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This section of the FEDERAL REGISTER contains documents other than rules or proposed rules that are applicable to the public. Notices of hearings and investigations, committee meetings, agency decisions and rulings, delegations of authority, filing of petitions and applications and agency statements of organization and functions are examples of documents appearing in this section.

DEPARTMENT OF AGRICULTURE

Animal and Plant Health Inspection Service

[Docket No. 95-016-2]

Availability of Determination of Nonregulated Status for Genetically Engineered Tomato Lines

AGENCY: Animal and Plant Health Inspection Service, USDA.

ACTION: Notice.

SUMMARY: We are advising the public of our determination that tomato lines developed by Zeneca Plant Science and Petoseed Company, Inc., designated as B, Da, and F that have been genetically engineered for suppressed polygalacturonase enzyme activity are no longer considered regulated articles under our regulations governing the introduction of certain genetically engineered organisms. Our determination is based on our evaluation of data submitted by Zeneca Plant Science and Petoseed Company, Inc., in their petition for a determination of nonregulated status, an analysis of other scientific data, and our review of comments received from the public in response to a previous notice announcing our receipt of the Zeneca Plant Science and Petoseed, Inc., petition. This notice also announces the availability of our written determination document and its associated environmental assessment and finding of no significant impact.

EFFECTIVE DATE: June 6, 1995.

ADDRESSES: The determination, an environmental assessment and finding of no significant impact, the petition, and all written comments received regarding the petition may be inspected at USDA, room 1141, South Building, 14th Street and Independence Avenue SW., Washington, DC, between 8 a.m. and 4:30 p.m., Monday through Friday, except holidays. Persons wishing to

inspect those documents are asked to call in advance of visiting at (202) 690-2817.

FOR FURTHER INFORMATION CONTACT: Dr. Subhash Gupta, Biotechnologist, Biotechnology Permits, BBEP, APHIS, 4700 River Road Unit 147, Riverdale, MD 20737-1237; (301) 734-7612. To obtain a copy of the determination or the environmental assessment and finding of no significant impact, contact Ms. Kay Peterson at (301) 734-7612.

SUPPLEMENTARY INFORMATION:

Background

On February 7, 1995, the Animal and Plant Health Inspection Service (APHIS) received a petition (APHIS Petition No. 94-290-01p) from Zeneca Plant Science of Wilmington, DE, and Petoseed Company, Inc., of Woodland, CA, (Zeneca/Petoseed) seeking a determination that tomato lines designated as B, Da, and F that have been genetically engineered for suppressed polygalacturonase (PG) enzyme activity do not present a plant pest risk and, therefore, are not regulated articles under APHIS' regulations in 7 CFR part 340.

On March 17, 1995, APHIS published a notice in the *Federal Register* (60 FR 14413-14414, Docket No. 95-016-1) announcing receipt of the Zeneca/Petoseed petition and announcing that the petition was available for public review. The notice also discussed the role of APHIS and the Food and Drug Administration in regulating the subject tomato lines and food products derived from them. In the notice, APHIS solicited written comments from the public as to whether the subject tomato lines posed a plant pest risk. The comments were to have been received by APHIS on or before May 16, 1995.

APHIS received five comments on the Zeneca/Petoseed petition, from a food company, a seed company, and State departments of agriculture. All the commenters supported the Zeneca/Petoseed petition for nonregulated status for the subject tomato lines.

Analysis

Zeneca/Petoseed's tomato lines B, Da, and F have been developed from an unmodified proprietary inbred tomato line coded as T7, that has been genetically engineered to contain a fragment of the tomato PG gene in the sense or antisense orientation.

Inhibition of the PG enzyme resulting from the transcription of the PG gene fragment results in an increased thickness of the tomato, which is a desired characteristic in processing tomatoes. The subject tomato lines also contain the bacterial neomycin phosphotransferase (*nptII*) gene that is used as a selectable marker. Tomato lines B, Da, and F were transformed through the use of disarmed vectors from a common soil-borne bacterium, the plant pathogen *Agrobacterium tumefaciens*. The subject tomato lines have been considered regulated articles, under APHIS' regulations in 7 CFR part 340 because they contain certain gene sequences derived from plant-pathogenic sources. However, evaluation of field data reports from field tests of the subject tomato lines conducted under APHIS permits or notifications since 1991 indicate that there were no deleterious effects on plants, nontarget organisms, or the environment as a result of the subject tomato plants' release into the environment.

Determination

Based on its analysis of the data submitted by Zeneca/Petoseed and a review of other scientific data, comments received from the public, and field tests of the subject tomato lines, APHIS has determined that tomato lines B, Da, and F: (1) Exhibit no plant pathogenic properties; (2) are no more likely to become a weed than tomatoes with suppressed PG activity developed by traditional breeding techniques; (3) are unlikely to increase the weediness potential of any other cultivated plant or wild species with which they can interbreed; (4) are unlikely to harm other organisms, such as bees, which are beneficial to agriculture; and (5) should not cause damage to processed agricultural commodities.

The effect of this determination is that tomato lines designated as B, Da, and F are no longer considered regulated articles under APHIS' regulations in 7 CFR part 340. Therefore, the permit and notification requirements pertaining to regulated articles under those regulations no longer apply to the field testing, importation, or interstate movement of the subject tomato lines or their progeny. However, the importation of the subject tomato lines or seeds capable of propagation is still subject to

the restrictions found in APHIS' foreign quarantine notices in 7 CFR part 319.

National Environmental Policy Act

An environmental assessment (EA) has been prepared to examine the potential environmental impacts associated with this determination. The EA was prepared in accordance with: (1) The National Environmental Policy Act of 1969 (NEPA) (42 U.S.C. 4321 *et seq.*), (2) Regulations of the Council on Environmental Quality for Implementing the Procedural Provisions of NEPA (40 CFR parts 1500-1508), (3) USDA Regulations Implementing NEPA (7 CFR part 1b), and (4) APHIS' NEPA Implementing Procedures (7 CFR part 372). Based on that EA, APHIS has reached a finding of no significant impact (FONSI) with regard to its determination that the subject tomato lines and lines developed from them are no longer regulated articles under its regulations in 7 CFR part 340. Copies of the EA and the FONSI are available upon request from the individual listed under FOR FURTHER INFORMATION CONTACT.

Done in Washington, DC, this 15th day of June 1995.

Lonnie J. King,

Acting Administrator, Animal and Plant Health Inspection Service.

[FR Doc. 95-15379 Filed 6-22-95; 8:45 am]

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USDA/APHIS Determination on a Petition 94-290-01 of Zeneca Plant
Science and Petoseed Company, Inc. Seeking Nonregulated Status for
Tomato Lines B, Da, and F with Suppressed Polygalacturonase
Enzyme Activity

Environmental Assessment and
Finding of No Significant Impact

June 1995

The Animal and Plant Health Inspection Service (APHIS) of the U. S. Department of Agriculture (USDA) has conducted an environmental assessment prior to issuing a determination of nonregulated status for genetically engineered tomato lines B, Da, and F with suppressed polygalacturonase (PG) enzyme activity. The genetic modification improves the processing characteristics of these tomato lines by suppressing PG enzyme activity. APHIS received a petition from the Zeneca Plant Science and Petoseed Company, Inc. regarding the status of the lines B, Da, and F as a regulated article under APHIS regulations at 7 CFR Part 340. APHIS has conducted an extensive review of the petition and supporting documentation, as well as other relevant scientific information. Based upon the analysis documented in this environmental assessment, APHIS has reached a finding of no significant impact on the environment from its determination that genetically modified tomato lines B, Da, and F shall no longer be a regulated article.



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Biotechnology, Biologics, and Environmental Protection
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U.S. Department of Agriculture
Date: JUN - 6 1995

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believe that having an increased fruit viscosity afforded by lower PG activity during fruit ripening will cause these tomato lines to become weed pests.

3. Tomato lines B, Da, and F are unlikely to increase the weediness potential of any, other cultivated or wild species with which they can interbreed.

4. Tomato lines B, Da, and F are unlikely to harm other organisms, such as bees, which are beneficial to agriculture.

5. Tomato lines B, Da, and F should not cause damage to processed agricultural commodities.

Therefore, after a review of the available evidence, APHIS believes that lines B, Da, and F will be just as safe to grow as traditionally-bred tomato varieties with similar characteristics that are not subject to regulation under 7 CFR Part 340. APHIS concludes that there will be no significant impact on the human environment if lines B, Da, and F are no longer considered regulated articles under regulations at 7 CFR Part 340.

II. BACKGROUND

Development of lines B, Da, and F. Zeneca Plant Science and Petoseed Company, Inc. have submitted a "Petition for Determination of Non-regulated Status" to the USDA, APHIS for tomato plants that contain a gene that increases the viscosity of tomatoes. Zeneca Plant Science and Petoseed Company, Inc. requested a determination from APHIS that the lines B, Da, and F, and any progeny derived from hybrid crosses between these lines and other non-transformed tomato varieties, no longer be considered regulated articles under 7 CFR Part 340.

The partial PG gene is either inserted in the antisense orientation or in the sense orientation between the 35S promoter from cauliflower mosaic virus and the termination sequence from the nopaline synthase gene (*nos*) from *Agrobacterium tumefaciens*. Both the antisense and sense genes down-regulate the endogenous PG gene in modified plants (Smith et al., 1988, Smith et al., 1990 a, b). This reduces the levels of the enzyme polygalacturonase. The enzyme is largely responsible for the breakdown of pectin in the tomato fruit. Pectin is a major contributor to viscosity characteristics of processed tomato products. Thus, the modified lines have improved consistency because of less breakdown of pectin.

Lines B, Da, and F have also been transformed with the *nptII* gene from *E. coli* that encodes the enzyme neomycin phosphotransferase II and serves as a selectable marker enabling identification of the transformed plant cells. This gene is fused to the *nos* promoter

sequence and octopine synthase termination sequence from *A. tumefaciens*, a known plant pest.

These two genes were introduced into lines B, Da, and F via an *Agrobacterium*-mediated transformation. This is a well-characterized procedure that has been used widely for over a decade to introduce various genes of interest directly into the plant genome.

Lines B, Da, and F have been field tested since 1991 in the major tomato growing regions of the United States under permits and acknowledgments of notifications by APHIS (USDA No. 91-205-02, 92-049-03, 92-352-01, 94-047-07, 94-047-08, 94-083-06). Lines B, Da, and F have been evaluated extensively in laboratory, greenhouse, and field experiments to confirm that they exhibit the desired agronomic characteristics and do not pose a plant pest risk. Although the field tests of lines B, Da, and F have been conducted in agricultural settings, the permit conditions for the tests have stipulated physical and reproductive confinement from other plants.

APHIS Regulatory Authority. APHIS regulations at 7 CFR Part 340, which were promulgated pursuant to authority granted by the Federal Plant Pest Act, (7 U.S.C. 150aa-150jj) as amended, and the Plant Quarantine Act, (7 U.S.C. 151-164a, 166-167) as amended, regulate the introduction (importation, interstate movement, or release into the environment) of certain genetically engineered organisms and products.

A genetically engineered organism is considered a regulated article if the donor organism, recipient organism, vector, or vector agent used in engineering the organism belongs to one of the taxa listed in the regulation and is also a plant pest, or if there is reason to believe that it is a plant pest. Lines B, Da, and F described in the Zeneca Plant Science and Petoseed Company, Inc. petition have been considered regulated articles because noncoding DNA regulatory sequences and portions of the plasmid vector are derived from plant pathogens.

Section 340.6 of the regulations, entitled "Petition Process for Determination of Nonregulated Status", provides that a person may petition the Agency to evaluate submitted data and determine that a particular regulated article does not present a plant pest risk and should no longer be regulated. If APHIS determines that the regulated article is unlikely to pose a greater plant pest risk than the unmodified organism, the Agency can grant the petition in whole or in part. As a consequence, APHIS permits would no longer be required for field testing, importation, or interstate movement of that article or its progeny.

III. PURPOSE AND NEED

APHIS has prepared this EA before making a determination on the status of lines B, Da, and F as regulated articles under APHIS regulations. The developers of lines B, Da, and F, Zeneca Plant Science and Petoseed Company, Inc., submitted a petition to USDA, APHIS requesting that APHIS make a determination that lines B, Da, and F shall no longer be considered regulated articles under 7 CFR Part 340.

This EA was prepared in compliance with the National Environmental Policy Act (NEPA) of 1969 (40 CFR 1500-1508) and the pursuant implementing regulations published by the Council on Environmental Quality (42 USC 4331 *et seq.*; 40 CFR 1500-1508; 7 CFR Part 1b; and 60 FR 6000-6005).

IV. ALTERNATIVES

A. No Action.

Under the Federal "no action" alternative, APHIS would not come to a determination that lines B, Da, and F are not regulated articles under the regulations at 7 CFR Part 340. Permits from APHIS would still be required for introductions of lines B, Da, and F. APHIS might choose this alternative if there were insufficient evidence to demonstrate the lack of plant pest risk from uncontained cultivation of lines B, Da, and F.

B. Determination that tomato lines B, Da, and F are no longer regulated articles.

Under this alternative, lines B, Da, and F would no longer be regulated articles under the regulations at 7 CFR Part 340. Permits from APHIS would no longer be required for introductions of lines B, Da, and F. A basis for this determination would include a "Finding of No Significant Impact" under the National Environmental Policy Act of 1969 (42 USC 4331 *et seq.*; 40 CFR 1500-1508; 7 CFR Part 1b; and 60 FR 6000-6005).

V. AFFECTED ENVIRONMENT AND POTENTIAL ENVIRONMENTAL IMPACTS

This EA addresses potential environmental impacts from a determination that lines B, Da, and F should no longer be considered regulated articles under APHIS regulations at 7 CFR Part 340. Previous EAs prepared by APHIS together with the issuance of permits for field tests of lines B, Da, and F, have addressed various attributes of these tomatoes. This EA discusses the genetic modification, and the potential environmental impacts that might be associated with the unconfined cultivation of lines B, Da, and F.

No other attributes of lines B, Da, and F tomato suggest that they will be any more "weedy" than the present tomato cultivars that are the result of traditional breeding. The lines B, Da, and F have retained the agronomic characteristics of the parental tomato. Zeneca Plant Science and Petoseed Company, Inc. have provided data regarding growth and yield characteristics, disease and pest susceptibilities, and fruit compositional analyses that support APHIS' conclusion that lines B, Da, and F are just as safe to grow as any other commercial fresh market tomato lines with reduced PG levels due to the introduction of the *rin* gene.

B. Potential impacts on the sexually-compatible relatives of tomato arising from pollination by lines B, Da, and F.

Tomato does not cross-pollinate with other plants in the United States without the intervention of man. Cultivated tomato is self-fertile and is almost exclusively self-pollinating, due, in part, to the presence of an inserted stigma developed through over 50 years of breeding (Rick, 1976). Cultivated tomato is not wind-pollinated and insect pollination is limited (Rick, 1976).

Many other members of the nightshade family are found as weeds in tomato fields. *L. esculentum* is sexually incompatible with all these weedy relatives (Rick, 1979). Two *Solanum* species, *S. lycopersicoides* and *S. rickii*, can be crossed with commercial tomato under specific, controlled conditions, but they do not naturally cross with *L. esculentum* (Stevens and Rick, 1986; De Verna et al., 1990). Neither of these *Solanum* species is a weed pest in the United States.

The cherry tomato, *L. esculentum* var. *cerasiforme*, was most likely the wild progenitor of the cultivated tomato (Rick, 1983). Some biotypes of *L. esculentum* var. *cerasiforme* are successful weeds that have spread throughout tropical America and into southern Texas and Florida (Rick, 1973). Cherry tomato, however, is not considered a weed pest. Although *L. esculentum* var. *esculentum* and var. *cerasiforme* can cross with either plant as male or female parent (Rick, 1979), the probability of lines B, Da, and F tomato naturally introgressing into var. *cerasiforme* in the United States is almost nil since the rate of outcrossing in var. *esculentum* is low (Rick, 1949; C. M. Rick, personal communication to M. Schechtman), and var. *cerasiforme* is not present in areas of the U.S. that are devoted to large scale cultivation of tomatoes (J. W. Scott, personal communication to M. Schechtman). There are no published reports that any visible traits of cultivated tomato have introgressed into var. *cerasiforme* from cultivated tomatoes in areas where the wild cherry tomato commonly grows.

Because tomato has no relatives other than itself with which it can naturally cross in the United States, and because commercial tomatoes are virtually exclusively self-pollinating, there is little possibility of a cross unaided by man between the lines B, Da, and F

C. Potential impacts on nontarget organisms, including beneficial organisms such as bees and earthworms.

Consistent with its statutory authority, APHIS evaluated whether lines B, Da, and F might indirectly harm plants or plant products (such as some agricultural commodities). APHIS considered the potential impacts that lines B, Da, and F might exert indirectly on organisms that are recognized as beneficial to agriculture. APHIS concludes that there is no reason to believe that the unconfined growth of lines B, Da, and F will pose any deleterious effects or significant impacts on nontarget organisms, including beneficial organisms. The reduced levels of PG enzyme in genetically modified lines B, Da, and F should not have any toxic properties.

APHIS believes that lines B, Da, and F will have no deleterious effects on organisms recognized as beneficial to agriculture (e.g., earthworms, honey bees). In addition, there is no reason to believe that the presence of lines B, Da, and F would have any adverse effect on other organisms, including any species recognized as threatened or endangered in the United States.

D. Potential impacts on agricultural practices.

The genetically modified tomato lines B, Da, and F should not have any major potential impacts on agricultural and cultivation practices. Any impacts should be comparable to those associated with the cultivation of other tomato cultivars.

E. Potential impact on processed agricultural commodities.

The release of lines B, Da, and F from regulation should have no adverse impact on agricultural commodities. The tomato lines B, Da, and F have been developed with reduced PG levels to improve processing qualities that are important in tomato paste products. These tomato lines do not appear to have any unique characteristics which would have an indirect plant pest effect on any processed plant commodity.

VI. CONCLUSION

APHIS has evaluated information from the scientific literature as well as data submitted by Zeneca Plant Science and Petoseed Company, Inc. that characterized lines B, Da, and F. After careful analysis, APHIS has identified no significant impact to the environment from issuance of a determination that lines B, Da, and F should no longer be regulated articles under APHIS regulations at 7 CFR Part 340.

APHIS has considered the foreseeable consequences of removing lines B, Da, and F from its regulation, and has reached the following conclusions:

1. Tomato lines B, Da, and F exhibit no plant pathogenic properties. Although pathogenic organisms were used in the development of lines B, Da, and F, these tomato plants are not infected nor can they cause disease in other plants.
2. Tomato lines B, Da, and F are no more likely to become weeds than tomatoes with suppressed PG activity developed by traditional breeding techniques. Tomato is not a weed pest, and there is no reason to believe that the introduced genetic constructs will provide a selective advantage sufficient enough to enable tomato lines expressing these genes to become weeds.
3. Tomato lines B, Da, and F are unlikely to increase the weediness potential of any other cultivated or wild species with which they can interbreed.
4. Tomato lines B, Da, and F are unlikely to harm other organisms, such as bees, which are beneficial to agriculture.
5. Tomato lines B, Da, and F should not cause damage to processed agricultural commodities.

Therefore, after review of the available evidence, APHIS concludes that lines B, Da, and F, and any progeny derived from hybrid crosses between these lines and other non-transformed tomato varieties will be just as safe to grow as traditionally-bred tomato lines that are not subject to regulation under 7 CFR Part 340. APHIS concludes that there should be no significant impact on the human environment if lines B, Da, and F were no longer considered regulated articles under its regulations (7 CFR Part 340).

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RESPONSE TO ZENECA PLANT SCIENCE AND PETOSEED COMPANY, INC. PETITION
FOR DETERMINATION OF NONREGULATED STATUS FOR
TOMATO LINES B, Da, AND F

Prepared by
United States Department of Agriculture
Animal and Plant Health Inspection Service
Biotechnology, Biologics, and Environmental Protection

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I. SUMMARY

Based on a review of scientific data, the Animal and Plant Health Inspection Service (APHIS) has determined that the tomato lines called B, Da, and F do not present a plant pest risk and are therefore no longer regulated articles under the regulations found at 7 CFR Part 340.6. Because of this determination, oversight under those regulations will no longer be required from APHIS for field testing, importation, or interstate movement of tomato lines B, Da, and F or their progeny. Importation of tomatoes derived from lines B, Da, and F and nursery stock or seeds capable of propagation is still, however, subject to the restrictions found in the foreign quarantine notices in 7 CFR Part 319.

This determination by APHIS has been made in response to a petition received from Zeneca Plant Science and Petoseed Company, Inc., dated October 14, 1994. The petition requested a determination from APHIS that tomato lines B, Da, and F do not present a plant pest risk and are therefore not regulated articles. On March 17, 1995, APHIS announced receipt of the Zeneca Plant Science and Petoseed Company, Inc., petition in the Federal Register (60 FR 14413) and stated that the petition was available for public view. APHIS invited written comments on this proposed action, to be submitted on or before May 16, 1995.

Lines B, Da, and F, as defined by its developers, Zeneca Plant Science and Petoseed Company, Inc., were engineered to produce tomato fruits with reduced polygalacturonase (PG) activity, an enzyme involved in the breakdown of pectin during fruit ripening. Tomato lines B, Da, and F have been modified by introducing the partial PG gene in either sense or antisense orientation and a marker gene neomycin phosphotransferase. Both the antisense and sense PG gene down-regulate the endogenous PG gene in modified plants. This reduces the level of the enzyme polygalacturonase. The enzyme is largely responsible for the breakdown of pectin in the tomato fruit. Pectin is a major contributor to viscosity characteristics of processed tomato products. Thus, the modified lines have improved consistency because of less breakdown of pectin. The introduced DNA that encodes genes also has accompanying DNA regulatory sequences that modulate their expression. The DNA regulatory sequences were derived from the plant pathogenic organisms, the bacterium *Agrobacterium tumefaciens* and cauliflower mosaic virus (CaMV).

APHIS regulations at 7 CFR Part 340, which were promulgated pursuant to authority granted by the Federal Plant Pest Act (FPPA), (7 U.S.C. 150aa-150jj) as amended, and the Plant Quarantine Act (PQA), (7 U.S.C. 151-164a, 166-167) as amended, regulate the introduction (importation, interstate movement, or release into the environment) of certain genetically engineered organisms and products. An organism is no longer subject to the regulatory requirements of 7 CFR Part 340 when it is demonstrated not to present a plant pest risk. Section 340.6 of the regulations, entitled, "Petition Process for Determination of

Nonregulated Status," provides that a person may petition the agency to evaluate submitted data and determine that a particular regulated article does not present a plant pest risk and should no longer be regulated. If the agency determines that the regulated article does not present a risk of introduction or dissemination of a plant pest, the petition would be granted, thereby allowing for unregulated introduction of the article in question.

Tomato lines B, Da, and F have been considered "regulated articles" under Part 340 of the regulations, in part, because lines B, Da, and F have been engineered using components from known plant pests. In addition, the vector system used to transfer the two genes into the recipient tomato was derived from the bacterial plant pathogen, *A. tumefaciens*. Also, certain noncoding regulatory sequences were derived from CaMV and *A. tumefaciens*.

Field testing of lines B, Da, and F have been done under APHIS oversight since 1992. All field trials were performed essentially under conditions of reproductive confinement.

This determination has been made based on an analysis that revealed that these tomato lines B, Da, and F: (1) exhibit no plant pathogenic properties; (2) are no more likely to become weeds than other tomatoes with suppressed PG activity developed by traditional breeding techniques; (3) are unlikely to increase the weediness potential for any other cultivated plant or native wild species with which the organisms can interbreed; (4) are unlikely to harm other organisms, such as bees, which are beneficial to agriculture; and 5) do not cause damage to processed agricultural commodities. APHIS has also concluded that there is no reason to believe that new progeny tomato varieties derived from tomato lines B, Da, and F will exhibit new plant pest properties, i.e., properties substantially different from any observed for the lines B, Da, and F tomato lines already field tested, or those observed for tomatoes in traditional breeding programs.

The potential environmental impacts associated with this determination have been examined in accordance with regulations and guidelines implementing the National Environmental Policy Act of 1969 (42 U.S.C. 4331 *et seq.*; 40 CFR 1500-1508; 7 CFR Part 1b; and 60 FR 6000-6005). An Environmental Assessment (EA) was prepared and a Finding of No Significant Impact (FONSI) was reached by APHIS for the determination that lines B, Da, and F are no longer regulated articles under its regulations at 7 CFR Part 340.

The body of this document consists of the following three parts: (1) background information that provides the legal framework under which APHIS has regulated the field testing, interstate movement, and importation of lines B, Da, and F; (2) a summary of and response to comments provided to APHIS on its proposed action during the public comment period; and (3) analysis of the key factors relevant to APHIS'

decision that the lines B, Da, and F do not present a plant pest risk.

II. BACKGROUND

Regulatory Authority. APHIS regulations, which were promulgated pursuant to authority granted by the Federal Plant Pest Act (FPPA), (7 U.S.C. 150aa-150jj) as amended, and the Plant Quarantine Act (PQA), (7 U.S.C. 151-164a, 166-167) as amended, regulate the introduction (importation, interstate movement, or release into the environment) of certain genetically engineered organisms and products.

Under § 340.0 of the regulations, a person is required to obtain a permit before introducing a regulated article. A genetically engineered organism is deemed a regulated article either if the donor organism, recipient organism, vector or vector agent used in engineering the organism belongs to one of the taxa listed in the regulation and is also a plant pest; or if APHIS has reason to believe that the genetically engineered organism presents a plant pest risk. Permission to conduct a field trial with an article regulated under 7 CFR Part 340 is granted when APHIS has determined that the conduct of the field trial, under the conditions specified by the applicant or stipulated by APHIS, does not pose a plant pest risk.

Before the introduction of a regulated article, a person is required under § 340.0 of the regulations to either (1) notify APHIS in accordance with § 340.3 or (2) obtain a permit in accordance with § 340.4. Introduction under notification (§ 340.3) requires that the introduction meets specified eligibility criteria and performance standards. The eligibility criteria impose limitations on the types of genetic modifications that qualify for notification, and the performance standards impose limitations on how the introduction may be conducted. Under § 340.4, a permit is granted for a field trial when APHIS has determined that the conduct of the field trial, under the conditions specified by the applicant or stipulated by APHIS, does not pose a plant pest risk.

The FPPA gives USDA, the authority to regulate plant pests and other articles to prevent direct or indirect injury, disease, or damage to plants and plant products. In addition, the PQA provides an additional level of protection by enabling USDA to regulate the importation and movement of nursery stock and other plants that may harbor injurious pests or diseases.

An organism is not subject to the regulatory requirements of 7 CFR Part 340 when it is demonstrated not to present a plant pest risk. Section 340.6 of the regulations, entitled "Petition Process for Determination of Nonregulated Status," provides that a person may petition the agency to evaluate submitted data and determine that a particular regulated article does not present a plant pest risk and should no longer be regulated. If the agency determines that the

regulated article does not present a risk of introduction or dissemination of a plant pest, the petition will be granted, thereby allowing for unregulated introduction of the article in question. A petition may be granted in whole or in part.

Lines B, Da, and F have been considered a "regulated articles" for field testing under Part 340 of the regulations in part because the vector system used to transfer the two genes into the tomato genome was derived from *A. tumefaciens*, a known plant pathogen. In addition, certain noncoding regulatory sequences were derived from plant pathogens, i.e., from CaMV and *A. tumefaciens*.

APHIS believes it prudent to provide assurance before commercialization that organisms such as lines B, Da, and F, that are derived at least in part from plant pests, do not pose any potential plant pest risk. Such assurance may aid the entry of new plant varieties into commerce or into breeding and development programs. The decision by APHIS that lines B, Da, and F are not regulated articles is based in part on evidence provided by Zeneca Plant Science and Petoseed Company, Inc., concerning the biological properties of lines B, Da, and F and their similarity to other varieties of tomato grown using standard agricultural practices for commercial sale or private use.

The fact that APHIS regulates genetically engineered organisms having plant pest components does not carry with it the presumption that the presence of part of a plant pest makes a whole plant a pest or that the plants or genes are pathogenic. The regulations instead have the premise that when plants are developed using biological vectors or material from pathogenic sources, or when pathogens are used as vector agents, they should be evaluated to assure that there is not a plant pest risk (McCammon and Medley, 1990). APHIS performs a review that allows a verification of the biology and procedures used; assesses the degree of uncertainty and familiarity; and allows the identification of any hazards, should they be present and predictable. The overall aims of APHIS regulations in the Code of Federal Regulations at 7 CFR Part 340 are to allow for the safe testing of genetically engineered organisms under an appropriate level of oversight, and to enable any issues of potential or hypothetical risks to be addressed early enough in the development of the new organisms to allow for the safe utilization of the technology in agriculture.

A certification that an organism does not present a plant pest risk means that there is reasonable certainty that the organism cannot directly or indirectly cause disease, injury, or damage either when grown in the field, or when stored, sold, or processed. APHIS' approach to plant pest risk is considerably broader than a narrow definition that encompasses only plant pathogens. Other traits, such as increased weediness, and harmful effects on beneficial organisms, such as earthworms and bees, are clearly subsumed within what is meant by direct or indirect plant pest risk. In APHIS' regulations at 7 CFR Part 340, a "plant pest" is defined as: "Any living stage (including

active and dormant forms) of insects, mites, nematodes, slugs, snails, protozoa, or other invertebrate animals, bacteria, fungi, other parasitic plants or reproductive parts thereof; viruses; or any organisms similar to or allied with any of the foregoing; or any infectious agents or substances, which can directly or indirectly injure or cause disease or damage in or to any plants or parts thereof, or any processed, manufactured, or other products of plants."

A determination that an organism does not present a plant pest risk can be made under this definition, especially when there is evidence that the plant under consideration: 1) exhibits no plant pathogenic properties; 2) is no more likely to become a weed than the non-modified parental variety; 3) is unlikely to increase the weediness potential of any other cultivated plant; 4) is unlikely to harm other organisms that are beneficial to agriculture; and 5) does not cause damage to processed agricultural commodities. Evidence has been presented by Zeneca Plant Science and Petoseed Company, Inc., that bears on these topics. In addition, because the Zeneca Plant Science and Petoseed Company, Inc., petition seeks a determination regarding new tomato varieties bred from these lines with suppressed PG activity, it should be established that there is no reason to believe that any new tomato varieties bred from lines B, Da, and F will exhibit plant pest properties substantially different from any observed for tomato in traditional breeding programs or as seen in the development of lines B, Da, and F already field tested.

III. RESPONSE TO COMMENTS

APHIS received 5 comments on the petition from a State department of agriculture, a food company, and a seed company. All comments supported the petition seeking nonregulated status for these tomato lines.

IV. ANALYSIS OF THE PROPERTIES OF THE LINES B, Da, AND F

A brief discussion of the biology of tomato is given in the next paragraph. This information is expanded in subsequent sections when it is relevant in addressing particular issues with respect to the tomato lines B, Da, and F.

The tomato, *L. esculentum* var. *esculentum*, is distributed worldwide and is grown commercially wherever agronomic conditions will permit an economic yield to be obtained. *Lycopersicon* is a genus of the large and diverse family Solanaceae, which also includes peppers, tobacco, and eggplant. The genus has been divided into two subgenera, the *esculentum* complex that contains those species that are easily crossed with commercial tomato, and the *peruvianum* complex that contain those species that are crossed with considerable difficulty (Stevens and Rick, 1986; Taylor, 1986). *Lycopersicon* species are native to Ecuador, Peru, and the Galapagos Islands; however, most evidence

suggests that the site of domestication of *L. esculentum* is Mexico (Taylor, 1986). The cultivated tomato is self-fertile and almost exclusively self-pollinating, generally requiring the intervention of man for cross-pollination. The only relative of *L. esculentum* var. *esculentum* that is found in the U.S. and with which var. *esculentum* is sexually compatible is *L. esculentum* var. *cerasiforme*. The cultivated tomato is a highly inbred perennial that is grown almost exclusively as an annual in the U.S. Of the over 500,000 acres of tomatoes that are grown annually in the U.S., approximately 40% are grown for fresh market consumption; the balance is grown for processing.

The Introduced Genes, Their Products, and the Added Regulatory Sequences Controlling Their Expression do not Present a Plant Pest Risk in lines B, Da, and F.

Lines B, Da, and F were produced using an *Agrobacterium*-mediated transformation protocol to transform tomato with a gene that suppresses the PG enzyme level and a selectable marker gene. The PG gene was isolated from *L. esculentum* Mill. variety Ailsa Craig. The partial gene was inserted either in sense or antisense orientation into tomato lines B, Da, and F. In both cases the gene down-regulates the endogenous PG gene in modified tomato plants.

The *nptII* gene, which acts as a marker to aid in laboratory selection of the transformed tomato cells, was also incorporated into the plant genome. The gene encodes the enzyme neomycin phosphotransferase (also called aminoglycoside 3'-phosphotransferase II), which confers resistance to the antibiotic kanamycin. This gene has been used extensively as a selectable marker in transformation experiments in bacterial, mammalian and plant cells. The *nptII* gene was derived from the bacterial transposon Tn5 isolated from a strain of *E. coli* K12 (Beck et al., 1982; Jorgensen et al., 1979). *E. coli*, a common enteric bacterium found in the human gut, is not a regulated article. The *nptII* gene has no involvement in plant disease or damage. Also, its use does not result in the presence of the antibiotic kanamycin in lines B, Da, and F, and its presence does not imply that kanamycin will be used in the cultivation of the tomatoes.

The introduced DNA that encodes the PG gene also has accompanying DNA regulatory sequences that modulate the expression of the gene. The DNA regulatory sequences were derived from plant pathogenic organisms: the bacterium *A. tumefaciens* and CaMV. Specifically, the DNA regulatory sequences associated with the truncated PG gene comprise the promoter derived from the 35S gene of CaMV, and the termination sequences derived from the nopaline synthase gene from *A. tumefaciens*. The DNA regulatory sequences associated with *nptII* comprise the tandem duplicated promoter region from nopaline synthase and the termination sequences from octopine synthase. Both regulatory sequences were from *A. tumefaciens*. Although these regulatory sequences were derived from plant pathogens, the regulatory sequences cannot cause plant disease by themselves or with the genes that they regulate.

Lines B, Da, and F were derived by transforming Petoseed proprietary commercial tomato inbred cultivar T7 via a well-characterized technique that uses DNA sequences from *A. tumefaciens* to introduce genes into the chromosome of the recipient plant (see reviews by Klee and Rogers, 1989; and Zambryski, 1988). Although some DNA sequences used in the transformation process were derived from the plant pathogen, *A. tumefaciens* (the causal agent of crown gall disease), the genes that cause crown gall disease were removed, and therefore the tomato plant does not develop crown gall disease. Once inserted into the chromosome of the tomato plant, the introduced genes are maintained and transmitted in the same manner as any other genes.

During extensive testing in the laboratory, greenhouse and in the field, plants of lines B, Da, and F exhibited the typical agronomic characteristics of the parent tomato. In APHIS' opinion, the components and processing characteristics of lines B, Da, and F reveal no differences in any component that could have an indirect plant pest effect on any processed plant commodity. The lines B, Da, and F have no plant pest characteristics.

Lines B, Da, and F have no significant potential to become successful weeds.

A study (National Research Council, 1989) produced for the National Academy of Sciences, entitled "Field Testing Genetically Modified Organisms: Framework for Decisions", identified the potential to inadvertently produce a new weed or increase the aggressiveness of existing weeds as "perhaps the single most commonly voiced concern about the introduction of genetically modified plants."

A weed pest is a plant that grows persistently in locations where it is unwanted. Tomato has been grown for centuries throughout the world without any reports that it is a serious weed pest. In the U.S., it is not listed under the Federal Noxious Weed Act. In fact, tomato, though an exotic species introduced into the United States, is not classified as a serious, principal, or common weed pest (Holm et al., 1979). Although tomato volunteers are not uncommon, they are easily controlled using herbicides or by mechanical means. Tomato possesses few of the characteristics of plants that are notably successful weeds, e.g., it does not produce abundant, long-lived seed (Keeler, 1989). It is a perennial crop that is grown almost exclusively as an annual crop in the U.S. Tomato is considered a highly domesticated, well-characterized crop plant that is not persistent in undisturbed environments without human intervention. Lines B, Da, and F are likely to be grown mostly in areas that are currently under tomato cultivation, i.e., in typical growing regions for the crop.

Zeneca Plant Science and Petoseed Company, Inc., have designed experiments and collected data from greenhouse and field trials that support the contention that tomato lines B, Da, and F have little potential to become a serious or successful weed. These observations

have shown that the lines B, Da, and F tomatoes have (1) agronomic and horticultural traits similar to those of traditionally bred tomatoes; (2) a range of seed germination rates and frequencies comparable to those of nontransformed tomatoes; and (3) no alterations in traits such as seed germination or dispersal that could confer a selective advantage and could enhance survival in the wild.

There are no morphological, physiological, or disease resistance characteristics of the lines B, Da, and F tomato that would entail the use of agricultural practices that vary from the traditional practices used today for the cultivation and propagation of tomatoes.

Lines B, Da, and F will not increase the weediness potential of any other plant with which it can interbreed.

Tomato does not cross-pollinate with other plants in the United States without the intervention of man. Cultivated tomato is self-fertile and is almost exclusively self-pollinating, due, in part, to the presence of an inserted stigma developed through over 50 years of breeding (Rick, 1976). Cultivated tomato is not wind pollinated and insect pollination is limited (Rick, 1976).

Many other members of the nightshade family are found as weeds in tomato fields. *L. esculentum* is sexually incompatible with all these weedy relatives (Rick, 1979). Two *Solanum* species, *S. lycopersicodes* and *S. rickii* can be crossed with commercial tomato under specific, controlled conditions, but they do not naturally cross with *L. esculentum* (Stevens and Rick, 1986; De Verna et al., 1990). Neither of these *Solanum* species is a weed pest in the United States.

The cherry tomato, *L. esculentum* var. *cerasiforme*, was most likely the wild progenitor of the cultivated tomato (Rick, 1983). Some biotypes of *L. esculentum* var. *cerasiforme* are successful weeds that have spread throughout tropical America and into southern Texas and Florida (Rick, 1973). Cherry tomato, however, is not considered a weed pest. Although *L. esculentum* var. *esculentum* and var. *cerasiforme* can cross with either plant as male or female parent (Rick, 1979), the probability of tomato lines B, Da, and F naturally introgressing into var. *cerasiforme* in the United States is almost nil since the rate of outcrossing in var. *esculentum* is low (Rick, 1949; C. M. Rick, personal communication), and var. *cerasiforme* is not present in areas of the U.S. that are devoted to large scale cultivation of tomatoes (J. W. Scott, personal communication to M. Schechtman). There are no published reports that visible traits of cultivated tomato have introgressed into var. *cerasiforme* from cultivated tomatoes in areas where the wild cherry tomato commonly grows.

Because tomato has no relatives other than itself with which it can naturally cross in the United States, and because commercial tomatoes are virtually exclusively self-pollinating, there is little possibility of a cross unaided by man between the lines B, Da, and F tomato and another plant. Therefore, there is no likelihood that

lines B, Da, and F tomato will increase the weediness potential of another plant. Cultivation of *L. esculentum* requires maintenance of genetic purity as a standard breeding practice. Regulations specifying procedures for the maintenance of genetic purity have been codified (See 7 CFR Part 201).

Even if an outcrossing event involving pollen from tomato lines B, Da, and F did occur, there is no reason to believe that the decreased PG activity brought about by the introduced truncated PG could affect seed persistence or weediness potential in progeny. Expression of the introduced PG and nptII gene constructs in any of the lines thus far tested has not changed any morphological or physiological characteristics that might affect pollination and there is also no reason to believe that this characteristic could be affected by the introduced genes.

Lines B, Da, and F should not cause damage to processed agricultural commodities.

The components and processing characteristics of lines B, Da, and F tomatoes reveal no apparent differences in any component that could have an indirect plant pest effect on any processed plant commodity. Tomatoes derived from the transgenic plants did not show an increase in disease, injury or damage compared to nontransgenic parent lines

Lines B, Da, and F should not be harmful to beneficial organisms, including bees.

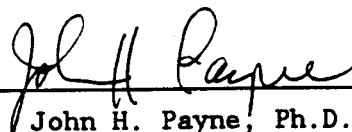
There is no reason to believe that deleterious effects on beneficial organisms could result specifically from the cultivation of lines B, Da, and F tomatoes. The conclusion is based on two lines of reasoning: (1) analysis of biochemical components of lines B, Da, and F tomatoes identified no toxic components of these tomatoes that are present in concentrations significantly different from the concentrations in nontransgenic tomatoes; and (2) no direct pathogenic properties, nor any hypothetical mechanisms for pathogenesis towards beneficial organisms such as bees and earthworms, were identified by Zeneca Plant Science and Petoseed Company, Inc., for lines B, Da, and F tomatoes. APHIS also cannot envision any plausible mechanisms for any hypothetical pathogenetic effect.

The definition of lines B, Da, and F encompass not only tomato lines that already have been field tested, but also new tomato lines produced through conventional breeding using lines B, Da, and F as one or both parents. APHIS believes that the analysis applied to lines B, Da, and F already field tested will apply equally well to these new tomato lines, and that the data provided by Zeneca Plant Science and Petoseed Company, Inc., justify the conclusion that such new lines derived from B, Da, and F will not present a plant pest risk. The variation in agronomic characteristics among the B, Da, and F lines that have been field tested does not differ significantly from that seen in commercial cultivars of tomato that have never been considered

regulated articles. Therefore, there is no reason to believe that any of the progeny of lines B, Da, and F will possess plant pest properties.

IV. CONCLUSION

APHIS has determined that tomato lines B, Da, and F that have previously been field tested under permit, will no longer be considered regulated articles under APHIS regulations at 7 CFR Part 340. Permits under those regulations will no longer be required from APHIS for field testing, importation, or interstate movement of those tomatoes or their progeny. Importation of lines B, Da, and F tomatoes and nursery stock or seeds capable of propagation is still, however, subject to the restrictions found in foreign quarantine notices in 7 CFR Part 319. This determination has been made based on data collected from these trials, laboratory analyses, and literature references presented herein which demonstrate that the lines B, Da, and F: 1) exhibit no plant pathogenic properties; 2) are no more likely to become weeds than a delayed-ripening tomato developed by traditional breeding techniques; 3) are unlikely to increase the weediness potential for any other cultivated or wild species with which it can interbreed; 4) are unlikely to harm other organisms, such as bees, that are beneficial to agriculture; and 5) should not cause damage to processed agricultural commodities. APHIS has also concluded that there is a reasonable certainty that new progeny of lines B, Da, and F or varieties bred from these lines will not exhibit new plant pest properties, i.e., properties substantially different from any observed for lines B, Da, and F already field tested, or those observed for tomatoes in traditional breeding programs.



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