press, St. Paul, Minnesota.

Ferro, D.N., B.J. Morzuch, and D. Margolies. 1983. Crop loss assessment of the Colorado potato beetle (Coleoptera: Chrysomelidae) on potatoes in western Massachusetts. J. Econ. Entomol. 76:349-356.

Fling, M., J. Kopf, and C. Richards. 1985. Nucleotide sequence of the transposon Tn7 gene encoding an aminoglycoside-modifying enzyme, 3'(9)-O-nucleotidyltransferase. Nucleic Acids Res. 13:7095-7106.

Fraley, R. T., S.G. Rogers, R.B. Horsch,, P.R. Sanders, J.S. Flick, S.P Adams, M.L. Bittner, L.A. Brand, C. Fink, J.S. Fry, G.R. Galluppi, S.B. Goldberg, N.L Hoffmann, and S.C. Woo. 1983. Expression of bacterial genes in plant cells. Proc. Natl. Acad. Sci. 80:4803-4807.

Fraley, R.T., R.B. Horsch, A. Matzke, M.D. Chilton, W.S. Chilton, and P.R. Sanders. 1984. In vitro transformation of petunia cells by an improved method of co-cultivation with A. tumefaciens strains. Plant Mol. Biol. 3:371-378.

Greene, A.E. and R.F. Allison. 1994. Recombination between viral RNA and transgenic plant transcripts. Science 263: 1423-1425.

Harcourt, D. G. 1971. Population dynamics of Leptinotarsa decemlineata (Say) in eastern Ontario. III. Major population processes. Can. Entomol. 103:1149-1161.

Hare, J. D. 1980. Impact of defoliation by Colorado potato beetle on potato yields. J. Econ. Entomol. 73:369-373.

Hawkes, J.G. 1990. The Potato: Evolution, Biodiversity and Genetic Resources. Smithsonian Institution Press, Washington, D.C.

Hawkes, J.G. 1978. Biosystematics of the potato. In The Potato Crop (P.M. Harris ed.). Chapman and Hall, New York.

Holland, J., K. Spindler, F. Horodyski, E. Gradau, S. Nichol, and S. van de Pol. 1982. Rapid evolution of RNA genomes. Science. 215:1577-1585.

Horsch, R.B., R.T. Fraley, S.G. Rogers, P.R. Sanders, P.R., Lloyd, and N. Hoffmann. 1984. Inheritance of functional foreign genes in plants. Science 223:496-498.

Huttner, S. L., C. Arntzen, R. Beachy, G. Breuning, E. Nester, C. Qualset and A. Vidaver. 1992. Revising oversight of genetically modified plants. Bio/Technology 10: 967-971.

Ishikawa, M., Kroner, P., Ahlquist, P., Mseshi, T. 1991. Biological activities of hybrid RNAs generated by 3'-end exchanges between tobacco mosaic and brome mosaic viruses. J. Virol. 65: 3451-3459.

Kaniewski, W., C. Lawson, B. Sammons, L. Haley, J. Hart, X. Delannay and N. Tumer. 1990. Field Resistance of Transgenic Russet Burbank Potato to Effects of Infection by Potato Virus X and Potato Virus Y. Biotechnology. 8: 750 - 754.

Kirkegaard, K. and D. Baltimore 1986. The mechanism of RNA recombination in poliovirus. Cell 47: 433-443.

Klee, H.J. and S.G. Rogers, S.G. 1989. Plant gene vectors and genetic transformation: plant transformation systems based on the use of Agrobacterium tumefaciens. Cell Culture and Somatic Cell Genetics of Plants. 6:1-23.

Koncz, C. and J. Schell. 1986. The promoter of T-DNA gene 5 controls the tissue-specific expression of chimeric genes carried by a novel type of Agrobacterium binary vector. Mol. Gen. Genet. 204:383-396.

Kononov, M.E., B. Bassuner, and S.B. Gelvin. 1997. Integration of T-DNA binary vector 'backbone' sequences into the tobacco genome: evidence for multiple complex patterns of integration. The Plant J. 11:945-957.

Krieg, A., A.M. Huger, G.A. Langenbruch, and W. Schnetter. 1983. Bacillus thuringiensis var. tenebrionis, a new pathotype effective against coleoptera. Z. Angew. Ent. 96:500-508.

Lai, M.M-C. 1992. RNA recombination in animal and plant viruses. Microbiol. Rev. 56:61-79.

Lawson, C., W. Kaniewski, L. Haley., R. Rozman, C. Newell, P. Sanders, and N. Tumer, N. 1990. "Engineering resistance to mixed virus infection in a commercial potato cultivar: resistance to potato virus X and potato virus Y in transgenic Russet Burbank." *Biotechnology.* 8: 127-134.

Lecoq, H., M. Ravelonand, R. Wipf-Scheibel, M. Monision, B. Raccah, and J. Dunez. 1993. Aphid transmission of a non-aphid transmissible strain of zucchini yellow mosaic potyvirus from transgenic plants expressing the capsid protein of plum poxvirus. Mol. Plant-Microbe Interact. 3:301-307.

Love, S. 1997. Personal communication.

MacIntosh, S.C., T.B. Stone, S.R. Sims, P.L. Hunst, P.L., J.T. Greenplate, P.G. Marrone, F.J. Perlak, D.A. Fischhoff, and R.L. Fuchs. 1990. Specificity and efficacy of purified Bacillus thuringiensis proteins against agronomically important insects. J. Invert. Path. 56:258-266.

McDonald, J.G. 1984. Viruses assocaited with mosaic symptoms in cultivar Russet Burbank potato Solanum tuberosum. Can. J. Plant. Path. 6:224-226.

McPherson, S.A., F. J. Perlak, R.L. Fuchs, P.G. Marrone, P.B. Lavrik, and D.A. Fischhoff. 1988. Characterization of the coleopteran-specific protein of *Bacillus thuringiensis* subsp. *tenebrionis*. Bio/Technol. 6:61-66.

Miller, W.A., G. Koev, and B.R. Mohan. 1997. Are there risks associated with transgenic resistance to luteoviruses? Plant Disease. 81:700-710.

Moyer, D. D. 1992. Fabrication and operation of a propane flamer for Colorado potato beetle control. Cornell Coop. Extension Bulletin, 7p.

Moyer, D., R. Kujawski, R. Derksen, R. Moeller, J.B. Sieczka, and W.M. Tingey. 1991. Development of a propane flamer for Colorado potato beetle control. Mimeo (also on videotape), Cornell Cooperative Extension, Suffolk County. Riverhead, New York.

Murphy, F. A., C.M. Fauquet, D.H.L. Bishop, S.A. Ghgabrial, A.W. Jarvis, G.P. Martelli, M.A. Mayo, M.D. Summers. 1995. Virus Taxonomy. Classification and Nomenclature of Viruses. Springer-Verlag Wien, New York.

National Potato Council. 1992. Potato Statistical Yearbook. Englewood, Colorado, pp. 26-27.

National Potato Council. 1996. Potato Statistical Yearbook. Englewood, Colorado, p. 56.

National Potato Council. 1997. Potato Statistical Yearbook. Englewood, Colorado.

Nagy, P.D., A. Dzianott, P. Ahlquist, J.J. Bujarski. 1995. Mutation of the helicase-like domain of of protein 1a alters the sites of RNA-RNA recombination in brome mosaic virus. J. Virol. 69: 2547-2556.

Nagy, P.D. and Bujarski, J.J. 1997. Engineering of homologous recombination hotspots with AU-rich sequences in brome mosaic virus. J. Virol. 71: 3799-3810.

Naumovich, L, and W. Kaniewski. 1994. The infection of the *Solanum tuberosum*, Russet Burbank potato, by PVX, PVY, and PLRV viruses during the cultivation of the tuber. Internal Monsanto Study.

Newell, C. A., R. Rozman, M.A. Hinchee, E.C. Lawson, L. Haley, P. Sanders, W. Kaniewski, N.E. Tumer, R.B. Horsch, and R.T. Fraley. 1991. Agrobacterium-mediated transformation of *Solanum tuberosum L.* cv. Russet Burbank. Plant Cell Rep. 10:30-34.

OECD. 1996. Consensus document on general information concerning the biosafety of crop plants made virus resistant through coat protein gene-mediated protection. OECD Series on Harmonization of Regulatory Oversight in Biotechnology, No. 5.

OECD. 1997. Consensus document on the biology of *Solanum tuberosum* subsp. *tuberosum* (potato). OECD Series on the Harmonization of Regulated Oversight in Biotechnology, No. 8.

Olkowski, W., N. Saiki, and S. Daar. 1992. IPM options for Colorado potato beetle. The IPM Practitioner 16:1-21.

Perlak, F.J., T.B. Stone, Y.M. Muskopf, L.J. Petersen, G.B. Parker, S.A. McPherson, J. Wyman, S. Love, G. Reed, D. Biever, and D.A. Fischhoff. 1993. Genetically Improved Potatoes: Protection from Damage by Colorado Potato Beetles. Plant Molecular Biology 22, 313-321.

Potato Growers of Michigan, Inc., and the Michigan Potato Industry Commission. 1992. December 1991 CPB Survey Results: Crop Years 1989-1991.

Preston, D. 1997. University of Minnesota and North Dakota State University, Cooperative Extension Service, personal communication.

Rao, A.L.N. and T.C. Hall. 1993. Recombination and polymerase error facilitate restoration of infectivity in brome mosaic virus. J. Virol. 67:969-979.

Raschke,E., G. Baumann,. and F. Schoffl. 1988. Nucleotide sequence analysis of soybean small heat shock protein genes belonging to two different multigene families. J. Mol. Biol. 199:549-557

Rich, A.E. 1983. *In* Potato Diseases, A.E. Rich (ed). Academic Press, NY.

Richins, R., H. Scholthof, and R. Shepard. 1987. Sequence of figwort mosaic virus DNA (caulimovirus group). Nucleic Acids Research, 15:8451-8466.

Robaglia, C., M. Durand-Tardif, M.Tronchet, G.Boudazin, S. Astier-Monifacier, and F. Casse-Delbart. 1989. Nucleotide sequence of potato virus Y (N strain) genomic RNA. Journal of General Virology. 70:935-947.

Robinson, D. J. 1996. Environmental risk assessment of releases of transgenic plants containing virus-derived inserts. Transgenic Research 5:359-362.

Rogan, G.J., A.M. Grace, and P.B. Lavrik. 1997. Assessment of the Equivalence of *B.t.t.* and NPTII Proteins Produced in *E. coli* and in Russet Burbank, HiLite and Shepody Potato Plant Lines Resistant to Potato Virus Y and Colorado Potato Beetle. MSL 15262

Roush, R. T., and W. M. Tingey. 1993. Strategies for management of insect resistance to synthetic and microbial insecticides. In press. In Advances in Potato Pest Management. American Phytopathological Society Press, Minneapolis.

Sambrook, J., Fritsch, E.F. and Maniatis, T. 1989. Molecular Cloning. A Laboratory Manual. 2nd Edition. Cold Spring Harbor Press.

Schoelz, J. E. and W.M. Wintermantel. 1993. Expansion of viral host range through complementation and recombination in transgenic plants. The Plant Cell 5: 1669-1679.

Shi X.M., J. Miller, J. Verchot, J.C. Carrington, and V.B. Vance. 1997. Mutations in the region encoding the central domain of helper component-proteinase (HC-Pro) elimate potato virus X/potyviral synergism. Virology. 231:35-42.

Shields, E.J. and J.A. Wyman. 1984. Effect of defoliation at specific growth stages on potato yields. J. Econ. Entomol. 77:1194-1199.

Simon, A. E., J.J. Bujarski. 1994. RNA-RNA recombination and evolution in virus-infected plants. Annual Review of Phytopathology 32:337-362.

Sims, S.R. 1993. Sensitivity of Selected Insect Species to the Colorado Potato Beetle Active Protein from Bacillus thuringiensis subsp. tenebrionis. Study No. 92-01-37-05. EPA MRID No. 42932207, an unpublished study conducted by Monsanto Company.

Slaney, A.C., H.L. Robbins, and L. English. 1992. Mode of action of Bacillus thuringiensis toxin Cry3A: An analysis of toxicity in Leptinotarsa decemlineata (Say) and Diabrotica undecimpunctata Howardii Barber. Insect Biochem. Molec. Biol. 22:9-18.

Stalker, D. M., C.M. Thomas, and D.R. Helinski. 1981. Nucleotide Sequence of the Region of the Origin of Replication of the Broad Host Range Plasmid RK2. Mol. Gen. Genetics 181:8-12.

Suidarsono, Woloshuk, S. L., Z. Xiong, G.M. Hellman, E.A. Wernsman, A.K. Wessinger, and S.A. Lommel. 1993. Nucleotide sequence of the capsid cistrons from six potato virus Y (PVY) isolates infecting tobacco. Archives of Virology 132:161-170.

Sutcliffe, J. G. 1978. Complete nucleotide Sequence of the Escherichia coli Plasmid pBR322. Symposia on Quantitative Biology 43:77-103.

Tynan, J.L., M.K. Williams, and A.J. Conner. 1990. Low frequency of pollen dispersal from a field trial of transgenic potatoes. J. Genet. and Breed. 44:303-306.

USDA. 1997. National Agriculture Statistics Service; http://www.usda.gov/nass.

USDA. 1996. Monsanto Company; Availability of Determination of Nonregulated Status for Potato Lines Genetically Engineered for Insect Resistance. 61:24755-24757

USDA. 1995. Availability of Determination of Nonregulated Status for Gentically Engineered Potato Lines. 60:13108-13109.

Vance, V. B. 1991. Replication of potato virus X RNA is altered in coinfections with potato virus Y. Virolog. 182:486-494.

Wang, K., S.E. Stachel, B. Timmerman, M. Van Montagu, and P.C. Zambryski. 1987. Site-specific nick in the T-DNA border sequence as a result of Agrobacterium vir gene expression. Science. 235:587-591.

Wong, E. Y., C.M. Hironaka, and D. Fischhoff. 1992. Arabidopsis thaliana Small Subunit Leader and Transit Peptide Enhances the Expression of Bacillus thuringiensis Proteins in Transgenic Plants. Plant Molec. Biol. 20:81-93.

# APPENDIX I

## Materials and Methods for Southern and PCR Analysis

(Russet Burbank RBMT15-101, Shepody SEMT15-02 and
Shepody SEMT15-15 Potato Lines are the subject of this petition.
Please disregard data on other lines.)

## APPENDIX I

### Materials and Methods for Southern and PCR Analysis

## DNA Molecular Analysis

*Note:* The Monsanto SOPs are documented laboratory protocols which are kept on file at Monsanto. These are available upon request.

*Plant tissue.* DNA was isolated from leaf tissue of NewLeaf Y potato lines as well as nontransformed control lines from each cultivar. Leaf tissue from the NewLeaf Y lines SEMT15-02 and SEMT15-15 was obtained from a 1996 field trial at Island Falls, ME. Leaf tissue from line HLMT15-46 was obtained from 1996 field trials at Hermiston, OR and New Denmark, New Brunswick, Canada. Leaf tissue from and line RBMT15-101 was obtained from a 1996 field trials at Lethbridge, Alberta, Canada and New Denmark, New Brunswick, Canada. For each line, the youngest fully expanded leaves were collected from six plants at approximately six weeks post-planting. For each plant line, the leaves were combined, frozen, and shipped on dry-ice to Monsanto Co., Chesterfield, MO. Upon receipt the leaves were stored at approximately -80°C.

*DNA isolation for PCR analysis.* Approximately 25-50 mg of frozen leaf tissue from each test and control samples (1 microgram) was utilized for PCR analysis. DNA isolations were carried out as per SOP No. BtP-PRO-083.

*DNA isolation for Southern blot analysis.* Approximately 1.5 g of frozen leaf tissue from each test and control samples was utilized for Southern blot analysis. DNA isolations were carried out as per SOP No. GEN-PRO-039-01.

*PCR analysis for detection of genetic elements.* PCR primer pairs were designed to determine the presence and integrity of the T-DNA within the transgenic plants' genome. Primer sets were designed to amplify the coding sequences of the *PVYcp*, *cry3A* and *nptII* genes.

The PVY1 and PVY2 primers anneal at the 5' and 3' ends of the *PVYcp* gene, respectively, and generate a 786 bp product following PCR amplification. B1 and B2 anneal at the 5' and 3' ends of the *cry3A* gene, respectively and generate a 1793 bp product following PCR amplification. N1 and N2 anneal at the 5' and 3' ends of the *nptII* gene, respectively and generate a 768 bp product after PCR amplification.

Other primer sets were designed to determine the linkage between these genetic elements. The linkage between *PVYcp* and *cry3A* was tested using the primers PVY1 and BC1. PVY1 anneals to the 5' end of *PVYcp* and BC1 anneals near the 3' end of *cry3A*. The product from amplification with these primers would be expected to be 2171 bp in length. This product would span within the T-DNA from the 5' end of *PVYcp* gene to the 3' end of *cry3A gene*. Two sets of primer pairs were used to determine the linkage between *cry3A* and *nptII*. PB2 and SS1 anneal at the 5' end of *cry3A* and within the P-Arab-SSU1A promoter, respectively, and would generate a 1665 bp product. N1 and SS2 anneal to the 5' end of *nptII* and within the P-Arab-SSU1A promoter, respectively, and a 1607 bp product is expected following amplification. The products from PB2/SS1 and N1/SS2 span the region of the T-DNA from 5' end of *cry3A* to the 5' end of *nptII*.

Each PCR reaction contained approximately 1 µg genomic DNA, 20 pMoles of each PCR primer, and PCR reagents. PCR reagents were obtained from GIBCO BRL (Gaithersburg, MD). The transformation vector, PV-STMT15, was used with all PCR primer sets as a positive control template. In these reactions the vector was mixed with genomic DNA isolated from nontransformed control tissue. PCR products obtained from these reactions were compared to products obtained from reactions with genomic DNA

from NewLeaf® Y lines. PCR oligonucleotides were obtained from GIBCO BRL (Gaithersburg, MD). PCR reactions were carried out in a Stratagene Robocycler Gradient 96 (Stratagene, La Jolla. CA). PCR products were subjected to agarose gel electrophoresis according to SOP No. GEN-PRO-003-01). The DNA molecular weight standards used in gel electrophoresis were obtained from Boehringer Mannheim.

*Selection of PCR amplification parameters.* In general, PCR conditions were as follows: 1 cycle of 94°C, 5 minutes; 35 cycles of 94°C, 45 seconds; "Anneal Temp", 30 seconds; 72°C, 3 minutes; 1 cycle of 72°C, 10 minutes. Following thermocycling samples were placed at 4°C until electrophoresis. At times, in order to optimize amplification, the "Anneal Temp" for different primer sets varied between 44°C and 64°C.

*Digestion of DNA samples for Southern analysis.* Approximately 10 µg of genomic DNA from each NewLeaf Y line and respective control of each cultivar were digested overnight with 10 units of *Bam*HI per µg of DNA in a final volume of 500 µL at 37°C according to SOP No. GEN-PRO-010-01 and as described by Sambrook, *et al.* (1989).

*DNA probes for Southern blot analysis.* Three different DNA probes were used to determine the presence in the NewLeaf® Y lines of the genetic elements outside the right and left border of plasmid PV-STMT15. These probes included the *aad*, *oriV* and *ori322* genetic regions. The probes were synthesized by polymerase chain reaction (PCR), essentially as described by Sambrook *et al.* (1989), using the plasmid PV-STMT15 as a template and gene specific primers that flanked the ends of the repective genetic elements. The PCR product was agarose gel purified (Qiagen, QIAEX II Gel Extraction Kit, Chatsworth, CA). The probes were radiolabeled using the RadPrime DNA Labeling Kit (GibcoBRL, Gaithersburgh, MD). The $^{32}$P dCTP used to label the probes was obtained from Amersham International (Buckinghamshire, England).

*Southern blot analysis.* Southern blot analysis was performed according to SOP No. PRO-025-02. The DNA digested with restiction endonucleases were separated based on size using a 0.8% agarose gel electrophoresis. Test, control and molecular weight standard were included in each gel. The DNA fragments were denatured and transferred onto Amersham Hybond®-N nylon membranes (Buckinhamshire, England) by capillary action and covalently crosslinked to the membrane with an ultraviolet Stratalinker® (Stratagene, LaJolla, CA). The blots were prehybridized at approximately 65°C in an aqueous solution of 0.5M sodium phosphate, 7% sodium dodecylsulfate (SDS) and 100 µg/mL tRNA. Hybridization solution was prepared using fresh prehybridization solution and the denatured radiolabeled probe. The blots were hybridized at approximately 65°C overnight. Membranes were washed in an aqueous solution of 2X SSC/0.1% SDS for 15 minutes at room temperature, 1X SSC/0.1% SDS for one 15 minute period at room temperature, 0.5X SSC/0.1% SDS for one 15 minute period at room temperature, and 0.1X SSC/0.1% SDS for two 15 minute periods at 65°C. Membranes were exposed to Kodak X-OMAT® film with intensifying screens at -80°C.

## References

Dean, D.A. 1993. Development and Validation of an Enzyme-Linked Immunosorbent Assay (ELISA) for Detection and Quantitation of Bacillus thuringiensis subsp. tenebrionis (B.t.t.) insecticidal protein in Colorado Potato Beetle Resistant Potatoes. Monsanto Technical Report, MSL No. 12341.

Grace, A. M. and Lavrik, P. B. 1996. Validation of a polyclonal antibody based enzyme linked immunosorbent assay (ELISA) for measurement of CP4 5-enoylpyruval-shikimate-3-phosphate synthase in potato plant tissues. Monsanto Technical Report, MSL No. 14906.

Sambrook, J., Fritsch, E. F., and Maniatis, T. 1989. "Molecular Cloning." Cold Spring Harbor Laboratory Press, 2nd Edition.

# APPENDIX II

## Expression Levels of *B.t.t.* and NPTII Proteins in Tissues Derived from Russet Burbank, Shepody and HiLite Potato Plants Resistant to Colorado Potato Beetle and Potato Virus Y

(Russet Burbank RBMT15-101, Shepody SEMT15-02 and
Shepody SEMT15-15 Potato Lines are the subject of this petition.
Please disregard data on other lines.)

[ CBI DELETED

]

Pages 55 through 195

# APPENDIX VI

## Agronomic Performance of NewLeaf Y Potato Lines

(Russet Burbank RBMT15-101, Shepody SEMT15-02 and
Shepody SEMT15-15 Potato Lines are the subject of this petition.
Please disregard data on HiLite lines.)

# Agronomic Performance of NewLeaf Y Potato Lines

December 1, 1997

Submitted by

Keith Reding, Ph.D. and Tom Salaiz
Monsanto
St. Louis, MO

Agronomic field trials were performed in 1996. The data demonstrate that the NewLeaf Y transgenic potato lines are substantially equivalent to the non-transformed parental lines.

**Table 1. Sample Date for NewLeaf Y Agronomic Field Trials**

| | Shepody NLY | | | | |
|---|---|---|---|---|---|
| | Date | | | | |
| | Lethbridge, Alberta | Parma, ID | Island Falls, ME | Lakeview, MI | Coloma, WI |
| **Planting** | 10/5/96 | 4/2296 | 3/6/96 | 5/13/96 | 4/22/96 |
| **Harvest** | NA[1] | 8/22/96 | 7/10/96 | 3/9/96 | 9/23/96 |
| **Stand Count** | NA | NA | 12/07/96 | 7/16/96 | 12/06/96 |
| **Vigor** | 2/8/1996 | NA | 12/07/96 | 7/16/96 | 12/06/96 |

| | RUSSETT BURBANK NL-Y | | | |
|---|---|---|---|---|
| | Date | | | |
| | Lethbridge, Alberta | Parma, ID | Lakeview, MI | Coloma, WI |
| **Planting** | 10/05/1996 | 4/22/96 | 5/13/96 | 4/22/96 |
| **Harvest** | NA | 9/20/96 | 03/09/96 | 9/23/96 |
| **Stand Count** | NA | NA | NA | 12/06/96 |
| **Vigor** | 02/08/1996 | NA | 7/16/96 | 12/06/96 |

| | Hilite NL-Y | | |
|---|---|---|---|
| | Date | | |
| | Island Falls, ME | Parma, ID | Hermiston, OR |
| **Planting** | 3/6/96 | 8/4/96 | 4/15/96 |
| **Harvest** | 7/10/96 | 8/22/96 | 8/26/96 |
| **Stand Count** | 12/07/96 | 5/17/96 | 06/06/96 |
| **Vigor** | 12/07/96 | 6/24/96 | 6/25/96 |

[1]Not Available

**Table 2. Agronomic Field Trial 1 Raw Data**

| Location | Line | Rep | % stand | Vigor (1-5) | Hollow Heart (#/10 tubers) | Brown Center (#/10 tubers) | Tubers (#/plot) | Total Yield (cwt/a) | Specific Gravity |
|---|---|---|---|---|---|---|---|---|---|
| Alberta | RBBT02-06 | 1 | 100 | 4.5 | 0 | 0 | 393 | 371 | 1.091 |
| Alberta | RBBT02-06 | 2 | 95 | 3 | 0 | 0 | 364 | 349 | 1.081 |
| Alberta | RBBT02-06 | 3 | 95 | 4 | 0 | 0 | 376 | 470 | 1.089 |
| Alberta | RBBT02-06 | 4 | 97.5 | 3.5 | 0 | 0 | 359 | 555 | 1.076 |
| Alberta | RBMT15-101 | 1 | 97.5 | 3 | 0 | 0 | 509 | 479 | 1.072 |
| Alberta | RBMT15-101 | 2 | 97.5 | 3.5 | 0 | 0 | 456 | 419 | 1.090 |
| Alberta | RBMT15-101 | 3 | 100 | 3 | 0 | 0 | 402 | 380 | 1.087 |
| Alberta | RBMT15-101 | 4 | 100 | 3 | 0 | 0 | 385 | 505 | 1.079 |
| Alberta | RB Control | 1 | 97.5 | 4 | 0 | 0 | 350 | 449 | 1.078 |
| Alberta | RB Control | 2 | 100 | 4 | 0 | 0 | 377 | 483 | 1.081 |
| Alberta | RB Control | 3 | 100 | 3 | 0 | 0 | 378 | 468 | 1.066 |
| Alberta | RB Control | 4 | 97.5 | 3.5 | 0 | 0 | 329 | 367 | 1.082 |
| Parma, ID | RBBT02-06 | 1 | . | . | 1 | 3 | 311 | 551.7 | 1.073 |
| Parma, ID | RBBT02-06 | 2 | . | . | 2 | 1 | 328 | 548.51 | 1.077 |
| Parma, ID | RBBT02-06 | 3 | . | . | 1 | 3 | 268 | 502.63 | 1.072 |
| Parma, ID | RBBT02-06 | 4 | . | . | 1 | 1 | 298 | 570 | 1.077 |
| Parma, ID | RBMT15-101 | 1 | . | . | 0 | 1 | 243 | 450.65 | 1.075 |
| Parma, ID | RBMT15-101 | 2 | . | . | 0 | 4 | 267 | 642.88 | 1.079 |
| Parma, ID | RBMT15-101 | 3 | . | . | 0 | 1 | 254 | 592.94 | 1.069 |
| Parma, ID | RBMT15-101 | 4 | . | . | 0 | 3 | 243 | 609.49 | 1.07 |
| Parma, ID | RB Control | 1 | . | . | 0 | 0 | 221 | 397.81 | 1.075 |
| Parma, ID | RB Control | 2 | . | . | 1 | 2 | 267 | 448.04 | 1.074 |
| Parma, ID | RB Control | 3 | . | . | 2 | 0 | 285 | 547.64 | 1.071 |
| Parma, ID | RB Control | 4 | . | . | 0 | 2 | 251 | 551.12 | 1.073 |
| Michigan | RBBT02-06 | 1 | . | 4 | 1 | 0 | 390 | 358.72 | 1.074 |
| Michigan | RBBT02-06 | 2 | . | 4 | 2 | 0 | 373 | 372.82 | 1.076 |
| Michigan | RBBT02-06 | 3 | . | 4 | 0 | 1 | 343 | 358.16 | 1.074 |
| Michigan | RBBT02-06 | 4 | . | 4 | 1 | 0 | 338 | 352.39 | 1.071 |
| Michigan | RBBT02-06 | 5 | . | 5 | 0 | 0 | . | 356.79 | 1.072 |
| Michigan | RBBT02-06 | 6 | . | 4 | 0 | 0 | . | 390.93 | 1.073 |

**Table 2 continued.**

| Location | Line | Rep | % stand | Vigor (1-5) | Hollow Heart (#/10 tubers) | Brown Center (#/10 tubers) | Tubers (#/plot) | Total Yield (cwt/a) | Specific Gravity |
|---|---|---|---|---|---|---|---|---|---|
| Michigan | RBBT02-06 | 7 | . | 4 | 0 | 0 | . | 346.62 | 1.073 |
| Michigan | RBBT02-06 | 8 | . | 5 | 1 | 0 | . | 420.49 | 1.075 |
| Michigan | RBMT15-34 | 1 | . | 3 | 0 | 0 | 331 | 329.87 | 1.074 |
| Michigan | RBMT15-34 | 2 | . | 3 | 0 | 0 | 349 | 353.91 | 1.077 |
| Michigan | RBMT15-34 | 3 | . | 3 | 0 | 1 | 298 | 326.11 | 1.072 |
| Michigan | RBMT15-34 | 4 | . | 4 | 0 | 0 | 325 | 370.66 | 1.071 |
| Michigan | RBMT15-34 | 5 | . | 4 | 0 | 0 | . | 354.71 | 1.073 |
| Michigan | RBMT15-34 | 6 | . | 4 | 0 | 0 | . | 387.64 | 1.074 |
| Michigan | RBMT15-34 | 7 | . | 3 | 0 | 0 | . | 370.41 | 1.073 |
| Michigan | RBMT15-34 | 8 | . | 5 | 1 | 0 | . | 435.96 | 1.073 |
| Michigan | RBMT15-101 | 1 | . | 4 | 0 | 0 | 391 | 361.20 | 1.077 |
| Michigan | RBMT15-101 | 2 | . | 5 | 1 | 0 | 391 | 398.30 | 1.076 |
| Michigan | RBMT15-101 | 3 | . | 4 | 1 | 0 | 414 | 400.62 | 1.075 |
| Michigan | RBMT15-101 | 4 | . | 5 | 0 | 0 | 377 | 395.57 | 1.071 |
| Michigan | RBMT15-101 | 5 | . | 5 | 0 | 0 | . | 370.17 | 1.071 |
| Michigan | RBMT15-101 | 6 | . | 4 | 0 | 0 | . | 423.14 | 1.072 |
| Michigan | RBMT15-101 | 7 | . | 3 | 0 | 0 | . | 409.76 | 1.075 |
| Michigan | RBMT15-101 | 8 | . | 4 | 0 | 0 | . | 478.02 | 1.075 |
| Michigan | RBMT15-123 | 1 | . | 3 | 0 | 0 | 394 | 314.33 | 1.077 |
| Michigan | RBMT15-123 | 2 | . | 3 | 1 | 0 | 380 | 353.43 | 1.073 |
| Michigan | RBMT15-123 | 3 | . | 4 | 0 | 0 | 321 | 370.25 | 1.073 |
| Michigan | RBMT15-123 | 4 | . | 4 | 1 | 0 | 407 | 364.25 | 1.073 |
| Michigan | RBMT15-123 | 5 | . | 4 | 0 | 0 | . | 352.95 | 1.073 |
| Michigan | RBMT15-123 | 6 | . | 3 | 0 | 0 | . | 363.68 | 1.075 |
| Michigan | RBMT15-123 | 7 | . | 3 | 0 | 0 | . | 301.75 | 1.076 |
| Michigan | RBMT15-123 | 8 | . | 4 | 1 | 1 | . | 333.48 | 1.074 |
| Michigan | RB-CONTROL | 1 | . | 4 | 0 | 0 | 293 | 364.25 | 1.075 |
| Michigan | RB-CONTROL | 2 | . | 4 | 0 | 1 | 300 | 354.15 | 1.074 |
| Michigan | RB-CONTROL | 3 | . | 4 | 0 | 0 | 282 | 356.31 | 1.072 |
| Michigan | RB-CONTROL | 4 | . | 5 | 0 | 1 | 314 | 406.31 | 1.078 |
| Michigan | RB-CONTROL | 5 | . | 5 | 1 | 0 | . | 377.79 | 1.049 |
| Michigan | RB-CONTROL | 6 | . | 5 | 0 | 0 | . | 375.14 | 1.073 |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Michigan | RB-CONTROL | 7 | . | 5 | 0 | 1 | . | 352.23 | 1.071 |
| Michigan | RB-CONTROL | 8 | . | 5 | 0 | 3 | . | 379.39 | 1.071 |
| Wisconsin | RBBT02-06 | 1 | 83 | 3 | 2 | 0 | 477 | 599.76 | 1.088 |
| Wisconsin | RBBT02-06 | 2 | 88 | 1.5 | 3 | 0 | 410 | 543.36 | 1.085 |
| Wisconsin | RBBT02-06 | 3 | 95 | 3 | 6 | 0 | 476 | 556.21 | 1.085 |
| Wisconsin | RBBT02-06 | 4 | 93 | 3 | 0 | 0 | 620 | 655.3 | 1.086 |
| Wisconsin | RBBT02-06 | 5 | 100 | 3 | 0 | 0 | 589 | 633.74 | 1.091 |
| Wisconsin | RBBT02-06 | 6 | 95 | 2.5 | 3 | 0 | 563 | 945.38 | 1.088 |
| Wisconsin | RBMT15-34 | 1 | 95 | 2 | 2 | 0 | 418 | 581.25 | 1.085 |
| Wisconsin | RBMT15-34 | 2 | 98 | 1.5 | 7 | 0 | 508 | 632.21 | 1.089 |
| Wisconsin | RBMT15-34 | 3 | 100 | 1.5 | 0 | 0 | 476 | 589.31 | 1.085 |
| Wisconsin | RBMT15-34 | 4 | 95 | 1 | 0 | 0 | 492 | 620.67 | 1.088 |
| Wisconsin | RBMT15-34 | 5 | 90 | 1.5 | 4 | 0 | 449 | 639.83 | 1.087 |
| Wisconsin | RBMT15-34 | 6 | 88 | 1.5 | 5 | 0 | 454 | 638.09 | 1.087 |
| Wisconsin | RBMT15-101 | 1 | 98 | 3 | 2 | 0 | 626 | 672.06 | 1.084 |
| Wisconsin | RBMT15-101 | 2 | 98 | 2 | 5 | 0 | 507 | 543.14 | 1.086 |
| Wisconsin | RBMT15-101 | 3 | 98 | 3 | 1 | 0 | 564 | 603.68 | 1.085 |
| Wisconsin | RBMT15-101 | 4 | 100 | 2 | 2 | 0 | 545 | 662.26 | 1.086 |
| Wisconsin | RBMT15-101 | 5 | 95 | 2.5 | 0 | 0 | 620 | 705.6 | 1.086 |
| Wisconsin | RBMT15-101 | 6 | 100 | 2.5 | 3 | 0 | 633 | 666.18 | 1.088 |
| Wisconsin | RBMT15-123 | 1 | 98 | 2 | 2 | 0 | 431 | 522.89 | 1.085 |
| Wisconsin | RBMT15-123 | 2 | 95 | 2 | 1 | 0 | 563 | 569.93 | 1.089 |
| Wisconsin | RBMT15-123 | 3 | 93 | 2 | 4 | 0 | 500 | 554.46 | 1.085 |
| Wisconsin | RBMT15-123 | 4 | 88 | 2 | 0 | 0 | 567 | 633.08 | 1.09 |
| Wisconsin | RBMT15-123 | 5 | 85 | 2 | 2 | 0 | 523 | 616.97 | 1.089 |
| Wisconsin | RBMT15-123 | 6 | 90 | 2 | 1 | 0 | 545 | 575.37 | 1.089 |
| Wisconsin | RB-CONTROL | 1 | 95 | 1.5 | 3 | 0 | 409 | 647.24 | 1.086 |
| Wisconsin | RB-CONTROL | 2 | 95 | 1.5 | 5 | 0 | 395 | 546.62 | 1.086 |
| Wisconsin | RB-CONTROL | 3 | 88 | 1 | 0 | 0 | 439 | 618.93 | 1.085 |
| Wisconsin | RB-CONTROL | 4 | 93 | 2 | 1 | 0 | 412 | 599.98 | 1.09 |
| Wisconsin | RB-CONTROL | 5 | 88 | 2 | 1 | 0 | 422 | 692.1 | 1.092 |
| Wisconsin | RB-CONTROL | 6 | 93 | 2 | 4 | 0 | 437 | 702.34 | 1.083 |

**Table 3. Agronomic Field Trial 1 LSD of Means.**

**Alberta,Canada**

| Line | %Stand | Vigor | Hollow Heart | Brown Center | Tubers (#/plot) | Total Yield (cwt/A) | Specific Gravity |
|---|---|---|---|---|---|---|---|
| SEMT15-02 | 96.87 | 3.75 | 0 | 0 | 373 b | 435.93 | 1.0842 |
| SEMT15-15 | 98.75 | 3.12 | 0 | 0 | 438 a | 445.61 | 1.082 |
| SE-CONTROL | 98.7 | 3.62 | 0 | 0 | 358 b | 441.84 | 1.0767 |
| LSD(0.05) | 3.75 | 0.9 | 0 | 0 | 55 | 140.12 | 0.015 |

**Parma,ID(Thill)**

| Line | %Stand | Vigor | Hollow Heart | Brown Center | Tubers (#/plot) | Total Yield (cwt/A) | Specific Gravity |
|---|---|---|---|---|---|---|---|
| RBBT02-06 | | | 12.5 a | 20 | 301 a | 543.21 | 1.0747 |
| RBMT15-101 | | | 0 b | 22.5 | 251 b | 573.99 | 1.0732 |
| RB-CONTROL | | | 7.5 ab | 10 | 256 b | 486.15 | 1.0732 |
| LSD (0.05) | | | 10.4 | 24.97 | 39 | 102.79 | 0.004 |

**Lakeview, MI**

| Line | %Stand | Vigor | Hollow Heart | Brown Center | Tubers (#/plot) | Total Yield (cwt/A) | Specific Gravity |
|---|---|---|---|---|---|---|---|
| RBBT02-06 | | 4.25 a | 6.25 a | 1.25 b | 361 a | 369.59 b | 1.0735 |
| RBMT15-34 | | 3.62 b | 1.25 b | 1.25 b | 325 b | 366.16 bc | 1.0733 |
| RBMT15-101 | | 4.25 a | 2.5 ab | 0 b | 393 a | 404.58 a | 1.074 |
| RBMT15-123 | | 3.5 b | 3.75 ab | 1.25 b | 375 a | 344.25 c | 1.0742 |
| RB-CONTROL | | 4.62 a | 1.25 b | 7.5 a | 297 b | 370.68 b | 1.0703 |
| LSD (0.05) | | 0.49 | 4.82 | 5.3 | 34.97 | 23.13 | 0.004 |

**Table 3 continued.**

| Coloma, WI Line | %Stand | Vigor | Hollow Heart | Brown Center | Tubers (#/plot) | Total Yield (cwt/A) | Specific Gravity |
|---|---|---|---|---|---|---|---|
| RBBT02-06 | 92.08 b | 2.66 a | 23.33 | 0 | 504 b | 585.09 ab | 1.0871 |
| RBMT15-34 | 94.16 ab | 1.5 c | 30 | 0 | 446 bc | 551.26 ab | 1.0868 |
| RBMT15-101 | 97.91 a | 2.5 a | 21.67 | 0 | 571 a | 605.16 a | 1.0858 |
| RBMT15-123 | 91.25 b | 2 b | 16.67 | 0 | 510 ab | 537.8 ab | 1.0878 |
| RB-CONTROL | 91.66 b | 1.66 bc | 23.33 | 0 | 387 c | 520.66 b | 1.087 |
| LSD (0.05) | 5.75 | 0.47 | 22.23 | 0 | 64 | 72.28 | 0.002 |

## Table 4. Agronomic Field Trial 1 ANOVA.

**Lethbridge, Alberta, Canada**

Respons % stand
Effect Test

| Source | DF | Sum of Squares | F Ratio | Prob>F |
|---|---|---|---|---|
| clone | 2 | 9.375 | 1 | 0.4219 |
| REP | 3 | 1.5625 | 0.1111 | 0.9504 |
| Error | 6 | 28.125 | | |
| C Total | 11 | 39.063 | | |

Response Vigor (1-5)
Effect Test

| Source | DF | Sum of Squares | F Ratio | Prob>F |
|---|---|---|---|---|
| Clone | 2 | 0.875 | 1.6154 | 0.2746 |
| Rep | 3 | 0.5 | 0.6154 | 0.6298 |
| Error | 6 | 1.625 | | |
| C Total | 11 | 3 | | |

Respons Tubers/plot
Effect Test

| Source | DF | Sum of Squares | F Ratio | Prob>F |
|---|---|---|---|---|
| Clone | 2 | 14341 | 7.0122 | 0.0269 |
| Rep | 3 | 5685.7 | 1.8534 | 0.2382 |
| Error | 6 | 6135.3 | | |
| C Total | 11 | 26162 | | |

**Parma, ID**

Respons Hollow Heart
Effect Test

| Source | DF | Sum of Squares | F Ratio | Prob>F |
|---|---|---|---|---|
| clone | 2 | 3.1667 | 4.3846 | 0.067 |
| REP | 3 | 1.3333 | 1.2308 | 0.3776 |
| Error | | | | |
| C Total | | | | |

Response Brown Center
Effect Test

| Source | DF | Sum of Squares | F Ratio | Prob>F |
|---|---|---|---|---|
| clone | 2 | 3.5 | 0.84 | 0.4768 |
| REP | 3 | 2.25 | 0.36 | 0.7845 |
| Error | 6 | 12.5 | | |
| C Total | 11 | 18.25 | | |

Respons Tubers/plot
Effect Test

| Source | DF | Sum of Squares | F Ratio | Prob>F |
|---|---|---|---|---|
| clone | 2 | 6021.2 | 5.8079 | 0.0395 |
| REP | 3 | 1419.3 | 0.9127 | 0.4889 |
| Error | 6 | 3110.2 | | |
| C Total | 11 | 10551 | | |

**Lakeview, MI**

Respons Vigor (1-5)
Effect Test

| Source | DF | Sum of Squares | F Ratio | Prob>F |
|---|---|---|---|---|
| clone | 4 | 7.15 | 7.7597 | 0.0002 |
| REP | 7 | 6.3 | 3.907 | 0.0043 |
| Error | 28 | 6.45 | | |
| C Total | 39 | 19.9 | | |

Response Hollow Heart
Effect Test

| Source | DF | Sum of Squares | F Ratio | Prob>F |
|---|---|---|---|---|
| clone | 4 | 1.4 | 1.5806 | 0.2069 |
| REP | 7 | 2.8 | 1.8065 | 0.1255 |
| Error | 28 | 6.2 | | |
| C Total | 39 | 10.4 | | |

Respons Brown Center
Effect Test

| Source | DF | Sum of Squares | F Ratio | Prob>F |
|---|---|---|---|---|
| clone | 4 | 2.85 | 2.6424 | 0.0546 |
| REP | 7 | 2.575 | 1.3642 | 0.2589 |
| Error | 28 | 7.55 | | |
| C Total | 39 | 12.975 | | |

**Coloma, WI**

Respons %Stand
Effect Test

| Source | DF | Sum of Squares | F Ratio | Prob>F |
|---|---|---|---|---|
| clone | 4 | 179.67 | 2.0817 | 0.1213 |
| REP | 5 | 35.467 | 0.3288 | 0.8896 |
| Error | 20 | 431.53 | | |
| C Total | 29 | 646.67 | | |

Response Vigor (1-5)
Effect Test

| Source | DF | Sum of Squares | F Ratio | Prob>F |
|---|---|---|---|---|
| clone | 4 | 6.2 | 10 | 0.0001 |
| REP | 5 | 1.0667 | 1.3763 | 0.275 |
| Error | 20 | 3.1 | | |
| C Total | 29 | 10.367 | | |

Respons Hollow Heart
Effect Test

| Source | DF | Sum of Squares | F Ratio | Prob>F |
|---|---|---|---|---|
| clone | 4 | 5.4667 | 0.4012 | 0.8055 |
| REP | 5 | 40.7 | 2.3894 | 0.0746 |
| Error | 20 | 68.133 | | |
| C Total | 29 | 114.3 | | |

**Table 5. Agronomic Field Trial 2 Raw Data.**

| location | Line | REP | % stand | * Vigor rating 1-3 scale at Wisconsin Vigor (1-5)* | Hollow Heart | Brown Center | Tubers (#/plot) | Total Yield (cwt/a) | Specific Gravity |
|---|---|---|---|---|---|---|---|---|---|
| Alberta, Canada | SEMT15-02 | 1 | 97.5 | 4 | 0 | 0 | 298 | 537 | 1.089 |
| Alberta, Canada | SEMT15-02 | 2 | 95 | 3 | 0 | 0 | 296 | 471 | 1.096 |
| Alberta, Canada | SEMT15-02 | 3 | 97.5 | 2 | 0 | 0 | 340 | 502 | 1.074 |
| Alberta, Canada | SEMT15-02 | 4 | 97.5 | 3 | 3 | 0 | 278 | 408 | 1.091 |
| Alberta, Canada | SEMT15-15 | 1 | 100 | 3 | 0 | 0 | 343 | 467 | 1.084 |
| Alberta, Canada | SEMT15-15 | 2 | 100 | 4 | 0 | 0 | 270 | 514 | 1.088 |
| Alberta, Canada | SEMT15-15 | 3 | 97.5 | 2 | 0 | 0 | 337 | 472 | 1.098 |
| Alberta, Canada | SEMT15-15 | 4 | 100 | 4 | 1 | 0 | 257 | 410 | 1.069 |
| Alberta, Canada | SHEPODY | 1 | 100 | 4 | 1 | 0 | 286 | 443 | 1.086 |
| Alberta, Canada | SHEPODY | 2 | 100 | 3 | 1 | 0 | 260 | 499 | 1.077 |
| Alberta, Canada | SHEPODY | 3 | 100 | 2 | 0 | 0 | 282 | 338 | 1.094 |
| Alberta, Canada | SHEPODY | 4 | 100 | 3 | 0 | 0 | 239 | 438 | 1.08 |
| Idaho, Thill | SEMT15-02 | 1 | . | . | 2 | 0 | 111 | 575 | 1.071 |
| Idaho, Thill | SEMT15-02 | 2 | . | . | 0 | 0 | 96 | 489 | 1.078 |
| Idaho, Thill | SEMT15-02 | 3 | . | . | 2 | 0 | 81 | 431 | 1.07 |
| Idaho, Thill | SEMT15-02 | 4 | . | . | 1 | 0 | 103 | 467 | 1.075 |
| Idaho, Thill | SEMT15-7 | 1 | . | . | 1 | 0 | 99 | 476 | 1.075 |
| Idaho, Thill | SEMT15-7 | 2 | . | . | 1 | 0 | 70 | 415 | 1.074 |
| Idaho, Thill | SEMT15-7 | 3 | . | . | 4 | 0 | 92 | 492 | 1.073 |
| Idaho, Thill | SEMT15-7 | 4 | . | . | 4 | 0 | 89 | 520 | 1.071 |
| Idaho, Thill | SEMT15-15 | 1 | . | . | 0 | 0 | 88 | 408 | 1.076 |
| Idaho, Thill | SEMT15-15 | 2 | . | . | 0 | 0 | 103 | 532 | 1.074 |
| Idaho, Thill | SEMT15-15 | 3 | . | . | 0 | 0 | 84 | 412 | 1.076 |
| Idaho, Thill | SEMT15-15 | 4 | . | . | 0 | 0 | 102 | 505 | 1.076 |
| Idaho, Thill | SE-Control | 1 | . | . | 0 | 0 | 117 | 620 | 1.074 |
| Idaho, Thill | SE-Control | 2 | . | . | 0 | 0 | 100 | 512 | 1.078 |
| Idaho, Thill | SE-Control | 3 | . | . | 1 | 0 | 112 | 585 | 1.071 |

**Table 5 continued.**

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Idaho, Thill | SE-Control | 4 | | | 1 | 0 | 120 | 558 | 1.074 |
| ME | SEMT15-02 | 1 | 100 | 4 | 2 | 1 | 316 | 361 | |
| ME | SEMT15-02 | 2 | 100 | 4 | 0 | 0 | 321 | 447 | |
| ME | SEMT15-02 | 3 | 100 | 4 | 1 | 0 | 375 | 444 | |
| ME | SEMT15-02 | 4 | 100 | 4 | 1 | 1 | 287 | 502 | |
| ME | SEMT15-02 | 5 | 97.5 | 5 | 1 | 0 | 313 | 406 | |
| ME | SEMT15-02 | 6 | 100 | 4 | 1 | 0 | 322 | 482 | |
| ME | SEMT15-7 | 1 | 100 | 4 | 0 | 1 | 259 | 382 | |
| ME | SEMT15-7 | 2 | 100 | 3 | 2 | 2 | 310 | 420 | |
| ME | SEMT15-7 | 3 | 100 | 4 | 1 | 2 | 251 | 404 | |
| ME | SEMT15-7 | 4 | 100 | 4 | 0 | 2 | 276 | 365 | |
| ME | SEMT15-7 | 5 | 97.5 | 3 | 0 | 0 | 288 | 377 | |
| ME | SEMT15-7 | 6 | 100 | 5 | 1 | 1 | 254 | 395 | |
| ME | SEMT15-15 | 1 | 100 | 3 | 0 | 0 | 322 | 425 | |
| ME | SEMT15-15 | 2 | 100 | 4 | 1 | 1 | 335 | 403 | |
| ME | SEMT15-15 | 3 | 100 | 3 | 3 | 1 | 268 | 362 | |
| ME | SEMT15-15 | 4 | 100 | 3 | 1 | 0 | 308 | 419 | |
| ME | SEMT15-15 | 5 | 97.5 | 3 | 3 | 0 | 290 | 396 | |
| ME | SEMT15-15 | 6 | 100 | 5 | 1 | 2 | 320 | 378 | |
| ME | SE-CONTROL | 1 | 97.5 | 4 | 5 | 1 | 283 | 426 | |
| ME | SE-CONTROL | 2 | 100 | 4 | 1 | 0 | 322 | 378 | |
| ME | SE-CONTROL | 3 | 100 | 4 | 1 | 1 | 302 | 471 | |
| ME | SE-CONTROL | 4 | 100 | 4 | 2 | 1 | 286 | 399 | |
| ME | SE-CONTROL | 5 | 100 | 4 | 2 | 1 | 316 | 514 | |
| ME | SE-CONTROL | 6 | 100 | 5 | 3 | 0 | 318 | 403 | |
| MI | SEMT15-02 | 1 | 78.75 | 5 | 2 | 0 | 250 | 385 | 1.079 |
| MI | SEMT15-02 | 2 | 62.5 | 5 | 0 | 0 | 245 | 362 | 1.074 |
| MI | SEMT15-02 | 3 | 57.5 | 4 | 2 | 0 | 220 | 343 | 1.077 |
| MI | SEMT15-02 | 4 | 67.5 | 4 | 2 | 0 | 264 | 414 | 1.072 |
| MI | SEMT15-02 | 5 | 75 | 3 | 0 | 0 | 233 | 324 | 1.079 |
| MI | SEMT15-02 | 6 | 70 | 3 | 2 | 0 | 251 | 388 | 1.077 |
| MI | SEMT15-7 | 1 | 62.5 | 3 | 0 | 0 | 251 | 311 | 1.077 |

**Table 5 continued.**

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| MI | SEMT15-7 | 2 | 55 | 4 | 0 | 0 | 218 | 355 | 1.077 |
| MI | SEMT15-7 | 3 | 57.5 | 4 | 1 | 0 | 213 | 344 | 1.076 |
| MI | SEMT15-7 | 4 | 58.75 | 3 | 2 | 0 | 205 | 341 | 1.076 |
| MI | SEMT15-7 | 5 | 50 | 5 | 2 | 0 | 191 | 330 | 1.073 |
| MI | SEMT15-7 | 6 | 53.75 | 4 | 0 | 0 | 189 | 309 | 1.072 |
| MI | SEMT15-15 | 1 | 57.5 | 5 | 1 | 0 | 242 | 353 | 1.077 |
| MI | SEMT15-15 | 2 | 75 | 5 | 0 | 0 | 243 | 365 | 1.079 |
| MI | SEMT15-15 | 3 | 68.75 | 5 | 1 | 0 | 201 | 373 | 1.075 |
| MI | SEMT15-15 | 4 | 71.25 | 5 | 2 | 0 | 245 | 386 | 1.075 |
| MI | SEMT15-15 | 5 | 60 | 5 | 3 | 0 | 259 | 384 | 1.075 |
| MI | SEMT15-15 | 6 | 78.75 | 4 | 0 | 0 | 292 | 324 | 1.084 |
| MI | SE-CONTROL | 1 | 56.25 | 4 | 2 | 0 | 269 | 343 | 1.069 |
| MI | SE-CONTROL | 2 | 68.75 | 5 | 3 | 0 | 259 | 406 | 1.080 |
| MI | SE-CONTROL | 3 | 81.25 | 5 | 3 | 0 | 254 | 383 | 1.073 |
| MI | SE-CONTROL | 4 | 73.75 | 5 | 1 | 0 | 228 | 374 | 1.072 |
| MI | SE-CONTROL | 5 | 72.5 | 4 | 3 | 0 | 260 | 394 | 1.077 |
| MI | SE-CONTROL | 6 | 86.25 | 4 | 4 | 0 | 276 | 412 | 1.074 |
| WI | SEMT15-02 | 1 | 97.5 | 3 | 0 | 0 | 250 | 453 | 1.081 |
| WI | SEMT15-02 | 2 | 97.5 | 2 | 0 | 0 | 238 | 391 | 1.085 |
| WI | SEMT15-02 | 3 | 92.5 | 2 | 1 | 0 | 267 | 453 | 1.084 |
| WI | SEMT15-02 | 4 | 97.5 | 3 | 1 | 0 | 264 | 440 | 1.082 |
| WI | SEMT15-02 | 5 | 100 | 3 | 0 | 0 | 292 | 483 | 1.084 |
| WI | SEMT15-7 | 1 | 100 | 2 | 0 | 0 | 103 | 407 | 1.086 |
| WI | SEMT15-7 | 2 | 92.5 | 1 | 0 | 0 | 190 | 389 | 1.08 |
| WI | SEMT15-7 | 3 | 90 | 1 | 0 | 0 | 261 | 452 | 1.077 |
| WI | SEMT15-7 | 4 | 92.5 | 1 | 0 | 0 | 250 | 448 | 1.085 |
| WI | SEMT15-7 | 5 | 97.5 | 1 | 0 | 0 | 197 | 444 | 1.082 |
| WI | SEMT15-15 | 1 | 100 | 3 | 0 | 0 | 220 | 428 | 1.084 |
| WI | SEMT15-15 | 2 | 100 | 2 | 0 | 0 | 162 | 326 | 1.08 |
| WI | SEMT15-15 | 3 | 100 | 2 | 1 | 0 | 283 | 469 | 1.078 |

**Table 5 continued.**

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| WI | SEMT15-15 | 4 | 100 | 2 | 0 | 0 | 272 | 495 | 1.083 |
| WI | SEMT15-15 | 5 | 95 | 3 | 0 | 0 | 300 | 505 | 1.083 |
| WI | SE-CONTROL | 1 | 100 | 2 | 1 | 0 | 212 | 426 | 1.078 |
| WI | SE-CONTROL | 2 | 92.5 | 2 | 0 | 0 | 220 | 430 | 1.081 |
| WI | SE-CONTROL | 3 | 87.5 | 2 | 1 | 0 | 219 | 438 | 1.077 |
| WI | SE-CONTROL | 4 | 97.5 | 3 | 0 | 0 | 231 | 441 | 1.08 |
| WI | SE-CONTROL | 5 | 87.5 | 2 | 0 | 0 | 249 | 466 | 1.073 |

**Table 6. Field Trial 2 LSD of Means.**

**Alberta,Canada**

| Line | %Stand | Vigor | HollowHeart | BrownCenter | Tubers/plot | Totalcwt/A | SpecificGravity |
|---|---|---|---|---|---|---|---|
| SEMT15-02 | 100.00 b | 3.9 | 7.5 | 0 | 303 a | 479.62 | 1.0875 |
| SEMT15-15 | 99.37 a | 4.1 | 2.5 | 0 | 301 a | 465.99 | 1.0847 |
| SE-CONTROL | 96.87 a | 4.0 | 5 | 0 | 266 b | 429.33 | 1.0842 |
| LSD(0.05) | 0.01 | ns | ns | ns | 31 | ns | ns |

**Parma,ID(Thill)**

| Line | %Stand | HollowHeart | BrownCenter | Tubers/plot | Totalcwt/A | SpecificGravity |
|---|---|---|---|---|---|---|
| SEMT15-02 | | 12.5 ab | 0 | 97 ab | 490.24 ab | 1.0735 |
| SEMT15-7 | | 25 a | 0 | 87 b | 475.72 ab | 1.0732 |
| SEMT15-15 | | 0 b | 0 | 94 b | 464.59 b | 1.0755 |
| SE-CONTROL | | 5 b | 0 | 112 a | 568.88 a | 1.0742 |
| LSD(0.05) | | 14.17 | ns | 16 | 93.86 | ns |

**Table 6 continued.**

**IslandFalls,ME**

| Line | %Stand | Vigor | HollowHeart | BrownCenter | Tubers/plot | Total cwt/A |
|---|---|---|---|---|---|---|
| SEMT15-02 | 100 | 4.16 | 10 ab | 3.33 b | 322 a | 440.27 |
| SEMT15-7 | 100 | 3.83 | 6.67 b | 13.33 a | 273 b | 390.41 |
| SEMT15-15 | 100 | 3.5 | 15 ab | 6.67 ab | 307 a | 397.25 |
| SE-CONTROL | 100 | 4.16 | 23.3 a | 6.67 ab | 304 a | 431.93 |
| LSD(0.05) | ns | ns | 15 | 8.89 | 29.68 | ns |

**Lakeview, MI**

| Line | %Stand | Vigor | HollowHeart | BrownCenter | Tubers/plot | Totalcwt/A | SpecificGravity |
|---|---|---|---|---|---|---|---|
| SEMT15-02 | 69 | 4 ab | 13.33 ab | 0 | 243 a | 369.55 a | 1.0763 |
| SEMT15-7 | 56 * | 2.83 b | 8.33 b | 0 | 211 b | 331.59 b | 1.0751 |
| SEMT15-15 | 69 | 4.83 a | 11.66 b | 0 | 247 a | 364.12 ab | 1.0775 |
| SE-CONTROL | 73 | 4.5 ab | 26.67 a | 0 | 257 a | 385.33 a | 1.0741 |
| LSD(0.05) | | 0.83 | 13.6 | ns | 26 | 32.67 | ns |

* = Significantly different from control using orthogonal contrast.

**Hancock, WI**

| Line | % Stand Count | Vigor | HollowHeart | BrownCenter | Tubers/plot | Totalcwt/A | Specific Gravity |
|---|---|---|---|---|---|---|---|
| SEMT15-02 | 97 ab | 2.6 a | 4 | 0 | 226 a | 319.91 a | 1.0832 a |
| SEMT15-7 | 94.5 ab | 1.2 b | 0 | 0 | 155 c | 252.1 b | 1.082 a |
| SEMT15-15 | 99 a | 2.4 a | 2 | 0 | 210 ab | 318.56 a | 1.0816 ab |
| SE-CONTROL | 93 b | 2.2 a | 4 | 0 | 175 bc | 255.54 b | 1.0778 b |
| LSD (0.05) | 4.8 | 0.59 | ns | ns | 47 | 55.7 | 0.003 |

## Table 7. Field Trial 2 ANOVA.

**Alberta, Canada**

Response: % stand
Effect Test

| Source | DF | Sum of Squares | F Ratio | Prob>F |
|---|---|---|---|---|
| clone | 2 | 21.875 | 9.3333 | 0.0081 |
| REP | 1 | 0 | 0 | 1 |
| Error | 8 | 9.375 | | |
| C Total | 11 | 31.25 | | |

Response: Vigor(1-5)
Effect Test

| Source | DF | Sum of Squar | F Ratio | Prob>F |
|---|---|---|---|---|
| clone | 2 | 0.16666667 | 0.1124 | 0.8951 |
| REP | 1 | 0.81666667 | 1.1011 | 0.3247 |
| Error | 8 | 5.9333333 | | |
| C Total | 11 | 6.9166667 | | |

Response: ollow Heart
Effect Test

| Source | DF | Sum of Sqs |
|---|---|---|
| clone | 2 | 0.5 |
| REP | 1 | 1.0666667 |
| Error | 8 | 7.4333333 |
| C Total | 11 | 9 |

**Parma, ID (Thill)** **No Brown center observed; therefore no ANOVA.

Response: Hollow Heart
Effect Test

| Source | DF | Sum of Squares | F Ratio | Prob>F |
|---|---|---|---|---|
| clone | 3 | 14.1875 | 6.0265 | 0.0155 |
| REP | 3 | 5.6875 | 2.4159 | 0.1337 |
| Error | 9 | 7.0625 | | |
| C Total | 15 | 26.9375 | | |

Response: Tubers/plot
Effect Test

| Source | DF | Sum of Squar | F Ratio | Prob>F |
|---|---|---|---|---|
| clone | 3 | 1309.6875 | 4.1641 | 0.0417 |
| REP | 3 | 517.6875 | 1.646 | 0.247 |
| Error | 9 | 943.5625 | | |
| C Total | 15 | 2770.9375 | | |

Response: Total Yield
Effect Test

| Source | DF | Sum of Squar |
|---|---|---|
| clone | 3 | 26732.069 |
| REP | 3 | 4517.34 |
| Error | 9 | 30987.467 |
| C Total | 15 | 62236.876 |

**Island Falls, ME**

Response: % stand
Effect Test

| Source | DF | Sum of Squares | F Ratio | Prob>F |
|---|---|---|---|---|
| clone | 3 | 0 | 0 | 1 |
| REP | 5 | 11.458333 | 3.6667 | 0.0229 |
| Error | 15 | 9.375 | | |
| C Total | 23 | 20.833333 | | |

Response: Vigor (1-5)
Effect Test

| Source | DF | Sum of Squar | F Ratio | Prob>F |
|---|---|---|---|---|
| clone | 3 | 1.8333333 | 1.9643 | 0.1627 |
| REP | 5 | 3.3333333 | 2.1429 | 0.1161 |
| Error | 15 | 4.6666667 | | |
| C Total | 23 | 9.8333333 | | |

Response: Hollow Heart
Effect Test

| Source | DF | Sum of Squar |
|---|---|---|
| clone | 3 | 9.4583333 |
| REP | 5 | 1.875 |
| Error | 15 | 22.291667 |
| C Total | 23 | 33.625 |

**Lakeview, MI** **No Brown center observed; therefore no ANOVA.

Response: % stand
Effect Test

| Source | DF | Sum of Squares | F Ratio | Prob>F |
|---|---|---|---|---|
| clone | 3 | 943.42448 | 4.2794 | 0.0227 |
| REP | 5 | 190.16927 | 0.5176 | 0.7592 |
| Error | 15 | 1102.2786 | | |
| C Total | 23 | 2235.8724 | | |

Response: Vigor (1-5)
Effect Test

| Source | DF | Sum of Squar | F Ratio | Prob>F |
|---|---|---|---|---|
| clone | 3 | 3.7916667 | 2.7246 | 0.0811 |
| REP | 5 | 2.2083333 | 0.9521 | 0.4767 |
| Error | 15 | 6.958333 | | |
| C Total | 23 | 12.958333 | | |

Response: Hollow Heart
Effect Test

| Source | DF | Sum of Squar |
|---|---|---|
| clone | 3 | 11.666667 |
| REP | 5 | 4 |
| Error | 15 | 18.333333 |
| C Total | 23 | 34 |

**Hancock, WI**

Response: % stand
Effect Test

| Source | DF | Sum of Squares | F Ratio | Prob>F |
|---|---|---|---|---|
| clone | 3 | 105.9375 | 2.9099 | 0.0781 |
| REP | 4 | 101.875 | 2.0987 | 0.144 |
| Error | 12 | 145.625 | | |
| C Total | 19 | 353.4375 | | |

Response: Vigor (1-5)
Effect Test

| Source | DF | Sum of Squar | F Ratio | Prob>F |
|---|---|---|---|---|
| clone | 3 | 5.8 | 10.546 | 0.0011 |
| REP | 4 | 1.8 | 2.4545 | 0.1024 |
| Error | 12 | 2.2 | | |
| C Total | 19 | 9.8 | | |

Response: Hollow Heart
Effect Test

| Source | DF | Sum of Squar |
|---|---|---|
| clone | 3 | 0.55 |
| REP | 4 | 1.5 |
| Error | 12 | 1.7 |
| C Total | 19 | 3.75 |

Table 8. Field Trial 3 Raw Data.

| Location | Line | Rep | % stand | Vigor (1-5) | Hollow Heart (#/10 tubers) | Brown Center (#/10 tubers) | Tubers (#/plot) | Total Yield (cwt/a) | Specific Gravity |
|---|---|---|---|---|---|---|---|---|---|
| Idaho | HLMT15-03 | 1 | 100 | 4 | 0 | 1 | 441 | 446.45 | 1.078 |
| Idaho | HLMT15-03 | 2 | 100 | 5 | 1 | 0 | 332 | 506.33 | 1.075 |
| Idaho | HLMT15-03 | 3 | 98 | 4 | 2 | 4 | 312 | 425.39 | 1.077 |
| Idaho | HLMT15-03 | 4 | 100 | 4 | 0 | 3 | 337 | 403.62 | 1.077 |
| Idaho | HLMT15-03 | 5 | 100 | 5 | 0 | 1 | 414 | 340.46 | 1.078 |
| Idaho | HLMT15-03 | 6 | 100 | 4 | 2 | 0 | 300 | 378.57 | 1.074 |
| Idaho | HLMT15-15 | 1 | 94 | 5 | 0 | 0 | 356 | 582.56 | 1.073 |
| Idaho | HLMT15-15 | 2 | 100 | 4 | 0 | 0 | 408 | 449.71 | 1.072 |
| Idaho | HLMT15-15 | 3 | 96 | 3 | 1 | 1 | 388 | 395.63 | 1.074 |
| Idaho | HLMT15-15 | 4 | 99 | 3 | 0 | 3 | 343 | 311.79 | 1.071 |
| Idaho | HLMT15-15 | 5 | 99 | 5 | 0 | 0 | 332 | 406.16 | 1.074 |
| Idaho | HLMT15-15 | 6 | 100 | 4 | 0 | 0 | 359 | 408.33 | 1.073 |
| Idaho | HLMT15-46 | 1 | 100 | 4 | 0 | 0 | 431 | 495.45 | 1.074 |
| Idaho | HLMT15-46 | 2 | 91 | 4 | 0 | 0 | 333 | 433.74 | 1.071 |
| Idaho | HLMT15-46 | 3 | 91 | 3 | 0 | 2 | 439 | 407.61 | 1.077 |
| Idaho | HLMT15-46 | 4 | 100 | 3 | 0 | 5 | 408 | 335.74 | 1.079 |
| Idaho | HLMT15-46 | 5 | 90 | 4 | 3 | 0 | 335 | 415.59 | 1.076 |
| Idaho | HLMT15-46 | 6 | 100 | 4 | 0 | 0 | 307 | 431.2 | 1.073 |
| Idaho | HL-CONTROL | 1 | 80 | 3 | 0 | 0 | 300 | 295.09 | 1.084 |
| Idaho | HL-CONTROL | 2 | 100 | 3 | 0 | 0 | 424 | 415.96 | 1.076 |
| Idaho | HL-CONTROL | 3 | 100 | 3 | 0 | 2 | 352 | 408.33 | 1.075 |
| Idaho | HL-CONTROL | 4 | 100 | 3 | 0 | 3 | 342 | 398.17 | 1.075 |
| Idaho | HL-CONTROL | 5 | 100 | 4 | 0 | 1 | 367 | 383.29 | 1.072 |
| Idaho | HL-CONTROL | 6 | 100 | 5 | 0 | 1 | 473 | 411.6 | 1.075 |
| Maine | HLMT15-03 | 1 | 100 | 4 | 0 | 0 | 490 | 370.98 | . |
| Maine | HLMT15-03 | 2 | 100 | 4 | 0 | 0 | 530 | 417.77 | . |
| Maine | HLMT15-03 | 3 | 100 | 4 | 0 | 0 | 596 | 403.03 | . |
| Maine | HLMT15-03 | 4 | 100 | 3 | 0 | 1 | 496 | 461.65 | . |
| Maine | HLMT15-15 | 1 | 100 | 5 | 0 | 0 | 548 | 381.62 | . |
| Maine | HLMT15-15 | 2 | 100 | 5 | 0 | 0 | 565 | 494.17 | . |

**Table 8 continued.**

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Maine | HLMT15-15 | 3 | 97.5 | 3 | 0 | 0 | 538 | 364.05 | . |
| Maine | HLMT15-15 | 4 | 100 | 4 | 0 | 0 | 499 | 290.26 | . |
| Maine | HLMT15-46 | 1 | 100 | 1 | 0 | 0 | 431 | 478.82 | . |
| Maine | HLMT15-46 | 2 | 100 | 1 | 0 | 0 | 469 | 372.72 | . |
| Maine | HLMT15-46 | 3 | 95 | 1 | 2 | 1 | 403 | 382.85 | . |
| Maine | HLMT15-46 | 4 | 100 | 1 | 0 | 0 | 436 | 315.67 | . |
| Maine | HL-CONTROL | 1 | 100 | 5 | 0 | 0 | 398 | 416.75 | . |
| Maine | HL-CONTROL | 2 | 100 | 5 | 0 | 0 | 360 | 393.81 | . |
| Maine | HL-CONTROL | 3 | 100 | 4 | 2 | 0 | 411 | 535.44 | . |
| Maine | HL-CONTROL | 4 | 100 | 4 | 2 | 0 | 374 | 279.12 | . |
| Oregon | HLMT15-03 | 1 | 100 | 5 | 0 | 0 | 354 | 409.67 | 1.07 |
| Oregon | HLMT15-03 | 2 | 100 | 5 | 0 | 0 | 403 | 491.05 | 1.07 |
| Oregon | HLMT15-03 | 3 | 100 | 5 | 0 | 0 | 444 | 500.22 | 1.073 |
| Oregon | HLMT15-03 | 4 | 100 | 5 | 0 | 0 | 378 | 443.18 | 1.073 |
| Oregon | HLMT15-03 | 5 | 97.5 | 5 | 0 | 0 | 416 | 510.99 | 1.068 |
| Oregon | HLMT15-03 | 6 | 97.5 | 5 | 0 | 0 | 417 | 514.98 | 1.072 |
| Oregon | HLMT15-15 | 1 | 100 | 4 | 0 | 0 | 425 | 478.28 | 1.075 |
| Oregon | HLMT15-15 | 2 | 100 | 4 | 0 | 0 | 393 | 427.22 | 1.076 |
| Oregon | HLMT15-15 | 3 | 100 | 5 | 0 | 0 | 421 | 438.39 | 1.075 |
| Oregon | HLMT15-15 | 4 | 100 | 5 | 0 | 0 | 439 | 425.23 | 1.073 |
| Oregon | HLMT15-15 | 5 | 97.5 | 4 | 0 | 0 | 409 | 450.76 | 1.074 |
| Oregon | HLMT15-15 | 6 | 97.5 | 4 | 0 | 0 | 440 | 463.12 | 1.073 |
| Oregon | HLMT15-46 | 1 | 100 | 5 | 0 | 0 | 310 | 436.4 | 1.07 |
| Oregon | HLMT15-46 | 2 | 100 | 5 | 0 | 0 | 346 | 467.51 | 1.069 |
| Oregon | HLMT15-46 | 3 | 100 | 5 | 0 | 0 | 349 | 501.42 | 1.07 |
| Oregon | HLMT15-46 | 4 | 100 | 5 | 0 | 0 | 349 | 480.28 | 1.068 |
| Oregon | HLMT15-46 | 5 | 100 | 4 | 0 | 0 | 353 | 500.62 | 1.07 |
| Oregon | HLMT15-46 | 6 | 97.5 | 4 | 0 | 0 | 335 | 445.17 | 1.073 |
| Oregon | HL-CONTROL | 1 | 100 | 5 | 0 | 0 | 315 | 524.15 | 1.069 |
| Oregon | HL-CONTROL | 2 | 100 | 5 | 0 | 0 | 332 | 483.07 | 1.072 |
| Oregon | HL-CONTROL | 3 | 100 | 5 | 0 | 0 | 343 | 497.43 | 1.069 |
| Oregon | HL-CONTROL | 4 | 97.5 | 5 | 0 | 0 | 322 | 481.87 | 1.07 |
| Oregon | HL-CONTROL | 5 | 100 | 5 | 0 | 0 | 332 | 471.9 | 1.072 |
| Oregon | HL-CONTROL | 6 | 100 | 5 | 0 | 0 | 365 | 536.92 | 1.069 |

Table 9. Field Trial 3 LSD of Means.

**Parma, ID**

| Line | %Stand | Vigor | Hollow Heart | Brown Center | Tubers (#/plot) | Total Yield (cwt/A) | Specific Gravity |
|---|---|---|---|---|---|---|---|
| HLMT15-03 | 79.66 | 4.3 a | 8.33 | 15 | 356 | 416.8 | 1.0765 a |
| HLMT15-15 | 78.33 | 4 ab | 1.66 | 6.67 | 364 | 425.69 | 1.0728 b |
| HLMT15-46 | 76.33 | 3.66 ab | 5 | 11.66 | 375 | 419.88 | 1.075 ab |
| HL-CONTROL | 77.33 | 3.5 b | 0 | 11.66 | 376 | 385.4 | 1.0761 a |
| LSD(0.05) | 4.94 | 0.69 | 10 | 9.06 | 74 | 73.65 | 0.003 |

**Island Falls, ME**

| Line | Vigor | Hollow Heart | Brown Center | Tubers (#/plot) | Total Yield (cwt/A) |
|---|---|---|---|---|---|
| HLMT15-03 | 3.75 a | 0 | 2.5 | 528 a | 413.35 |
| HLMT15-15 | 4.25 a | 0 | 0 | 537 a | 382.52 |
| HLMT15-46 | 1 b | 5 | 2.5 | 434 b | 387.51 |
| HL-CONTROL | 4.5 a | 10 | 0 | 385 b | 406.27 |
| LSD(0.05) | 0.79 | 10.99 | 5.96 | 53.79 | 122.23 |

**Hermiston, OR**

| Line | %Stand | Vigor | Hollow Heart | Brown Center | Tubers (#/plot) | Total Yield (cwt/A) | Specific Gravity |
|---|---|---|---|---|---|---|---|
| HLMT15-03 | 99.16 | 5 a | 0 | 0 | 402 a | 478.35 ab | 1.071 b |
| HLMT15-15 | 99.16 | 4.33 b | 0 | 0 | 421 a | 447.17 b | 1.0743 a |
| HLMT15-46 | 99.58 | 4.66 ab | 0 | 0 | 340 b | 471.9 ab | 1.07 b |
| HL-CONTROL | 99.58 | 5 a | 0 | 0 | 334 b | 499.22 a | 1.0701 b |
| LSD (0.05) | 1.21 | 0.41 | 0 | 0 | 23.31 | 38.47 | 0.002 |

# Table 10 Field Trial 3 ANOVA

**Parma, ID**

**Response: %Stand**
**Effect Test**

| Source | DF | Sum of Squares | F Ratio | Prob>F |
|---|---|---|---|---|
| Clone | 3 | 61.83333 | 0.809 | 0.5084 |
| Rep | 5 | 115.8333 | 0.9093 | 0.5008 |
| Error | 15 | 382.1667 | | |

**Response: Vigor (1-5)**
**Effect Test**

| Source | DF | Sum of Squares | F Ratio | Prob>F |
|---|---|---|---|---|
| Clone | 3 | 2.458333 | 2.5652 | 0.0934 |
| Rep | 5 | 5.375 | 3.3652 | 0.0309 |
| Error | 15 | 4.791667 | | |

**Response: Hollow Heart**
**Effect Test**

| Source | DF | Sum of Squares | F Ratio | Prob>F |
|---|---|---|---|---|
| Clone | 3 | 2.458333 | 1.138 | 0.3652 |
| Rep | 5 | 2.375 | 0.6602 | 0.659 |
| Error | 15 | 10.79167 | | |

**Island Falls, ME**

**Response: %Stand**
**Effect Test**

| Source | DF | Sum of Squares | F Ratio | Prob>F |
|---|---|---|---|---|
| Clone | 3 | 4.296875 | 1 | 0.4363 |
| Rep | 3 | 10.54688 | 2.4545 | 0.1298 |
| Error | 9 | 12.89063 | | |

**Response: Vigor (1-5)**
**Effect Test**

| Source | DF | Sum of Squares | F Ratio | Prob>F |
|---|---|---|---|---|
| Clone | 3 | 31.25 | 41.6667 | <.0001 |
| Rep | 3 | 2.25 | 3 | 0.0677 |
| Error | 9 | 2.25 | | |

**Response: Hollow Heart**
**Effect Test**

| Source | DF | Sum of Squares | F Ratio | Prob>F |
|---|---|---|---|---|
| Clone | 3 | 2.75 | 1.9412 | 0.1936 |
| Rep | 3 | 2.75 | 1.9412 | 0.1936 |
| Error | 9 | 4.25 | | |

**Hermiston, OR**

**Response: %Stand**
**Effect Test**

| Source | DF | Sum of Squares | F Ratio | Prob>F |
|---|---|---|---|---|
| Clone | 3 | 1.041667 | 0.3571 | 0.7847 |
| Rep | 5 | 12.5 | 2.5714 | 0.0715 |
| Error | 15 | 14.58333 | | |

**Response: Vigor (1-5)**
**Effect Test**

| Source | DF | Sum of Squares | F Ratio | Prob>F |
|---|---|---|---|---|
| Clone | 3 | 1.833333 | 5.5 | 0.0094 |
| Rep | 5 | 1 | 1.8 | 0.1734 |
| Error | 15 | 1.666667 | | |

**No Hollow Heart nor Brown Center detected, hence no ANOVA**
**Response: Hollow Heart**
**Effect Test**

| Source | DF | Sum of Squares | F Ratio | Prob>F |
|---|---|---|---|---|
| Clone | | | | |
| Rep | | | | |
| Error | | | | |

# APPENDIX VII

## Virus Challenge Experiment Data
## and
## Data from Seed Certification Trials

(Russet Burbank RBMT15-101, Shepody SEMT15-02 and
Shepody SEMT15-15 Potato Lines are the subject of this petition.
Please disregard data on other lines.)

**Evaluation of NewLeaf® Y Potato Lines for Resistance to Potato Virus Y - Greenhouse Challenge Experiments 1994-1995 HAWAII**

## INTRODUCTION

To evaluate the potato virus Y resistance of NewLeaf Y potato lines of the varieties Russet Burbank (RBMT15-101) and Shepody (SEMT15-02, SEMT15-15), greenhouse experiments were conducted over several years on Hawaii. These experiments, conducted by [ CBI DELETED ], were designed to simulate worst case potato Y virus (PVY) pressure through direct mechanical inoculation. The virus infection was determined through visual observation of symptoms and through use of enzyme linked immunosorbant assays (ELISA) for both NewLeaf Y potato lines and non-transformed (non-transgenic) control lines of the same variety.

## METHODS

The plants were grown from virus-free plantlets, planted one per slot in Speedling trays under greenhouse conditions. Six plants of each line were selected for use in this PVY challenge experiment and transferred to 6 inch grow bags. One plant was held as a non inoculated "healthy" control and 5 (group) were mechanically inoculated with PVY using virus infected tobacco, supplied by Monsanto, as the source. In each trial, one or two of the transgenic lines were compared to non-transgenic control lines of the same variety. At approximately 4 weeks following inoculation, all plants, control and transgenic, were visually observed for symptoms and tested by ELISA for the presence of PVY. Transgenic plants, only, were checked once more, at approximately 8 weeks, for signs of infection.

ELISA tests were conducted at approximately four weeks on all plant groups. Composite samples for conducting ELISA assays were created by selecting one young leaf from each of the five inoculated plants and pooling them together for testing. Symptomless plant groups were tested twice by ELISA for the presence of PVY. The second, confirmatory ELISA was conducted one week after the first to confirm negative results.

The transgenic NewLeaf Y potato lines RBMT15-101, SEMT15-02, and SEMT15-15 were all tested in this series of greenhouse trials.

Protocols as provided by [ CBI DELETED ] are included as Attachment 1.

® Registered Trademark of Monsanto Company, St. Louis, MO 63198

## RESULTS

Results for each of the 5 trials conducted are shown in Tables 1-5. Overall, all plants grew normally. The "healthy" controls of transgenic lines and non-transgenic control lines did not exhibit signs of virus infection nor test positive with ELISA (data not shown in this report). In every trial, the NewLeaf Y potato lines showed no visible symptoms of PVY infection and did not test positive for virus infection using ELISA's. Control non-transgenic lines exhibited more PVY mosaic symptoms and had ELISA titers much higher than those for the transgenic lines.

## DISCUSSION

In this greenhouse testing, where extreme virus exposure was imposed through mechanical inoculation, transformed potato lines RBMT15-101, SEMT15-02, and SEMT15-15 showed resistance to infection by PVY. Control lines of the same varieties were infected using the same procedure and exhibited both visual symptoms and positive ELISA results. It can be concluded that these NewLeaf Y potato lines are highly resistant to infection by potato virus Y and are not symptomless carriers of the virus.

**TABLE 1. Greenhouse Virus Challenge Experiments Using PVY - Hawaii**

Five virus-free plants of each line were mechanically inoculated with PVY , grown under greenhouse conditions and observed and tested for signs of PVY infection. Plants were inoculated in two groups, one on 8/4/94 and the second on 8/10/94. Non-transgenic control plants and transgenic plants were treated the same. The table shows results for NewLeaf potato line SEMT15 -02 and SEMT15-15 and eight lines of non-transgenic control.

| Line # | ELISA Reading* (O.D.) | Symptoms Observed | ELISA Reading* (O.D.) | Symptoms Observed |
|---|---|---|---|---|
| Date: | 9/15/94 | 9/15/94 | 10/26/94 | 10/26/94 |
| | | | | |
| | | | | |
| SEMT15-02 | 0.154 | NONE | 0.094 | NONE |
| SEMT15-15 | 0.122 | NONE | 0.091 | NONE |
| | | | | |
| SE CONTROL #1 | 2.342 | NONE | | |
| SE CONTROL #2 | 0.992 | NONE | | |
| SE CONTROL #3 | 1.926 | NONE | | |
| SE CONTROL #4 | 1.991 | NONE | | |
| SE CONTROL #5 | 0.604 | NONE | | |
| SE CONTROL #6 | 2.571 | NONE | | |
| SE CONTROL #7 | 2.646 | NONE | | |
| SE CONTROL #8 | 3.000 | NONE | | |

Line # - The tested line of transformed Shepody (SE) and control lines. ELISA's were conducted on composite samples of one leaf per plant. Eight lines of non-transgenic control were tested in this test.

* A second ELISA reading was done to confirm the first reading if the first reading was negative.

## TABLE 2. Greenhouse Virus Challenge Experiments Using PVY - Hawaii

Five virus-free plants of each line were mechanically inoculated with PVY on 3/23/95, grown under greenhouse conditions and observed and tested for signs of PVY infection. One plant of each line was held as an uninoculated control. Plants of non-transgenic control lines and transgenic lines were treated the same. The table shows results for NewLeaf potato line SEMT15-15 and the non-transgenic control line.

| Line # | ELISA Reading* (O.D.) | Symptoms Observed | ELISA Reading* (O.D.) | Symptoms Observed |
|---|---|---|---|---|
| **Date:** | **4/17/95** | **4/17/95** | **5/22/95** | **5/22/95** |
| | | | | |
| SEMT15-15 | 0.115 | NONE | 0.113 | NONE |
| | | | | |
| SECRTL 005 #1 | 2.591 | M | | |
| SECRTL 005 #2 | 2.626 | M | | |
| SECRTL 005 #3 | 2.420 | M | | |
| SECRTL 005 #4 | 2.579 | M | | |
| SECRTL 005 #5 | 2.402 | M | | |

Line # - The tested line of transformed Shepody (SE) and control line. ELISA's were conducted on composite samples of one leaf per plant. Five groups of the single non-transgenic control line were used in this study.

Symptom - M = Leaf mottling due to mosaic

* A second ELISA reading was done to confirm the first reading if the first reading was negative.

## TABLE 3. Greenhouse Virus Challenge Experiments Using PVY - Hawaii

Five virus-free plants of each line were mechanically inoculated with PVY on 5/16/95, grown under greenhouse conditions and observed and tested for signs of PVY infection. Non-transgenic control plants and transgenic plants were treated the same. The table shows results for NewLeaf potato lines SEMT15-02 and RBMT15-101 and non-transgenic control lines.

| Line # | ELISA Reading* (O.D.) | Symptoms Observed | ELISA Reading* (O.D.) | Symptoms Observed |
|---|---|---|---|---|
| **Date:** | **6/6/95** | **6/6/95** | **6/26/95** | **6/26/95** |
| RBMT15-101 | 0.170 | None | 0.119 | None |
| RBCRTL 0011 #1 | 1.047 | M,P | | |
| RBCRTL 0011 #2 | 1.998 | M,P | | |
| RBCRTL 0011 #3 | 2.636 | M,P | | |
| RBCRTL 0011 #4 | 2.441 | M,P | | |
| RBCRTL 0011 #5 | 2.664 | M,P | | |
| RBCRTL 0012 #1 | 0.341 | M,P | | |
| RBCRTL 0012 #2 | 0.604 | M,P | | |
| RBCRTL 0012 #3 | 1.171 | M,P | | |
| RBCRTL 0012 #4 | 0.733 | M,P | | |
| RBCRTL 0012 #5 | 0.600 | M,P | | |
| RBCRTL 0013 #1 | 0.935 | M,P | | |
| RBCRTL 0013 #2 | 0.742 | M,P | | |
| RBCRTL 0013 #3 | 0.835 | M,P | | |
| RBCRTL 0013 #4 | 1.629 | M,P | | |
| RBCRTL 0013 #5 | 0.473 | M,P | | |
| SEMT15-02 | 0.113 | None | 0.126 | None |
| SECRTL 001 #1 | 2.456 | M | | |
| SECRTL 001 #2 | 2.608 | M | | |
| SECRTL 001 #3 | 2.458 | M | | |
| SECRTL 001 #4 | 2.635 | M | | |
| SECRTL 001 #5 | 2.586 | M | | |

Line # - The tested line of transformed Shepody (SE), Russet Burbank (RB) and control lines. ELISA's were conducted on composite samples of one leaf per plant. Five groups of each of the non-transgenic control lines were included in this study.

Symptom - M = Leaf mottling due to mosaic; P = leaf puckering.

* A second ELISA reading was done to confirm the first reading if the first reading was negative.

## TABLE 4. Greenhouse Virus Challenge Experiments Using PVY - Hawaii

Five virus-free plants of each line were mechanically inoculated with PVY ON 6/7/95, grown under greenhouse conditions and observed and tested for signs of PVY infection. Control non-transgenic and transgenic plants were treated the same. The table shows results for NewLeaf potato line SEMT15-02 and SEMT15-15 and non-transgenic control lines.

| Line # | ELISA Reading* (O.D.) | Symptoms Observed | ELISA Reading* (O.D.) | Symptoms Observed |
|---|---|---|---|---|
| **Date:** | **6/26/95** | **6/26/95** | **7/26/95** | **7/26/95** |
| | | | | |
| SEMT15-02 | 0.129 | NONE | 0.105 | NONE |
| SEMT15-15 | 0.133 | NONE | 0.109 | NONE |
| | | | | |
| SECTRL 005 #1 | 1.345 | M | | |
| SECTRL 005 #2 | 2.023 | M | | |
| SECTRL 005 #3 | 1.279 | M | | |
| SECTRL 005 #4 | 1.746 | M | | |
| SECTRL 005 #5 | 1.998 | M | | |
| | | | | |
| | | | | |
| RBMT15-101 | 0.135 | NONE | 0.107 | NONE |
| | | | | |
| RBCTRL 12 #1 | 1.196 | M,P | | |
| RBCTRL 12 #2 | 1.311 | M,P | | |
| RBCTRL 12 #3 | 1.171 | M,P | | |
| RBCTRL 12 #4 | 1.607 | M,P | | |
| RBCTRL 12 #5 | 1.077 | M,P | | |

Line # - The tested line of transformed Shepody (SE), Russet Burbank (RB) and control lines. ELISA's were conducted on composite samples of one leaf per plant. Five lines of non-transgenic control were tested.

Symptom - M = Leaf mottling due to mosaic; P = Leaf puckering

* A second ELISA reading was done to confirm the first reading if the first reading was negative.

## TABLE 5. Greenhouse Virus Challenge Experiments Using PVY - Hawaii

Five virus-free plants of each line were mechanically inoculated with PVY on 2/27/97, grown under greenhouse conditions and observed and tested for signs of PVY infection. Plants were inoculated in two groups, one on 3/17/97 and the second on 4/22/97 Control non-transgenic and transgenic plants were treated the same. The table shows results for NewLeaf potato lines RBMT15-101, SEMT15 -02 and SEMT15-15 and non-transgenic control lines.

| Line # | ELISA Reading* (O.D.) | Symptoms Observed | ELISA Reading* (O.D.) | Symptoms Observed |
|---|---|---|---|---|
| **Date:** | **3/17/97** | **3/17/97** | **4/22/97** | **4/22/97** |
| | | | | |
| RBMT15-101 | 0.154 | NONE | 0.140 | NONE |
| | | | | |
| RBCTRL #11 | 0.429 | VC, M | | |
| RBCTRL #12 | 0.411 | VC, M | | |
| | | | | |
| SEMT15-2 | 0.141 | NONE | 0.116 | NONE |
| SEMT15-15 | 0.136 | NONE | 0.118 | NONE |
| | | | | |
| SECTRL #5 | 0.583 | VC, M | | |

Line # - The tested line of transformed Shepody (SE), Russet Burbank (RB) and control lines. ELISA's were conducted on composite samples of one leaf per plant. The non-transgenic control lines (one group each) were tested in the same fashion.

Symptom - VC = Vein clearing; M = Leaf mottling due to mosaic

* A second ELISA reading was done to confirm the first reading if the first reading was negative.

p. 224

**Evaluation of NewLeaf® Y Potato Lines for Resistance to Potato Virus Y - Field Challenge Experiments 1995-1998 Prince Edward Island, Canada**

## INTRODUCTION

To evaluate the virus resistance of NewLeaf Y potato lines of the varieties Russet Burbank (RBMT15-101) and Shepody ( SEMT15-02, SEMT15-15), field experiments were conducted over three growing seasons on Prince Edward Island. These experiments, conducted by [ CBI DELETED ] Prince Edward Island (PEI), Canada, were designed to simulate worst case potato virus Y (PVY) pressure under field conditions. Virus infection was determined through visual observation and use of enzyme linked immunosorbant assays (ELISA) on both NewLeaf Y potato lines and non-transformed commercial lines of the same variety.

## METHODS

In 1995, the multi-season field experiment was initiated by planting virus-free plantlets of each transformed line and two control non-transformed lines of the same cultivar. The plants were interspersed within the plots with virus susceptible non-transformed potato plants infected with potato virus Y to increase overall virus exposure. The ratio of infected to non-infected plants was approximately 3 to 1. Potato virus Y is largely aphid transmitted so cultural practices were implemented and maintained through the growing season to enhance aphid populations. The procedure for enhancing virus Y pressure was enacted only during the first year (1995) of the study. The level of potato leaf roll infection was not deliberately enhanced and is assumed to be that occurring naturally in the area; however, practices enhancing aphid populations are likely to increase the exposure pressure to this virus as well.

At the end of the 1995 season, tubers from the plants were harvested and saved. A sample of the tubers was shipped to Florida in December/January to be planted in the field and visually rated for virus symptoms when the plants were large enough to rate; approximately 4 to 6 weeks after planting. The other tubers were retained for planting on PEI the following growing season (1996). Procedures followed in the Florida testing were those normally used to certify seed potatoes for Can idian authorities and were conducted by professionals in seed certification. Virus infection was determined by visual observation of symptoms (mosaic).

® Registered Trademark of Monsanto Company, St. Louis, MO 63198

In 1996, up to 40 tubers of each line saved from the previous season were planted in the field and harvested at the end of the growing season. It was anticipated that the virus pressure would increase in subsequent seasons as infection would now be present in the non-transformed lines. A portion of the harvested tubers were evaluated in Florida tests in January/February, 1997. The remaining tubers harvested in 1996 were planted and grown to maturity on PEI in 1997. All of the 1997 harvested tubers were retained and planted back at PEI in the spring of 1998, using a randomized block design, with 3 replications of single row plots 20 feet in length. This plot design allowed a total of 50 to 60 plants per line to be observed for virus symptoms. Ten leaves were collected from each transgenic line, in July, to conduct ELISA's. Both observed symptoms and ELISA results were recorded and are reported herein.

## RESULTS

Samples from NewLeaf Y lines RBMT15-101, SEMT15-02 and SEMT15-15 were consistently lower in total virus infected plants than the control lines of potatoes (Tables 1-3). In all cases, the transformed lines showed no evidence of potato virus Y infection. Lines SEMT15-02 and SEMT15-15 showed no mosaic symptoms over the course of the study and there was a complete absence of the potato virus Y when tested with ELISA at the end of the 3 years of trials. In contrast, control shepody plants were 100% infected by the end of the trials. Russet burbank lines also showed substantial differences in potato virus Y infection between the RBMT15-101 line and the two control lines. RBMT15-101 was free from infection by virus Y. The transformed and control lines in each variety were comparable for observed potato leaf roll virus infection as the inserted genes do not confer resistance to any virus disease other than potato virus Y.

## DISCUSSION

In three seasons of field testing under extreme potato virus Y exposure, transformed potato lines RBMT15-101, SEMT15-02 and SEMT15-15 showed high resistance to infection by this virus. Control lines of the two varieties, russet burbank and shepody, were highly infected by the end of the field testing. It can be concluded that these three transformed potato lines are highly resistant, with a stable and persistent trait, to infection by potato virus Y and are not symptomless carriers of the virus.

## TABLE 1 . Field Virus Challenge Trial - Prince Edward Island

Field results of trials conducted in 1995-1996 - Potatoes were planted as virus free plantlets in the field at the beginning of the 1995 growing season, challenged with potato virus Y and subjected to natural infection pressure for potato leafroll virus. Tubers from the resulting plants were grown out in Florida early in 1996 and visually rated for virus. These are the results from the visual ratings.

| Line # | # PLT | % LR | % MOS | % TOTAL VIRUS |
|---|---|---|---|---|
| | | | | |
| RBMT15-101 | 39 | 5 | 0 | 5 |
| | | | | |
| RB CONTROL 11 | 34 | 0 | 29 | 29 |
| RB CONTROL 12 | 40 | 2.5 | 2.5 | 5 |
| | | | | |
| | | | | |
| SEMT15-02 | 11 | 0 | 0 | 0 |
| SEMT15-15 | 24 | 0 | 0 | 0 |
| | | | | |
| | | | | |
| SE - CONTROL 5 | 11 | 0 | 18 | 18 |
| SE - COMMERCIAL CONTROL | 47 | 0 | 19 | 19 |
| | | | | |

Line # - The tested line of transformed Russet Burbank (RB) , Shepody (SE) and control lines of each variety. There is no difference between a numbered control and a commercial control. Both are non-transgenic, but simply come from different sources.

#PLT - Number of tubers planted, representative of harvest from individual plantlets planted initially in the field trials

%LR - Percent of plants infected with potato leafroll virus (visual rating)

%MOS - Percent of plants showing visual symptoms of potato virus Y (mosaic)

%TOTAL VIRUS - Percent of plants infected with potato virus Y or potato leafroll virus.

## TABLE 2. Field Virus Challenge Trial - Prince Edward Island

Field results of trials conducted in 1996-1997 - Potato tubers, saved from the plants grown in the field in 1995, were planted in the field at the beginning of the 1996 growing season, managed to enhance potato virus Y pressure and subjected to natural infection pressure for potato leafroll virus. Tubers from the resulting plants were grown out in Florida early in 1997 and visually rated for virus. These are the results from the visual ratings.

| LINE # | # PLT | % LR | % MOS | % TOTAL VIRUS |
|---|---|---|---|---|
| | | | | |
| RBMT15-101 | 26 | 8 | 0 | 8 |
| | | | | |
| RB CONTROL 11 | 27 | 0 | 41 | 41 |
| RB CONTROL 12 | 28 | 4 | 25 | 29 |
| | | | | |
| | | | | |
| SEMT15-2 | 22 | 5 | 0 | 5 |
| SEMT15-15 | 28 | 0 | 0 | 0 |
| | | | | |
| | | | | |
| SE - CONTROL 5 | 26 | 0 | 50 | 50 |
| SE - COMMERCIAL CONTROL | 19 | 0 | 95 | 95 |
| | | | | |

Line # - The tested line of transformed Russet Burbank (RB), Shepody (SE) and control lines of each variety. There is no difference between a numbered control and a commercial control. Both are non-transgenic, but simply come from different sources.

#PLT - Number of tubers planted that were saved from the plants grown from tubers of plantlets initially used in the field trials

%LR - Percent of plants infected with potato leafroll virus (visual rating)

%MOS - Percent of plants showing visual symptoms of potato virus Y (mosaic)

%TOTAL VIRUS - Percent of plants infected with potato virus Y or potato leafroll virus.

## TABLE 3. Field Virus Challenge Trial - Prince Edward Island

Results of tests conducted in 1997-1998 - Potato tubers, saved from the plants grown in the field in 1996, were planted in the field at the beginning of the 1997 growing season and subjected to natural infection pressure for potato viruses. Tubers from the resulting plants were grown out on PEI in the summer of 1998 and rated for virus using ELISA detection methods for potato leafroll virus and potato virus Y. The 1998 plot was a randomized block design, with 3 replications of plots of 20 feet of row. This plot design allowed a total of 50 to 60 plants to be observed for virus symptoms. In July 1998, 10 leaves from 10 individual plants were collected from each transgenic line to conduct ELISA's. Results are the percent of the ten sampled plants with positive infections as determined from ELISA's.

| Line # | % LR | % MOS | % TOTAL VIRUS |
|---|---|---|---|
| | | | |
| RBMT15-101 | 20 | 0 | 20 |
| | | | |
| RB CONTROL 11 | 40 | 20 | 60 |
| RB CONTROL 12 | 60 | 30 | 90 |
| | | | |
| SEMT15-02 | 10 | 0 | 10 |
| SEMT15-15 | 20 | 0 | 20 |
| | | | |
| SE CONTROL 5 | 10 | 100 | 100 |
| SE COMMERCIAL CONTROL | 20 | 100 | 100 |

Line # - The tested line of transformed Russet Burbank (RB), Shepody (SE) and control lines of each variety. There is no difference between a numbered control and a commercial control. Both are non-transgenic, but simply come from different sources.

%LR - Percent of plants infected with potato leafroll virus as determined by ELISA testing of 10 leaf samples per line

%MOS - Percent of plants with ELISA determined virus Y infection as determined by 10 leaf samples per line

%TOTAL VIRUS - Percent of 10 sampled plants infected with potato virus Y or potato leafroll virus.

# Certification of NewLeaf Y Potato Lines in the U.S. and Canadian Seed Certification Programs - Revised to include 1997 Data

## Summary

NewLeaf Y potato lines were evaluated in the U.S. and Canadian Seed Certification programs in 1995, 1996 and 1997. The certification data demonstrates that the transgenic potato lines are equivalent to non-transgenic Russet Burbank potato with regard to variety characteristics and susceptibility to potato pathogens, except that the transgenic lines are resistant to the Potato virus Y and express the Btt gene for Colorado Potato Beetle resistance.

## Background

### *Description of the Seed Potato Certification Program*

Production of seed potatoes, including transgenic potatoes, is under the oversight of seed potato certification agencies in the U.S. and Canada. Certification of seed potatoes is a regulatory activity conducted by government agencies or universities and is focused on ensuring that seed potatoes are suitable (free from disease, true-to-type) for replanting to produce seed and commercial crops. This approach to seed production is necessary because commercial potatoes are produced through vegetative propagation. Because the cleansing effect relative to plant disease which occurs via seed production does not occur in vegetatively propagated potato, it is imperative that diseases not be introduced or maintained, even at very low levels, to avoid affecting the productivity of a potato crop. This requirement is the basis for the development of seed potato certification programs.

The certification process occurs as follows:

- Certified potato seed growers apply to the agencies asking for certification evaluation of individual seed lots of potatoes.

    1. Growers provide a history of the individual seed lot, including the variety, the source of the originating seed potatoes and the previous production location.
    2. The grower source of seed potatoes provides a seed health certificate from a certification agency documenting disease incidence, varietal purity, generation, certification number (ID) from the previous season, and plant characteristics if unique.

- The certification agency monitors the location (maps, description of location) of seed lots (field, storage), acres and quantity produced, lot segregation, disease incidence and varietal purity through the following actions:

    1. Field inspections are scheduled to evaluate the crop during the growing season for: 1) signs of disease, noting type and incidence; 2) varietal uniformity or purity (true-to-type); and 3) cultural conditions. The inspector notes off-type plants, unsatisfactory cultural or crop conditions as well as symptoms of disease.
    2. Harvest/storage inspections are done to determine tuber condition, establish lot identification and verify lot segregation.
    3. Post-harvest tests (field, greenhouse or laboratory assay) are conducted to document that disease incidence did not increase during the growing season to levels unacceptable for reproduction of another generation; serological assays are used routinely to confirm the disease diagnosis based on visible symptoms. At the end of post-harvest testing, potatoes are designated as CERTIFIED SEED if all requirements have been met. **For transgenic potatoes this designation also indicates that the potatoes are "true-to-type" for the specific variety transformed.**
    4. A shipping point inspection is conducted prior to seed delivery to ensure that the customer is receiving the correct seed lot and that the quality meets the requirements of certified seed for that jurisdiction.

Unsuitable seed potatoes (disease, varietal mixture, chemical/physical damage) are disqualified as certified seed and used for processing, consumption or destroyed (non-registered product).

Seed potato certification programs have a history of more than 80 years in the U.S. and Canada. They continue to improve with adoption of new technologies. Processes for initiating seed potatoes and inspecting/testing the crop at various production stages now employ tissue culture and sensitive pathogen testing techniques in modern facilities.

Many seed potato inspectors have 20-40 years of field experience. They can verify that the varieties and the pathogens which impact the certification system have changed little in the 80 years although the certification program itself has grown more sophisticated.

In certified seed production, the multiplication rate (bulk-up) is very important. It is another consideration in the containment of transgenic potatoes. For seed potatoes this is generally in the range of 10-12X increase each year. This is meager compared to true seed crops (50-150X) and necessitates a period of 4 or more years before quantities adequate for commercial production are available. Therefore, seed potato multiplication is a relatively slow process, with 4 years required to expand production from one acre to seed quantities adequate for 1% of the potato acres in North America (Table 1).

Table 1. Seed Potato Production - 4 years of increase (starting with plants or minitubers)

| Years of Field Production | 1 | (X) | 2 | (X) | 3 | (X) | 4 |
|---|---|---|---|---|---|---|---|
| Generation | FG1 | | FG2 | | FG3 | | FG4 |
| cwt[1] | 200 | 12 | 2,400 | 12 | 28,800 | 12 | 345,600 |
| Acres(from cwt) | 10 | | 120 | | 1,440 | | 17,280 |

[1]cwt; centiweight or 100 pounds

***NewLeaf Y Russet Burbank, Shepody and Seed Certification***

In 1995, 1996, and 1997, seed potato certification programs in the U.S. and Canada evaluated seed lots of NewLeaf Y potatoes produced by seed growers. The seed potatoes were entered into the programs and evaluated as any other variety grown for seed. The process included field inspections, harvest/storage inspections, post-harvest seed trials, as well as additional testing by ELISA of plants with viral disease symptoms. These ELISA test assays were conducted by Idaho Crop Improvement Association on samples from the post-harvest test site in California (1995, Dr. Richard Clarke, Area Manager; 1996 and 1997, Dr. Jonathon Whitworth, Area Manager).

If during inspections or the post-harvest test, a certification agency determines that plants and/or tubers in a seed lot are not typical or the response to virus and bacterial disease is atypical for the variety transformed, then the lot is disqualified from certification and required to be destroyed. Designation of seed lots as certified seed of NewLeaf Y by the certification programs signifies that each program found the plants, tubers and disease response to be typical of Russet Burbank with the added advantage that the NewLeaf Y plants are resistant to the Colorado Potato Beetle and to the Potato Leafroll virus. At NatureMark's propagation and production facility/farm in Maine where screening of new transformed lines occurs, all potato production in the field is subjected to the seed certification process. When off-type transformed plants in a line (lot) are detected or a question arises about disease susceptibility, the line is destroyed at the direction of the certification agency. Each year since 1993, the Maine Seed Potato Certification program (lines were inspected by Reginald Brown, Supervisor and field inspectors, inspected under certification numbers 639 and/or 645) has evaluated and certified several thousand lines (Table 2) of transformed potatoes derived from up to eight different parental varieties at NatureMark's Maine site. During those inspections only a small percentage (< 1 %) of lines were designated as unsatisfactory on the basis of plant type. These plants were destroyed to comply with the certification process.

Table 2. Number of NewLeaf Potato lines evaluated for certified seed.

| Year | Number of Lines |
|---|---|
| 1993 | 1749 |
| 1994 | 2124 |
| 1995 | 2714 |
| 1996 | 2348 |

During 1995 and 1996 there were 141 seed lots (18 lines including SEMT15-02, SEMT15-07, SEMT15-15, RBMT15-101) of Shepody, Russet Burbank and Hi-Lite Russet NL Y grown by 45 seed growers and evaluated by 12 different certification systems in the U.S. and Canada. A summary of the results and observations follows (Hi-Lite lines are not included):

1. Shepody and Russet Burbank NL Y are equivalent to unimproved Shepody and Russet Burbank in terms of plant habit, tuber type, and phenotypic stability as shown by acceptance of these lines into certification programs.

2. Shepody and Russet Burbank NL Y resistance to infection by PVY is substantial, as illustrated by total freedom from infection in NLY seed lots, as determined by observations in field inspections and post-harvest tests.

3. The susceptibility and reaction of NLY lines to other potato viruses (symptoms and incidence of infection) are comparable to the parent lines of Shepody and Russet Burbank as demonstrated by the certification results for these lines.

Table 3 summarizes post-harvest test data for sites where comparisons can be made between Shepody and Russet Burbank NLY (with PVY resistance) and Shepody (unimproved) , Russet Burbank (unimproved) and Russet Burbank NewLeaf (Russet Burbank with CPB resistance) without PVY resistance. The resistance to infection by PVY has been complete at all seed sites as documented in the table. Reaction to other potato viruses, particularly PLRV and PVA, has been comparable to the unimproved varieties, both in incidence as well as symptom expression.

**Table 3. Summary of Post-Harvest Test results for Shepody and Russet Burbank NewLeaf Y compared to unimproved potatoes of the same varieties grown on the same farms, where available.**

| State/ Province | Crop Year | # Sites | Product | SE, RB NL Y # samples | SE, RB NL Y # samples with PLRV | SE, RB NL Y # samples with Mosaic* | | Variety | SE, RB (unimproved) # samples | SE, RB (unimproved) # samples with PLRV | SE, RB (unimproved) # samples with Mosaic* |
|---|---|---|---|---|---|---|---|---|---|---|---|
| STATES | | | | | | | | | | | |
| ND | 1996 | 1 | SE NL Y | 2 | 1 | 0 | | SE | 0 | 1 | 0 |
| | | 1 | RB NL Y | 3 | 1 | 0 | | RB | 18 | 3 | 0 |
| | 1997 | 3 | SE NL Y | 9 | NA | 0 | | SE | 0 | NA | 0 |
| | | | | | | | | RB | 2 | NA | 2 |
| ME | 1995 | 1 | SE NL Y | 6 | 1 | 0 | | SE | 2 | 0 | 0 |
| | | 1 | RB NL Y | 5 | 0 | 0 | | RB | 3 | 0 | 0 |
| | 1996 | 2 | SE NL Y | 13 | 0 | 0 | | SE | 2 | 0 | 0 |
| | | 3 | RB NL Y | 9 | 0 | 1 | ** | RB | 6 | 0 | 2 |
| | 1997 | 12 | SE NL Y | 35 | NA | 0 | ** | | 8 | NA | 0 |
| | | 2 | RB NL Y | 8 | NA | 2 | | RB | 17 | NA | 1 |
| WI | 1995 | 1 | SE NL Y | 1 | 0 | 0 | | SE | 0 | NA | 0 |
| | 1996 | 4 | SE NL Y | 7 | 0 | 0 | | SE | 1 | 0 | 0 |
| | | 2 | RB NL Y | 3 | 0 | 0 | | RB | 1 | 1 | 0 |
| | 1997 | 2 | SE NL Y | 3 | NA | 0 | | SE | 0 | NA | 0 |
| ID | 1995 | 2 | SE NL Y | 2 | 0 | 0 | | SE | 5 | 0 | 3 |
| | | | | | | | | RB | 10 | 4 | 1 |
| | 1996 | 6 | SE NL Y | 15 | 0 | 0 | | SE | 1 | 0 | 0 |
| | | 1 | RB NL Y | 1 | 0 | 0 | | RB | 9 | 2 | 3 |
| | 1997 | 6 | SE NL Y | 21 | NA | 1 | *** | SE | 0 | NA | 0 |
| | | | | | | | | RB | 12 | NA | 9 |
| MN | 1997 | 3 | SE NL Y | 5 | NA | 1 | **** | SE | 0 | NA | 0 |
| MT | 1995 | 2 | SE NL Y | 2 | 0 | 0 | | SE | 3 | 0 | 1 |
| | 1996 | 4 | SE NL Y | 8 | 0 | 0 | | SE | 0 | 0 | 0 |
| | | 1 | RB NL Y | 1 | 0 | 0 | | RB | 3 | 1 | 0 |
| | 1997 | 2 | SE NL Y | 8 | NA | 0 | | SE | 0 | NA | 0 |
| | | | | | | | | RB | 3 | NA | 0 |

| State/ Province | Crop Year | # Sites | Product | SE, RB NL Y # samples | # samples with PLRV | # samples with Mosaic* | | Variety | SE, RB (unimproved) # samples | # samples with PLRV | # samples with Mosaic* | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| PROV | | | | | | | | | | | | |
| AB | 1996 | 4 | SE NL Y | 7 | 1 | 0 | | | | | | |
| | | 2 | RB NL Y | 2 | NA | 0 | | | | | | |
| | 1997 | 3 | SE NL Y | 10 | NA | 0 | | | | | | |
| BC | 1996 | 1 | SE NL Y | 3 | 0 | 0 | | | | | | |
| | 1997 | 1 | SE NL Y | 1 | NA | 0 | | | | | | |
| MAN | 1995 | 1 | SE NL Y | 1 | 0 | 0 | | | | | | |
| | 1996 | 1 | SE NL Y | 3 | 0 | 0 | | | | | | |
| | 1997 | 1 | SE NL Y | 4 | NA | 0 | | | | | | |
| PEI | 1995 | 1 | SE NL Y | 2 | 0 | 0 | | | | | | |
| | | 1 | RB NL Y | 1 | 0 | 0 | | | | | | |
| | 1996 | 1 | SE NL Y | 8 | 2 | 0 | | | | | | |
| | | 2 | RB NL Y | 4 | 1 | 0 | | | | | | |
| | 1997 | 4 | SE NL Y | 16 | NA | 0 | | | | | | |
| | | 2 | RB NL Y | 3 | NA | 1 | ** | RB | 5 | NA | 2 | |
| NB | 1996 | 5 | SE NL Y | 7 | 0 | 0 | | SE | 0 | 2 | 0 | |
| | | 1 | RB NL Y | 1 | 0 | 0 | | RB | 0 | 1 | 0 | |
| | 1997 | 10 | SE NL Y | 16 | NA | 0 | | | | | | |
| | | 2 | RB NL Y | 4 | NA | 0 | | RB | 1 | NA | 1 | |
| PQ | 1997 | 2 | SE NL Y | 11 | NA | 0 | | | | | | |
| SK | 1996 | 1 | SE NL Y | 2 | NA | 0 | | | | | | |
| | 1997 | 1 | SE NL Y | 7 | NA | 1 | ** | | | | | |
| TOTAL | | 110 | | 281 | 7 | 7 | a | | 114 | 15 | 25 | * |

mosaic* = mosaic disease incited by either PVY or PVA
** mosaic disease caused by PVA as determined by ELISA
*** retested and determined to be a false reading
**** tested for Bt gene expression and found to be negative so sample was contaminated with non-transgenic seed
a = No transgenic was found to be susceptible to PVY disease as determined by ELISA's
SE NL Y = Shepody resistant to CPB and PVY
RB NL Y = Russet Burbank resistant to CPB and PVY

(l) Seed growers collect random samples of tubers (200-400) from each lot as it is harvested and submit the samples to the certification agencies for planting in a post-harvest field trial in either Florida or California. In plots managed by [ CBI DELETED ] each plant with symptoms is flagged when inspected and a sample from that plant is tested by ELISA for the presence of the potato viruses PVX, PVY, PVA and PLRV. Other agencies will test plants with questionable symptoms.

# APPENDIX VIII

## Safety, Compositional, and Nutritional Aspects of NewLeaf Y Russet Burbank and Shepody Potatoes: Conclusion Based on Studies and Information Evaluated According to FDA'S Policy on Foods from New Plant Varieties

(Russet Burbank RBMT15-101, Shepody SEMT15-02 and
Shepody SEMT15-15 Potato Lines are the subject of this petition.
Please disregard data on other lines.)

[ CBI DELETED

]

Pages 235 through 269

# APPENDIX IX

## Determination of the relative amounts of coat protein mRNA in genetically modified NewLeaf Y potato varieties compared with coat protein RNA from non-modified potato varieties naturally infected by PVY

Glen Rogan, Maor Bar-Peled, Larry Turner and Heather Seitsinger

(Russet Burbank RBMT15-101, Shepody SEMT15-02 and
Shepody SEMT15-15 Potato Lines are the subject of this petition.
Please disregard data on other lines.)

[ CBI DELETED

]

Pages 271 through 289

# APPENDIX X

## Pest Management for Greatest NewLeaf Potatoes Value

(Russet Burbank RBMT15-101, Shepody SEMT15-02 and
Shepody SEMT15-15 Potato Lines are the subject of this petition.
Please disregard data on other lines.)

## Pest Management for Greatest NewLeaf® Potatoes Value

NewLeaf potatoes provide season-long control of Colorado potato beetles and can be grown successfully with fewer insecticide inputs. For management of other insect pests, NatureMark® recommends that you follow these guidelines:

---

**1. Do Not Apply Insecticides for Potato Beetles**
NewLeaf potatoes provide all the Colorado potato beetle control you'll need, replacing foliar and systemic insecticides for beetle control. For maximum economic benefit, target insect pests with foliar insecticide applications as-needed.

**2. Scout Your Crop for Flea Beetles**
NewLeaf potatoes provide suppression of potato flea beetles. Scout your crop and treat only if flea beetles increase to damaging levels.

**3. Monitor for European Corn Borer**
When adult corn borer activity has been confirmed in your area, monitor for cream colored eggs on the underside of mid-lower leaves.

**3. Use Selective, Non-Persistent Insecticides When Possible**
Insecticides such as dimethoate and malathion provide inexpensive control of flea beetles and plant bugs. Since dimethoate is more persistent, it will give a longer period of control. Malathion is less harmful to beneficial insects so it may be a better choice for late season flea beetle or plant bugs control (after July 15).

**4. Use Pirimor (Pirimicarb) For Control of Aphid Pests**
Pirimor is very selective and does not harm beneficial insects. Because it is so selective, it is also safer for applicators and handlers.

**5. Scout Your Crop for Beneficial, as Well as Pest Insects.**
Spiders, lady beetles and other beneficial "natural enemies" can provide complete control of aphids when they are allowed to exist in your potato fields. Look for these beneficial insects and give them an opportunity to do their work before stepping in with an insecticide for control of aphid or other pests.

---

**6. For additional information specific to your farm or area, call NatureMark at 1-800-3TATERS, or call your Provincial pest management specialist.**

NewLeaf potatoes are the first step toward effective and environmentally sound insect control. By choosing the right materials for control of other pests, you can achieve the maximum economic benefit from this product.

97-339-01P

MONSANTO
*Food · Health · Hope*

MONSANTO COMPANY
700 CHESTERFIELD PARKWAY NORTH
ST. LOUIS, MISSOURI 63198
PHONE (314) 694-1000
http://www.monsanto.com

January 14, 1999

Dr. David Heron
USDA, APHIS, PPQ, BSS
Unit 147
4700 River Road
Riverdale, MD 20737-1236

Dear Dr. Heron,

**SUBJECT:** **USDA Petition 97-339-01P**
Petition for Determination of Regulatory Status
for NewLeaf® Y Potatoes

Enclosed is a supplemental submission for the above mentioned petition. We are submitting this information in the interest of harmonization of reviews between USDA and CFIA. This supplemental information is molecular characterization by Southern blot for potato lines RBMT15-101, SEMT15-02, and SEMT15-15. The data was generated to satisfy a request by CFIA authorities in Canada. The data does not contradict molecular characterization conclusions that are included in the above mentioned petition. It does provide additional information on the number of and nature of the insertion loci in each of the lines. Data support the conclusion of no safety issues for these potato lines.

Included in each of the three binders are the following:

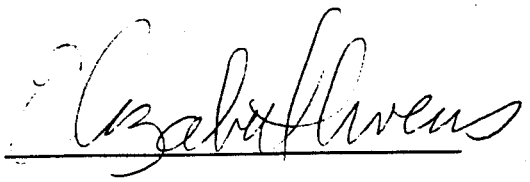
**Tab 1:** **Text** describing the molecular characterization as submitted to CFIA (pages 15-16 of the application to CFIA submitted in December, 1998)

**Tab 2:** **Monsanto Study No. 98-01-37-26** "Characterization...of RBMT-101 and SEMT15-15..." (Appendix XI of application to CFIA)

**Tab 3:** **Monsanto Study No. 98-01-37-29** "Characterization ...of SEMT15-02..." (Appendix XII of application to CFIA)

1/21/99
Kelly

If you have questions please contact me at your convenience.

Signature 

Elizabeth D. Owens, Ph.D.
Regulatory Affairs Manager
Monsanto Company
700 Chesterfield Parkway North, BB1K
St. Louis, MO 63198

Phone: 314/ 737-5721
Fax: 314/ 737-7085
Email: elizabeth.d.owens@monsanto.com

cc: Russell Schneider, Monsanto
Robert Ingratta, Monsanto (letter only)

Enclosures

97-339-01P

# PETITION FOR DETERMINATION OF REGULATORY STATUS FOR NEWLEAF® Y POTATO
Lines RBMT15-101, SEMT15-02, and SEMT15-15

**USDA Petition No. 97-339-01P**

## Supplemental Information

**January 14, 1999**

**Submitted by**

**Elizabeth D. Owens, Ph.D.**
**Monsanto Company**

**97-357U3**

**Contains No Confidential Business Information**



1/21/99

**USDA Petition No. 97-339-01P**

**Supplemental Information**
**January 14, 1999**

# Table of Contents

b) Southern Analysis

*Insert Integrity and Number of Inserts (Lines RBMT15-101 and SEMT15-15)*

As described above, previous PCR studies demonstrated that the T-DNA inserts present in each line were intact, that each line contained a complete set of all three genes and that the genes were linked. However, due to the limitations of PCR, data on loci (number of insertion events within the genome) and gene integrity could not be evaluated. Therefore, further studies were performed using Southern blot analysis to estimate the number of loci, and the integrity of the genes that were incorporated into each line. Details and copies of the Southern blots may be found in Appendix XI, "Characterization of T-DNA Inserts Present in NewLeaf® Y Potato Line Nos. RBMT15-101and SEMT15-15 by Southern Blot Analysis".

Genomic DNA was isolated from young leaf tissue from the two NewLeaf Y lines and control Russet Burbank and Shepody lines. The isolated DNA was digested with restriction enzymes that cleaved the DNA into smaller fragments, which were subjected to Southern blot analysis. The analysis defined the genetic elements which were transferred from plasmid PV-STMT15 to the genome of the potato lines. In addition, based on the number and sizes of DNA fragments generated from the restriction enzyme cleavage reactions and the known locations of the restriction sites within the plant vector PV-STMT15, the analysis provided information on the number of loci and insert integrity of the DNA introduced in the NewLeaf Y potato lines.

Southern blot analyses indicate that for Line No. RBMT15-101, insertion of the T-DNA occurred at three to four loci. At least one locus contains two copies of the T-DNA organized in inverted orientations. For two copies of the T-DNA, transfer of the T-DNA resulted in incomplete resolution of the right border, leaving incomplete copies of the *FMV* promoter associated with the *PVYcp* coding region. Since the Northern blot performed using mRNA from this line shows the presence of a single band, it is concluded that no gene product other than the expected mRNA and PVYcp is produced as a result of insertion of the T-DNA's in this fashion. One of the *cry3A* genes lacks the *Arabidopsis* small subunit promoter and a portion of the 5-prime end of the gene. This insert contains a portion of the *cry3A* gene associated with the *NOS* terminator. No protein product was expected to be produced from this region and no Cry3A immuno-cross reactive protein was detected. One of the T-DNA's has an incomplete *NOS* promoter region associated with an intact *nptII* coding region. No detectable NPTII protein was produced in this line, therefore no detectable NPTII protein was produced as a result of this promoter. The coding regions of all of the other genetic elements are intact. Southern blot analysis shows that no plasmid sequences beyond the left and right borders were inserted. See Appendix XI for detailed descriptions and methods of analysis by Southern blot.

Southern blot analyses indicate that for Line No. SEMT15-15 insertion of the T-DNA occurred at four to five loci. At least one locus contains copies of the T-DNA organized in inverted orientations. For two copies of the T-DNA, transfer of the T-DNA resulted in incomplete resolution of the right border, leaving incomplete copies of the *FMV* promoter associated with the *PVYcp* coding region. Since the Northern blot performed using mRNA from this line shows the presence of a single band, it can be concluded that no gene product other than the expected mRNA and PVYcp is produced as a result of insertion of the T-DNA's in this fashion. One of the T-DNA's in this line has an incomplete *NOS* promoter region associated with an intact *nptII* coding region. No detectable NPTII protein was produced in this line, therefore no detectable NPTII protein was produced as a result of this promoter. The coding regions of all of the genetic elements are intact. Southern blot analysis showed that plasmid sequences beyond the left and right borders, which include the *aad* gene and the *oriV* and *ori322* plasmid elements, were inserted into this line as well. See Appendix XI for detailed descriptions and methods of analysis by Southern blot.

*Insert Integrity and Number of Inserts (Line SEMT15-02)*

As described above, previous PCR studies demonstrated that the T-DNA inserts present in each line were intact, that each line contained a complete set of all three genes and that the genes were linked. However, due to the limitations of PCR, data on loci (number of insertion events within the genome) and gene integrity could not be evaluated. Therefore, further studies were performed using Southern blot analysis to estimate the number of loci, and the integrity of the genes that were incorporated into each line. Details and copies of the Southern blots may be found in Appendix XII, "Characterization of T-DNA Inserts Present in NewLeaf® Y Potato Line No. SEMT15-02 by Southern Blot Analysis".

Southern blot analyses indicates that for Line No. SEMT15-02, insertion of the T-DNA occurred at four to five loci. At least one locus contains two copies of the T-DNA organized in inverted orientations and one locus contains two T-DNA's linked by a complete copy of the plasmid backbone. For seven copies of the T-DNA, transfer of the T-DNA resulted in incomplete resolution of the right border leaving incomplete copies of the *FMV* promoter associated with the *PVYcp* coding region. Characterization of the *PVYcp* RNA produced by this line using Northern blot analysis was performed. A single RNA band of the expected size was observed. Therefore, no gene product other than the expected RNA was detected as a result of insertion of the T-DNA's in this fashion. One of the T-DNA's in this line has an incomplete *NOS* promoter region associated with an intact *nptII* coding region. One of the *nptII* genes has a truncation within the coding region. All full length and less than full length copies of the *nptII* gene are associated with *NOS* terminators. No detectable NPTII protein was produced in this line, therefore no detectable NPTII protein was produced as a result of the insertion of copies of T-DNA in this fashion. The coding regions of all other genetic elements are intact. Southern blot analysis showed that plasmid sequences beyond the left and right borders which include the *aad* gene and the *oriV* and *ori322* plasmid elements were inserted into this line. Integration of complete backbone elements occurred in two different ways: at one locus two T-DNA's are linked by a complete copy of the backbone; at two other loci, backbone integration is not associated with the left border flanking the *NOS* promoter of the *nptII* gene.

## c) Conclusions

- The *PVYcp*, *cry3A* and *nptII* genes were inserted in the genome of all three NewLeaf Y lines, as determined by PCR analysis and confirmed by Southern blot.
- Multiple inserts are present in lines RBMT15-101, SEMT15-15 and SEMT15-02, with between three to five loci, as determined by Southern blot.
- Integrity of the linkages between the genetic elements was maintained for at least one of the inserts in lines RBMT15-101, SEMT15-15 and SEMT15-02, however Southern blot analyses indicated at least one locus in each line contains copies of the T-DNA organized in tandem and/or inverted orientations.
- Southern blot analyses also indicated that no genetic elements outside the left and right borders of plasmid PV-STMT15 were transferred to the genome of line RBMT15-101. For lines SEMT15-02 and SEMT15-15, insertion of the DNA was not delimited by the right border; therefore, in addition to the elements between the left and right borders, these lines also contain the *aad*, *ori322*, and *oriV* genetic elements.

[ CBI DELETED

]

Pages 298 through 396