RESPONSE TO DNA PLANT TECHNOLOGY CORPORATION PETITION FOR DETERMINATION OF NONREGULATED STATUS FOR TOMATO LINE 1345-4

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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 line called 1345-4 does not represent a plant pest risk and is therefore not a regulated article 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 line 1345-4 tomatoes or their progeny.

This determination by APHIS has been made in response to a petition received from DNA Plant Technology Corporation (DNAP) dated August 16, 1994. The petition requested a determination from APHIS that the tomato line 1345-4 does not present a plant pest risk and is therefore not a regulated article. On September 26, 1994, APHIS announced receipt of the DNAP petition in the Federal Register (59 FR 49055) and stated that the petition was available for public view. APHIS invited written comments on this proposed action, to be submitted on or before November 25, 1994.

Line 1345-4, as defined by its developer, the DNA Plant Technology Corporation Company, was engineered to produce tomato fruits whose ripening is delayed, even though the fruits are left on the vine or in storage for long periods of time. Tomato line 1345-4 has been modified by introducing a truncated version of an aminocyclopropane carboxylate (ACC) synthase gene and a marker gene neomycin phosphotransferase. The presence of the truncated gene inhibits the expression of the unmodified gene normally found in tomato, which is necessary for the production of the ripening agent ethylene. 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 line 1345-4 has been considered a "regulated article" for field testing under Part 340 of the regulations, in part, because line 1345-4 has 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 line 1345-4 has been done under APHIS oversight in 1992 and 1993, and is continuing in 1994. All field trials were performed essentially under conditions of reproductive confinement.

APHIS has determined that the tomato line 1345-4 does not pose a direct or indirect plant pest risk and therefore will no longer be considered a regulated article under APHIS regulations at 7 CFR Part 340. Oversight under those regulations will no longer be required by APHIS for field testing, importation, or interstate movement of these tomatoes or their progeny. (Importation of tomatoes derived from line 1345-4 [and nursery stock or seeds capable of propagation] is still, however, subject to the restrictions found in the Foreign Quarantine Notice regulations at 7 CFR Part 319.)

This determination has been made based on an analysis that revealed that this tomato line 1345-4: (1) exhibit no plant pathogenic properties; (2) is no more likely to become a weed than other delayed-ripening tomatoes developed by traditional breeding techniques; (3) is unlikely to increase the weediness potential for any other cultivated plant or native wild species with which the organisms can interbreed; (4) should not cause damage to processed agricultural commodities; and (5) is unlikely to harm other organisms, such as bees, which are beneficial to agriculture. APHIS has also concluded that there is no reason to believe that new progeny tomato varieties derived from line 1345-4 will exhibit new plant pest properties, i.e., properties substantially different from any observed for the line 1345-4 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 1500-1509; 7 CFR Part 1b; 44 FR 50381-50384; and 44 FR 51272-51274). An Environmental Assessment (EA) was prepared and a preliminary Finding of No Significant Impact (FONSI) was reached by APHIS for the determination that line 1345-4 is no longer a regulated article under its regulations at 7 CFR Part 340.

The body of this document consists of the following two parts: (1) background information that provides the legal framework under which APHIS has regulated the field testing, interstate movement, and importation of line 1345-4 and a summary of and response to comments provided to APHIS on its proposed action during the public comment

period; and (2) analysis of the key factors relevant to APHIS' decision that the line 1345-4 does 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.

Line 1345-4 has been considered a "regulated article" 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 line 1345-4, 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 line 1345-4 is not a regulated article is based in part on evidence provided by DNAP concerning the biological properties of line 1345-4 and its 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) exhibit 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) does not cause damage to processed agricultural commodities; and 5) is unlikely to harm other organisms that are beneficial to agriculture. Evidence has been presented by DNAP that bears on these topics. In addition, because the DNAP petition seeks a determination regarding new tomato varieties containing the delayed ripening phenotype, it should be established that there is no reason to believe that any new tomato varieties bred from line 1345-4 will exhibit plant pest properties substantially different from any observed for tomato in traditional breeding programs or as seen in the development of line 1345-4 already field tested.

III. RESPONSE TO COMMENTS

APHIS received 7 comments on the petition from the following: a food company (1); state departments of agriculture (2); and universities (4). All comments were favorable to substance of the petition.

IV. ANALYSIS OF THE PROPERTIES OF THE LINE 1345-4

A brief discussion of the biology of tomato follows in the next paragraph to help inform the subsequent analysis. This information is expanded in subsequent sections when it is relevant in addressing particular issues with respect to the line 1345-4 tomato.

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* be 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 line 1345-4

Line 1345-4 was produced using an *Agrobacterium*-meditated transformation protocol to transform tomato with a gene designed to confer the delayed ripening phenotype and a selectable marker gene. The ACC synthase gene was isolated from *L. esculentum*. The gene, which was inserted into tomato line 91103-114, was truncated, i.e., it was shortened and contains approximately 75 percent of the nucleotide sequences of the complete gene. The truncated ACC synthase gene does not encode a functional enzyme.

Neomycin phosphotransferase, which acts as a marker to aid in laboratory selection of the transformed tomato cells, was also incorporated into the plant genome. This gene was obtained from the common bacterium *E. coli*. The gene encodes the enzyme neomycin phosphotransferase (also called aminoglycoside 3'-phosphotransferase II), which confers resistance to the antibiotic kanamycin. This gene was introduced as a marker, i.e., as a tag enabling identification of tomato cells that had concomitantly taken up the truncated ACC synthase gene. The marker gene was isolated from a transposon contained in 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 gene has no involvement in plant disease or damage. Also, its use does not result in the presence of the antibiotic kanamycin in line 1345-4 and its presence does not imply that kanamycin will be used in the cultivation of the tomatoes.

The introduced DNA that encodes the truncated ACC synthase gene also has accompanying DNA regulatory sequences that modulate the expression of the gene. The DNA regulatory sequences were derived from three organisms two of which are plant pathogens: the bacterium *A. tumefaciens* and CaMV. Specifically, the DNA regulatory sequences associated with the truncated ACC synthase gene comprise the promoter derived from the 35S gene of CaMV, the translational leader sequences of the chlorophyll a/b binding protein from *Petunia hybrida*, and the termination sequences derived from the nopaline synthase gene from *A. tumefaciens*. The DNA regulatory sequences associated with neomycin phosphotransferase 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.

Line 1345-4 plants were derived by transforming tomato line 91103-114 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. The sexual process by which tomato plants pass their genes to their progeny involves almost exclusively self pollination, or pollination of other tomato plants or sexually compatible relatives with human intervention. The presence of the truncated gene inhibits the expression of the unmodified gene normally found in tomato, which is necessary for the production of the ripening agent ethylene.

During extensive testing in the laboratory, greenhouse and in the field, line 1345-4 plants exhibited the typical agronomic characteristics of the parent tomato. In APHIS' opinion, the components and processing characteristics of line 1345-4 reveal no differences in any component that could have an indirect plant pest effect on any processed plant commodity. The line 1345-4 has no plant pest characteristics.

Line 1345-4 has no significant potential to become a successful weed

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. Line 1345-4 is likely to be grown mostly in areas that are currently under tomato cultivation, i.e., in typical growing regions for the crop.

DNAP has designed experiments and collected data from greenhouse and field trials that support the contention that tomato line 1345-4 has little potential to become a serious or successful weed. These observations have shown that the line 1345-4 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 line 1345-4 tomato that would entail the use of agricultural practices that vary from the traditional practices used today for the cultivation and propagation of tomatoes. To achieve optimal flavor, however, line 1345-4 tomatoes may be left to ripen in the field longer than conventional tomatoes.

Line 1345-4 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 line 1345-4 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), 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 line 1345-4 tomato and another plant. Therefore, there is no likelihood that the line 1345-4 tomato will increase the weedy 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 a line 1345-4 tomato did occur, there is no reason to believe that the delayed ripening brought about by the truncated ACC synthase could affect seed persistence or weediness potential in progeny. Expression of the two gene 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.

Line 1345-4 should not cause damage to processed agricultural commodities

The components and processing characteristics of line 1345-4 tomatoes reveal no apparent differences in any component that could have an indirect plant pest effect on any processed plant commodity.

Line 1345-4 should not be harmful to beneficial organisms, including bees

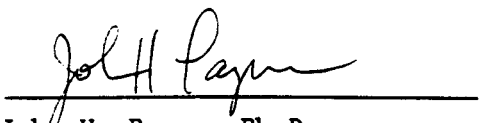
There is no reason to believe that deleterious effects on beneficial organisms could result specifically from the cultivation of line 1345-4 tomatoes, based on two lines of reasoning: (1) analysis of biochemical components of line 1345-4 tomatoes identified no toxic components of these tomatoes that 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 DNAP for line 1345-4 tomatoes. APHIS also cannot envision any plausible mechanisms for any hypothetical pathogenetic effect.

The definition of line 1345-4 encompasses not only tomato lines that already have been field tested, but also new tomato lines produced through conventional breeding using line 1345-4 as one or both parents. APHIS believes that the analysis applied to line 1345-4 already field tested will apply equally well to these new tomato lines, and that the data provided by DNAP justify the conclusion that such new 1345-4 lines will not present a plant pest risk. The variation in agronomic characteristics among the 1345-4 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. While it is impossible to predict the exact agronomic characteristics of the progeny of a cross between a line 1345-4 and another tomato cultivar, cross-breeding between well-characterized tomato varieties is the traditional means by which new and improved tomato varieties are created. These crosses have often used as one parent tomato cultivars that are considerably more genetically different from standard commercial cultivars than are line 1345-4 tomatoes, i.e., other members of the genus. Breeders then select and continue to breed the progeny which display the desired agronomic characteristics. The general range of phenotypes expected from such crosses is well known. There is no reason to believe that any of the progeny of line 1345-4 will possess plant pest properties.

IV. CONCLUSION

APHIS has determined that tomato line 1345-4 that has 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 line 1345-4 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 demonstrate that the line 1345-4: 1) exhibits no plant pathogenic properties. Although pathogenic organisms were used in the development of this line, these tomato plants are not infected nor can they incite disease in other plants; 2) is no more likely to become a weed that a delayed-ripening tomato developed by traditional breeding techniques; 3) is unlikely to increase the weediness potential for any other cultivated or wild species with which it can interbreed; 4) is 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 line 1345-4 tomato varieties bred from these lines will not exhibit new plant pest properties, i.e., properties substantially different from any observed for line 1345-4 already field tested, or those observed for tomatoes in traditional breeding programs.



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