Notices

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Animal and Plant Health Inspection Service

[Docket No. 95-097-2]

Agritope, Inc.; Availability of Determination of Nonregulated Status for Cherry Tomato Line Genetically Engineered for Modified Fruit Ripening

AGENCY: Animal and Plant Health Inspection Service, USDA.

ACTION: Notice.

SUMMARY: We are advising the public of our determination that a cherry tomato line developed by Agritope, Inc., designated as 35-1-N that has been genetically engineered for modified fruit ripening is no longer considered a regulated article under our regulations governing the introduction of certain genetically engineered organisms. Our determination is based on our evaluation of data submitted by Agritope, Inc., in its 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 Agritope, 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: March 27, 1996.

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. Ved Malik, 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; e-mail: mkpeterson@aphis.usda.gov.

SUPPLEMENTARY INFORMATION:

Background

On November 20, 1995, the Animal and Plant Health Inspection Service (APHIS) received a petition (APHIS Petition No. 95–324–01p) from Agritope, Inc., (Agritope) of Beaverton, OR, seeking a determination that a cherry tomato line designated as 35–1–N that has been genetically engineered for modified fruit ripening does not present a plant pest risk and, therefore, is not a regulated article under APHIS' regulations in 7 CFR part 340.

On January 23, 1996, APHIS published a notice in the Federal Register (61 FR 1743–1744, Docket No. 95–097–1) announcing that the Agritope petition had been received and was available for public review. The notice also discussed the role of APHIS and the Food and Drug Administration in regulating the subject tomato line and food products derived from it. In the notice, APHIS solicited written comments from the public as to whether the subject tomato line posed a plant pest risk. The comments were to have been received by APHIS on or before March 25, 1996.

During the designated 60-day comment period, APHIS received a total of 21 comments on the petition for cherry tomato line 35–1–N from individuals, a seed company, a State department of agriculture, and a university. All of the comments were in support of the subject petition.

Analysis

Cherry tomato line 35–1–N has been genetically engineered to contain the sam-k gene derived from Escherichia coli bacteriophage T3 that encodes an enzyme, S-adenosylmethionine hydrolase (SAMase), which alters the ethylene biosynthetic pathway and delays ripening of the tomato on the vine. When exposed to exogenous ethylene the fruit of line 35–1–N ripen normally. The subject cherry tomato line also contains the nptII gene from the prokaryotic transposon Tn5, which encodes the enzyme neomycin phosphotransferase II and is used as a selectable marker for transformation. Expression of the introduced genes is controlled in part by the 3' region of the nopaline synthase gene from the plant pathogen Agrobacterium tumefaciens. The A. tumefaciens vector system was used to transfer the added genes into the Large Red Cherry parental line.

Cherry tomato line 35–1–N has been considered a regulated article under APHIS' regulations in 7 CFR part 340 because it contains regulatory gene sequences derived from the plant pathogen *A. tumefaciens*. However, evaluation of field data reports from field tests of the subject tomato line conducted under APHIS permits or notifications since 1992 indicates 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 Agritope and a review of other scientific data, comments received, and field tests of the subject tomato line, APHIS has determined that cherry tomato line 35–1–N: (1) Exhibits no plant pathogenic properties; (2) is no more likely to become a weed than cherry tomato cultivars 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) will not harm threatened or endangered species or other organisms, such as bees, that are beneficial to agriculture; and (5) will not cause damage to raw or processed agricultural commodities. Therefore, APHIS has concluded that cherry tomato line 35–1–N and any progeny derived from hybrid crosses with other nontransformed tomato varieties will be just as safe to grow as traditionally bred cherry tomato lines that are not regulated under 7 CFR part 340.

The effect of this determination is that Agritope's cherry tomato line designated as 35–1–N is no longer considered a regulated article under APHIS' regulations in 7 CFR part 340. Therefore, the notification requirements pertaining to regulated articles under those regulations no longer apply to the field testing, importation, or interstate movement of the subject tomato line or its progeny. However, the importation of cherry tomato line 35–1–N 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 Agritope's cherry tomato line 35–1–N and lines developed from it 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 4th day of April 1996.

Lonnie J. King,

Administrator, Animal and Plant Health Inspection Service.

[FR Doc. 96–8904 Filed 4–9–96; 8:45 am]

BILLING CODE 3410–34–P

USDA/APHIS Determination on a Petition 95-324-01p of Agritope, Inc. Seeking Nonregulated Status for Delayed-Ripening Cherry Tomato Line 35-1-N

Environmental Assessment and Finding of No Significant Impact

The Animal and Plant Health Inspection Service (APHIS) of the U. S. Department of Agriculture (USDA) has prepared an environmental assessment prior to issuing a determination of nonregulated status for a genetically engineered delayed-ripening cherry tomato line 35-1-N. The genetic modification causes expression of the enzyme S-adenosylmethionine hydrolase which metabolizes the precursor of ethylene and thereby delays the ripening process of tomato fruit. APHIS received a petition from Agritope, Inc. regarding the status of line 35-1-N 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 prepared a finding of no significant impact on the environment from its determination that the genetically modified tomato line 35-1-N shall no longer be a regulated article. As a result of the preparation of a finding of no significant impact, the preparation of an environmental impact statement is not required.

John H. Payne

MAR 27 1996
DATE

JOHN H. PAYNE
ACTING DIRECTOR
BIOTECHNOLOGY, BIOLOGICS, AND
ENVIRONMENTAL PROTECTION
ANIMAL AND PLANT HEALTH INSPECTION SERVICE

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I. Summary

APHIS prepared an Environmental Assessment (EA) prior to making its determination on the regulated status of the genetically engineered delayed-ripening (DR) tomato line 35-1-N. The developer of line 35-1-N, the Agritope, Inc. Company, (hereafter referred to as Agritope), has petitioned APHIS for a determination that line 35-1-N does not present a plant pest risk and should therefore no longer be a regulated article under the APHIS regulations found at 7 CFR Part 340. As a regulated article under these regulations, interstate movements and field tests of line 35-1-N have been conducted under permits issued or notifications acknowledged by APHIS.

The line 35-1-N has been developed in an effort to allow the harvest of vine-ripened tomatoes with extended market and shelf life to supply fruit of good flavor quality to the consumer. Mature fruits, harvested for fresh market production, ripen more slowly, and remain firmer longer than the nonmodified recipient tomato fruits. The gene conferring this characteristic was introduced via recombinant DNA techniques. These techniques enabled the developer to insert two transgenes into the genome of a "Large Red Cherry" tomato variety (1) a gene sequence encoding the enzyme S-adenosylmethionine hydrolase that metabolizes the precursor of the fruit ripening hormone ethylene, and (2) a gene encoding the selectable marker neomycin phosphotransferase (NPTII). The enzyme NPTII confers resistance to certain antibiotics used to select transformed cells. Line 35-1-N is a regulated article under APHIS regulations because some DNA regulatory sequences accompanying the introduced genes were derived from known plant pests, and their introduction into the plant genome was mediated via a well-characterized plant pest vector system.

Previous EAs, prepared before granting permits for field trials of line 35-1-N, addressed questions pertinent to plant pest risk issues concerning the conduct of such trials under physical and reproductive confinement. These, however, do not address several issues relevant to the unconfined growth of line 35-1-N. With respect to these new issues, APHIS concludes that line 35-1-N: (1) exhibits no plant pathogenic properties; (2) is no more likely to become a weed than other cherry tomato cultivars developed by traditional breeding techniques; (3) is unlikely to increase the weediness potential or negatively impact the biodiversity of any other cultivated or wild species with which it can interbreed; (4) will not harm threatened or endangered species or organisms that are beneficial to agriculture; and (5) should not cause damage to raw or processed agricultural commodities.

APHIS believes that the DR tomato line 35-1-N and its progeny will be just as safe to grow as traditionally bred cherry tomato varieties 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 line 35-1-N and its progeny are no longer considered regulated articles under 7 CFR Part 340.

II. Background

A. Development of Line 35-1-N

Agritope has submitted a "Petition for Determination of Nonregulated Status" to APHIS for a cherry tomato line designated 35-1-N that has been genetically engineered to exhibit delayed ripening (DR) of fruit. Agritope. requested a determination from APHIS that line 35-1-N, and any progeny derived from hybrid crosses between these lines and other nontransformed tomato varieties including both cherry and noncherry types, no longer be considered regulated articles under 7 CFR Part 340.

DR tomato line 35-1-N was developed by using standard *Agrobacterium* binary vectors for introducing into the genome of Large Red Cherry a *S*-adenosylmethionine hydrolase (SAMase) encoding gene derived from *E. coli* bacteriophage T3. This results in transformed cherry tomato plants that exhibit significantly reduced levels of *S*-adenosylmethionine (SAM), the substrate for conversion (through ACC synthase) to 1-aminocyclopropane-1-carboxylic acid (ACC) which is the first committed step in ethylene biosynthesis. Ethylene is an endogenous plant hormone known to play an important role in fruit ripening of climacteric fruits. Lack of a sufficient pool of SAM for conversion to ACC in fruit results in tomato fruit with significantly reduced ethylene biosynthetic capabilities and a modified ripening phenotype. In the case of line 35-1-N the phenotype is characterized by fruit in which ripening on the vine is delayed while ripening off the vine is essentially suspended. However, in either case, tomato fruits expressing SAMase ripen normally when exposed to exogenous ethylene.

The cherry tomato line for which Agritope is requesting this determination, line 35-1-N, contains a version of the *sam-k* gene modified in the 5' region of the gene with a Kozak consensus sequence. This construct encodes a functional SAMase protein. Since SAM plays a central role in numerous biosynthetic pathways in plants, expression of *sam-k* gene is under the control of an organ specific (fruit) and temporally regulated (post-climacteric) promoter. The efficacy of this strategy is demonstrated by the fact that the organ specific and temporal expression pattern of ethylene biosynthesis precisely matches the SAMase expression kinetics (ethylene synthesis is inversely correlated to SAMase expression) and provides an explanation of the observed modified ripening.

Tomato fruit expressing SAMase have been field tested since 1992 in the principal tomato growing regions of the United States. These tests were carried out under field release permits and/or notifications granted by APHIS (USDA permits Nos. 92-085-01, 93-49-01N, 93-050-01, 93-176-01N, 93-340-02N, 93-361-01-N, 940048-01N, 94-143-03-N, 94-353-01N, 95-121-03N, 95-121-04N). Further tests are currently being conducted in additional locations in Mexico (permission granted by Sanidad Vegetal, May 4, 1995 and December 4, 1995). Data collected from these trials as well as from laboratory analyses and literature references presented in this petition demonstrate that SAMase expressing cherry tomato line 35-1-N exhibits no plant pathogenic properties, is no more likely to become a weed than the nontransgenic parental variety, is unlikely to increase the weediness potential of any other cultivated plant or native wild species, does not damage or cause to be damaged

processed agricultural commodities and finally is unlikely to harm other organisms that are beneficial to agriculture.

B. APHIS Regulatory Authority

APHIS regulations under 7 CFR Part 340, 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), pertain to 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 regulations and is also a plant pest or there is reason to believe that it is a plant pest. DR cherry tomato line 35–1–N has been considered a regulated article because some noncoding DNA regulatory sequences and portions of the plasmid vector are derived from plant pathogens and the transformation vector is a plant pathogen.

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 will 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 of nonregulated status of DR cherry tomato line 35–1–N and its progeny under APHIS regulations. Agritope., the developer of line 35–1–N, submitted a petition requesting that APHIS make a determination that cherry tomato line 35–1–N and its progeny should no longer be considered regulated articles under 7 CFR Part 340.

This EA was prepared in compliance 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; 60 FR 6000-6005, February 1, 1995).

IV. Alternatives

A. No Action

Under the "no action" alternative, APHIS would not come to a determination that line 35–1–N and its progeny were not regulated articles under the regulations at 7 CFR Part 340.

Permits from APHIS would still be required for the introduction of line 35-1-N and its progeny. APHIS would choose this alternative if there were insufficient evidence to demonstrate lack of plant pest risk from the uncontained cultivation of line 35-1-N and its progeny.

B. Proposed Action: Determination of Nonregulated Status

Under this alternative, cherry tomato line 35-1-N and its progeny would no longer be considered regulated articles under 7 CFR Part 340. Permits or acknowledged notifications from APHIS would no longer be required for the introduction of line 35-1-N or its progeny. A basis for this determination would include a "Finding of No Significant Impact" under NEPA.

V. Potential Environmental Impacts

This EA addresses potential environmental impacts from a determination that line 35-1-N and its progeny should no longer be considered regulated articles under 7 CFR Part 340. Previous EAs prepared by APHIS for the issuance of permits for field tests of line 35-1-N and backcross progeny have addressed various properties of these tomatoes. This EA discusses the genetic modification and potential environmental impacts associated with the unconfined cultivation and distribution of line 35-1-N. These environmental impacts are compared to the environmental impacts posed by the unconfined cultivation and distribution of cherry tomatoes not subject to APHIS regulations.

Additional technical information and literature citations included in the determination document (Appendix A) in this EA are incorporated by reference. These include detailed discussions of the biology of tomato including cherry types, the genetic components used in the construction of line 35-1-N, the genotypic and phenotypic differences between the recipient and line 35-1-N, and the analyses that lead to the conclusion that line 35-1-N does not pose a plant pest risk.

A. Increased Weediness

Various definitions of the term "weed" have been proposed. The salient point is that a plant can be considered a weed when it is growing where humans do not want it (Baker, 1965; de Wet and Harlan, 1975; Muenscher, 1980). Baker (1965) lists common attributes that can be used to assess the likelihood that a plant species will behave as a weed. Keeler (1989) and Tiedje et al. (1989) have adapted and analyzed these characteristics to develop guidelines to assess the weediness potential of transgenic plants; both authors emphasize the importance of evaluating the parent plant and the nature of the specific genetic changes.

The parent of line 35-1-N is the nontransformed cherry tomato line Large Red Cherry (*Lycopersicon esculentum* Mill. var. *cerasiforme*). Large Red Cherry is an open pollinated line developed by Petoseed, Inc. and available in the public domain through various commercial outlets. It is characterized as an indeterminate small fruited cherry type variety with an average fruit size of 1.5 inches in diameter. Fruit ripen to a deep red approximately

70 days post transplanting and are borne in clusters on highly productive plants. Large Red Cherry tomato line exhibits no appreciable weedy characteristics. All cultivated forms of tomato including cherry tomato belong to the species *L. esculentum*. As a crop, cherry tomatoes are grown commercially wherever environmental conditions permit the production of an economically viable yield. In the United States, the principal cherry tomato growing regions are Florida and California. Cherry tomatoes are available in the United States year-round although the greatest supply is from June through October. Cherry tomatoes are consumed in many forms. They are eaten whole, or cooked as an ingredient in many prepared foods. Due to the versatility of all types of tomato and its ability to be consumed in so many various forms, tomato has become one of the most widely consumed vegetable crops with worldwide consumption estimated at upwards of 50 million metric tons (Tigchelaar, 1986).

Tomato has been grown for centuries throughout the world without any report that it is a serious weed pest. Cherry tomato is not classified as a serious, principal, or common weed pest in the U.S. (Holm et al., 1979), and it is not listed under the Federal Noxious Weed Act (7 CFR Part 360). Although cherry tomato volunteers are common, they are easily controlled with herbicides or by mechanical means.

The genetically engineered *sam-k* gene in line 35-1-N results in a reduction in the biosynthesis of the plant hormone ethylene. Because ethylene can effect many growth, developmental, and disease processes in plants, Agritope. submitted results of an extensive field study of line 35-1-N and its parental line that was designed to examine parameters that have been proposed as indicators of weediness potential. Thus, there is no indication that the genetic modification will convert line 35-1-N into a weed.

The introduction and expression of the selectable marker gene *(nptII)* into tomato plants also does not affect their weediness characteristics. This gene facilitates the selection of transformed cells in the laboratory, but provides no selective advantage in agricultural environments. This gene is not involved in plant disease or damage. Also, its use does not result in the presence of the antibiotics in tomatoes and does not imply that antibiotics will be used in the cultivation of the tomatoes.

No other characteristics of line 35-1-N reported by Agritope., including disease and pest susceptibilities, fruit ripening characteristics or the number of volunteers in field trials, suggest that line 35-1-N will be any more "weedy" than present cherry tomato cultivars derived from traditional breeding.

B. Gene Transfer to Tomato Relatives and Other Organisms

APHIS considered the potential for genes to be transferred from line 35-1-N to other sexually compatible relatives of cultivated tomato (*Lycopersicon esculentum* var *esculentum* Mill. *Lycopersicon esculentum* Mill. var *cerasiforme*), and the impact that such introgression would have on the weediness potential and biodiversity of progeny. Cherry tomato generally does not cross-pollinate with other plants in the U.S. without human intervention. Cultivated tomato is self-fertile and almost exclusively self-pollinating, due, in part, to the presence of an inserted stigma (Rick, 1976). Tomato is not wind-pollinated and insect pollination is limited

(Rick, 1976). Agritope. reported no differences in flower morphology between line 35-1-N and the nonmodified recipient, therefore outcrossing in line 35-1-N is naturally limited by its reproductive biology.

Because commercial tomatoes are almost exclusively self-pollinating and the seed is sold commercially, crosses unaided by humans between the line 35-1-N cherry tomato and another plant are highly unlikely. Therefore, there is no likelihood that line 35-1-N will negatively impact the biodiversity of other plant species in the United States. Although limited by high self-pollination rates, outcrossing of line 35-1-N to primitive tomato cultivars and wild or weedy relatives (particularly those in the *esculentum*-complex) is possible in Mexico, Central America, and northwestern South America. This is discussed in further detail in the determination. Our analysis of the biology of cherry tomato and its relatives leads us to predict that the environmental impacts of cultivation of line 35-1-N anywhere in the world would be no different from such impacts attributable to similar varieties produced with traditional breeding techniques. We note that any international traffic in line 35-1-N would be fully subject to national and regional phytosanitary standards promulgated under the International Plant Protection Convention (IPPC).

Even if an outcrossing event involving pollen from line 35-1-N did occur, there is no reason to believe that the progeny would be any more weedy than progeny from crosses resulting from pollination by other traditionally bred tomato cultivars. The minor genotypic differences observed in line 35-1-N are not expected to confer a selective advantage that would increase their weediness potential relative to other commercial cherry tomato cultivars, nor would they be expected to increase the weediness potential if introgressed into other sexually compatible plants.

Horizontal gene transfer of transgenes from genetically engineered plants to other organisms is not well documented; however, horizontal gene transfer of transgenes from higher transgenic plants to a soil microorganism has been reported (Hoffmann et al., 1994). Because SAMase activity and kanamycin resistance have already been characterized in many soil microorganisms (Tran and Kretzmer, 1993; Henschke and Schmidt, 1990), APHIS anticipates that horizontal gene transfer into soil microorganisms of the plant transgenes conferring these traits should pose no greater plant pest risk or risk to biodiversity than would direct or horizontal gene transfer among soil microorganisms.

C. Nontarget Species

Consistent with its statutory authority and requirements under NEPA, APHIS evaluated the potential for line 35-1-N, and plant products derived from it, to have damaging or toxic effects directly or indirectly on nontarget organisms, particularly those that are recognized as beneficial to agriculture and to those which are recognized as threatened or endangered in the United States.

Agritope. analysis of biochemical components of line 35-1-N tomato fruit identified no toxic components that are present in concentrations significantly different from the concentrations in nontransgenic tomatoes. The genetic modification in line 35-1-N does not result in the

production of new proteins, enzymes or metabolites in the plant that are known to have toxic properties. The plants also do not exhibit any pathogenic properties.

APHIS concludes that the unconfined growth of line 35-1-N, and products derived from it, will have no deleterious effects on organisms recognized as beneficial to agriculture (e.g., earthworms, honey bees) or on other organisms, including any species recognized as threatened or endangered in the United States.

D. Agricultural Practices

Cherry tomato line 35-1-N exhibits the typical agronomic characteristics of the parent line with the exception of ripening phenotype. APHIS believes that the DR phenotype in line 35-1-N does not pose a plant pest risk nor will it have a significant impact on agricultural practices. As a result of the DR phenotype, fruit from fresh market varieties developed from line 35-1-N could be harvested at less frequent intervals than they might be otherwise. This may broaden or delay the harvesting period as well as possibly reduce labor costs. Tomatoes from line 35-1-N are expected to have a longer shelf life, and may not need to be refrigerated during storage. The DR trait is also expected to delay over-ripening of processing tomatoes that may be developed from line 35-1-N. This could provide growers with more flexibility in harvest dates and potentially reduce losses caused by ripe-rot diseases.

APHIS concludes that the DR tomato line 35-1-N should not have any major potential impacts on agricultural and cultivation practices.

E. Raw or Processed Agricultural Commodities

A determination of nonregulated status by APHIS under Section 340.6 includes an evaluation of whether the regulated article causes disease, injury, or damage to raw or processed agricultural products. Safety concerns for human and animal consumption of products of new plant varieties, however, are addressed by the FDA. Their policy statement concerning regulation of products derived from new plant varieties, including those genetically engineered, was published in the Federal Register on May 29, 1992, at 57 FR 22984-23005. Agritope. has satisfactorily completed a voluntary food safety consultation with the FDA consistent with this policy statement. Agritope. provided to the FDA extensive composition studies of tomato fruits and processed tomato products derived from line 35-1-N which revealed that they are not materially different from those of the parental cultivar.

SAMase is not expected to cause disease, injury, or damage to agricultural commodities because it is not associated with plant pathogenicity. The enzymatic reaction it catalyzes and the products of that reaction are not expected to cause damage or injury to tomato fruit. APHIS has previously determined that expression of *nptII* gene in certain genetically engineered tomato lines does not have an adverse impact on agricultural commodities (see APHIS EA and Determination documents for petitions 92-196-01p and 94-290-01p). The DR phenotype is expected to increase the quality of fruit harvested from commercial fresh market cherry tomatoes which may be developed from line 35-1-N.

APHIS concludes that the cherry tomato line 35-1-N does not have any unique characteristics, compared to commercially cultivated tomatoes, that would pose a direct or indirect plant pest risk or have an adverse impact on any raw or processed agricultural plant commodity.

VI. Conclusion

APHIS has evaluated the scientific literature as well as data submitted by Agritope. relevant to cherry tomato line 35-1-N. APHIS has considered the foreseeable consequences of a determination of nonregulated status for tomato line 35-1-N and its progeny, and has reached the following conclusions:

1. Cherry tomato line 35-1-N exhibits no properties of plant pathogens. Although pathogenic organisms were used in the development of this line, these tomato plants are not infected, nor can they cause disease in other plants.

2. Cherry tomato line 35-1-N is no more likely to become a weed than other tomato lines developed by traditional breeding techniques. Tomato is not a weed pest, and there is neither reason to believe nor data to indicate that the introduced genetic constructs will provide a selective advantage sufficient to enable tomato lines expressing these genes to become weeds.

3. The potential for unintended gene introgression from cherry tomato line 35-1-N into sexually compatible weedy or wild relatives by outcrossing or into other organisms by horizontal gene transfer is limited. Furthermore, gene introgression from line 35-1-N into sexually compatible relatives would be no more likely to increase their weediness potential than would gene introgression from other cultivated tomatoes developed by traditional breeding methods.

4. Cherry tomato line 35-1-N will not have deleterious effects on threatened or endangered species or other organisms which are beneficial to agriculture.

5. Cherry tomato line 35-1-N should not cause disease, damage, or injury to raw or processed agricultural commodities.

Therefore, APHIS concludes that tomato line 35-1-N, and any progeny derived from hybrid crosses between these lines and other nontransformed tomato varieties, will be just as safe to grow as traditionally bred tomato lines that are not regulated under 7 CFR Part 340. APHIS concludes that there should be no significant impact on the human environment if line 35-1-N and its progeny were no longer considered regulated articles under 7 CFR Part 340.

VII. Preparers and Reviewers

Terry L. Medley, J.D., Associate Administrator
Animal and Plant Health Inspection Service

Biotechnology, Biologics, and Environmental Protection

John Payne, Ph.D., Associate Director
(Acting Director, Biotechnology, Biologics, and Environmental Protection)

Biotechnology Permits

Arnold Foudin, Ph.D., Deputy Director
Subhash Gupta, Ph.D., Biotechnologist (reviewer)
David S. Heron, Ph.D., Biotechnologist
Susan Koehler, Ph.D., Biotechnologist
James Lackey, Ph.D., Biological Safety Officer
Vedpal Malik, Ph.D., Biotechnologist (preparer, petition coordinator)
H. Keith Reding, Ph.D., Biotechnologist
Sivramiah Shantharam, Ph.D., Team Leader, Biotechnologist
James L. White, Ph.D., Team Leader, Biotechnologist

Biotechnology Coordination and Technical Assistance

Michael A. Lidsky, J.D., L.L.M., Deputy Director
Shirley P. Ingebritsen, M.A., Regulatory Analyst
L. Val Giddings, Ph.D., International Policy Team Leader (reviewer)
Quentin Kubicek, Ph.D., Plant Pathologist
Michael Schechtman, Ph.D., Domestic Policy Team Leader
Frank Y. Tang, Ph.D., J.D., Animal Physiologist/Biotechnologist

Environmental Analysis and Documentation

Carl Bausch, J.D., Deputy Director

VIII. Agency Contact

Ms. Kay Peterson, Regulatory Analyst
Biotechnology, Biologics, and Environmental Protection
USDA, APHIS
4700 River Road, Unit 147
Riverdale, MD 20737-1237

301-734-7612 PHONE
301-734-8669 FAX

Appendix A **Determination: Response to the Agritope, Inc. Company Petition for Determination of Nonregulated Status for Delayed-Ripening Tomato Line 35-1-N**

Determination: Response to the Agritope, Inc. Company Petition for Determination of Nonregulated Status for Delayed-Ripening Tomato Line 35-1-N

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I. Summary

Based on a review of scientific data and literature, the Animal and Plant Health Inspection Service (APHIS) has determined that the delayed-ripening (DR) cherry tomato line 35-1-N does not present a plant pest risk, and therefore this line, and any progeny derived from crosses involving this line, will no longer be considered regulated articles under 7 CFR Part 340. Consequently, oversight under those regulations will no longer be required from APHIS for field testing, importation, or interstate movement of cherry tomato line 35-1-N or its progeny. Importation of cherry tomatoes derived from line 35-1-N and nursery stock or seeds capable of propagation, however, remains subject to 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 the Agritope, Inc. (Agritope) dated November 20, 1995 that requested a determination from APHIS that cherry tomato line 35-1-N does not present a plant pest risk and therefore should no longer be considered a regulated article. On January 23, 1996, APHIS announced receipt of this petition in the Federal Register (61 FR 1743-1744) and stated that the petition was available for public view. APHIS invited written comments on this proposed action to be submitted by March 25, 1996. Cherry tomato line 35-1-N was developed by introducing a gene into the genome of a cherry tomato that encodes an enzyme capable of degrading *S*-adenosylmethionine (SAM). Production of this enzyme, *S*-adenosylmethionine hydrolase (SAMase) in fruit alters the ethylene biosynthetic pathway and causes a modified fruit ripening phenotype. Agritope, Inc. requests a determination from APHIS that the ***Sam-k*** containing cherry tomato line 35-1-N and any progeny derived from hybrid crosses between this line and any other nontransformed cherry tomato varieties, no longer be considered a regulated article under 7 CFR Part 340.

Using standard *Agrobacterium* binary vectors, Agritope scientists have introduced a ***sam-k*** gene derived from *E. coli* bacteriophage T3 into the cherry tomato genome. This results in transformed cherry tomato plants that exhibit significantly reduced levels of *S*-adenosylmethionine (SAM), the substrate for conversion (through ACC synthase) to 1-aminocyclopropane-1-carboxylic acid (ACC) which is the first committed step in ethylene biosynthesis. Ethylene is an endogenous plant hormone known to play an important role in fruit ripening of climacteric fruits. Lack of a sufficient pool of SAM for conversion to ACC in fruit results in cherry tomatoes with significantly reduced ethylene biosynthetic capabilities and delayed ripening (DR) phenotype. In the case of line 35-1-N the phenotype is characterized by fruit in which ripening on the vine is delayed while ripening off the vine is essentially suspended. However, in either case, cherry tomato fruits expressing ***sam-k*** ripen normally when exposed to exogenous ethylene.

Agritope requests that USDA, APHIS based on data presented in this document, determine that DR cherry tomatoes, defined as a cherry tomato cultivar or progeny of a cultivar genetically engineered using the following binary vector: pAG 5420 containing the ***sam-k*** gene and its associated promoter and terminator, do not represent a plant pest risk, are not otherwise deleterious to the environment and are therefore not a regulated article.

Agritope, Inc. believes that the observed ripening phenotype has numerous commercial applications in the current fresh cherry tomato production and distribution system. These include but are not limited to the following:

1. Reduction in producer losses through reduced harvest of immature and/or over mature fruit.
2. Improved production dynamics and reduced harvest frequency.
3. Reduced spoilage and loss through out the distribution system.
4. Enhanced quality due to harvest of more physiologically mature fruit.

Cherry tomato line 35-1-N has been considered a "regulated article" under Part 340 of the regulations, in part, because it has been engineered using components from known plant pests. Some of the DNA regulatory sequences were derived from the plant pathogens *Agrobacterium tumefaciens* (Zambryski, 1988). In addition, the vector system used to transfer the two genes into the recipient cherry tomato was derived from *A. tumefaciens*. Field testing of line 35-1-N has been conducted since 1992 under APHIS permits or notifications using conditions of reproductive confinement as stipulated in the principal cherry tomato growing regions of the United States. These tests were carried out under field release permits and/or notifications granted by APHIS (USDA permits 92-085-01, 93-49-01N, 93-050-01, 93-176-01N, 93-340-02N, 93-361-01-N, 940048-01N, 94-143-03-N, 94-353-01N, 95-121-03N, 95-121-04N) and further tests are currently being conducted in additional locations in Mexico (permission granted by Sanidad Vegetal, May 4, 1995). Data collected from these trials as well as from laboratory analyses and literature references presented in this petition demonstrate that *sam-k* expressing cherry tomato line 35-1-N exhibits no plant pathogenic properties, is no more likely to become a weed than the nontransgenic parental variety, is unlikely to increase the weediness potential of any other cultivated plant or native wild species, does not damage or cause to be damaged processed agricultural commodities and finally is unlikely to harm other organisms that are beneficial to agriculture.

This determination has been made based on an analysis that reveals that line 35-1-N (1) exhibits no properties of plant pathogens; (2) is no more likely to become a weed than the nonmodified parental variety or other cherry tomatoes developed by traditional breeding techniques; (3) is unlikely to increase the weediness potential or adversely impact the biodiversity of any other cultivated plant or native wild species with which the organism can interbreed; (4) is unlikely to harm other organisms, such as bees, that are beneficial to agriculture; and (5) will 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 line 35-1-N will exhibit new plant pest properties; i.e., properties substantially different from those observed for cherry tomato line 35-1-N already field tested, or those observed for cherry tomatoes derived from traditional breeding programs.

The potential environmental impacts associated with this determination have been examined 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; 60 FR 6000-6005, February 1, 1995). An environmental assessment (EA) and Finding of No Significant Impact (FONSI) was prepared by APHIS for its determination that line 35-1-N and its progeny are no longer considered regulated articles under 7 CFR Part 340.

The body of this document consists of three parts: (1) background information that provides the legal framework under which APHIS has regulated the introduction of line 35-1-N, (2) a summary of comments provided to APHIS during the public comment period and APHIS' response to those comments' and (3) an analysis of the key factors relevant to APHIS' decision that line 35-1-N does not present a plant pest risk.

II. Regulatory Authority

APHIS regulations at 7 CFR Part 340, promulgated pursuant to 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), pertain to the introduction (importation, interstate movement, or release into the environment) of certain genetically engineered organisms and products. The FPPA provides the authority to regulate plant pests and other articles to prevent direct or indirect injury, disease, or damage to plants and plant products. The PQA also enables USDA to regulate the importation and movement of nursery stock and other plants that may harbor injurious pests or diseases.

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 if the donor organism, recipient organism, vector or vector agent used in engineering the organism belongs to one of the taxa listed in the regulations 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 of an article regulated under 7 CFR Part 340 is granted when APHIS has determined that the conduct of the field trial 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 (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 specified eligibility criteria and performance standards be met. The eligibility criteria set 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.

An organism is no longer subject to 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 is granted, thereby allowing for unregulated introduction of the article. A petition may be granted in whole or in part.

Cherry tomato line 35-1-N has been considered a "regulated article" under Part 340 of the regulations, in part, because some of the noncoding DNA regulatory sequences were derived from plant pathogens, i.e., the bacterium *Agrobacterium tumefaciens*. In addition, the vector system used to transfer the two genes into the recipient cherry tomato was derived from *A. tumefaciens*. APHIS believes it prudent to provide assurance before commercialization that organisms such as DR cherry tomato line 35-1-N, that are derived in part from plant pests, do not pose a 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 35-1-N is no longer a regulated article is based, in part, on evidence provided by Agritope, Inc. concerning the biological properties of cherry tomato line 35-1-N and its similarity to other varieties of cherry tomato grown under standard agricultural practices for commercial sale or private use.

A determination 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' definition of 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 properties of plant pathogens; (2) is no more likely to become a weed than the nonmodified 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 Agritope, Inc. that bears on these topics. In addition, because the petition also seeks a determination on any progeny derived from crosses between DR cherry tomato line 35-1-N and traditional cherry tomato varieties, it should be established that such progeny will not exhibit plant pest properties substantially different from those observed for cherry tomatoes in traditional breeding programs or as seen in the development of line 35-1-N.

III. Response to Comments

APHIS received a total of 21 comments on the petition for cherry tomato line 35-1-N from individuals, a seed company, a state department of agriculture, and a university. All of the comments were in support of the subject petition.

IV. Analysis of the Properties of Cherry Tomato Line 35-1-N

In order to establish that cherry tomato line 35-1-N or varieties derived from it do not pose a plant pest risk greater than or different from that presented by traditionally bred cherry tomatoes grown in the United States, APHIS considered, among other things, the biology, cultivation, storage, and processing of the nonmodified recipient cherry tomato, and the plant pest risks associated with the modified organism and these activities. A brief discussion follows of these issues and the biology, development, and intended use of cherry tomato line 35-1-N or varieties derived from it. This information provides background for the plant pest issues and is expanded in subsequent sections, when relevant to address particular issues related to line 35-1-N.

Agritope developed Line 35-1-N by genetically engineering the Large Red Cherry tomato line (*Lycopersicon esculentum* Mill. var *cerasiforme*) to express a gene conferring the DR phenotype and a kanamycin resistance gene as a selectable marker for transformation. Large Red Cherry is an open pollinated line developed by Petoseed, Inc. and available through various commercial outlets. It is a highly inbred perennial in its native tropical habitat and is a member of the genus *Lycopersicon*, species *esculentum* (Rick, 1979). The genus is native to South America and the natural distribution ranges from northern Chile to southern Columbia, westward to the Pacific Ocean and eastward to the foothills of the Andes range. The wild source of cultivated tomato must certainly have been *Lycopersicon esculentum* var *cerasiforme,* which previously had been carried from the Andean center of origin of the genus through northern South America, across the Panamanian Isthmus to Central America and Southern Mexico (Rick 1983).

Tomato has been grown for food for hundreds of years and continues to be a popular commodity both for commercial production and also in home gardens. In the United States alone, per capita consumption is approximately 25.5 kg/year (Rick 1978).

Tomato is a simple diploid species with twelve pairs of highly differentiated chromosomes. The genome size of tomato is estimated at 7.1×10^8 bp per haploid genome (Galbraith et al., 1983). Cultivated tomato is self fertile and for all practical purposes, under commercial and home garden cultivation it is exclusively self pollinating (Rick, 1979; Taylor, 1986). Cross pollination normally requires human intervention and in general is only carried out in variety development and/or hybrid seed production programs. Interspecific hybrids generally occur only as a result of new variety development efforts and are carried out via hand pollination.

To this end, tomato serves as a classical example of the use of interspecific hybridization to improve the characteristics of the cultivated species. Wild species represent an excellent germplasm pool for new genetic material aimed at addressing the problems of disease resistance, insect resistance and tolerance to environmental stresses such as drought, poor soil conditions and cool temperatures (Rick et al, 1987). While the efficacy of such wide crosses in variety improvement programs is indisputable, the natural occurrence of such crosses appears to be very limited.

The closest genetic relatives to the cultivated tomato (outside of the genus *Lycopersicon*) are found in the genus *Solanum*. Hybrids have been obtained between *S. lycopersicoides* and *L. esculentum* and gene transfer has been accomplished between the two with limited success (Stevens et al., 1986; DeVerna et al., 1990). Such hybrids however were only possible using specialized laboratory techniques. Hybrids have also been obtained between *S. rickii* and *L. esculentum* using a sesquidipoloid bridging species (DeVerna et al., 1990) but no other member of the genus including *S. nigrum*, a common weed in commercial tomato fields, are reported to have produced any viable hybrids with cultivated tomato (Taylor, 1986). Table 2 summarizes the occurrence of interspecific hybrids with *L. esculentum*.

Production systems for fresh market cherry tomatoes are based on multiple hand harvests of fruit at the mature green stage to breaker. Fruit are then shipped to local packing houses for packing and distribution. In contrast, processing tomatoes are generally mechanically harvested at a fully ripened stage, and shipped directly to a processing plant. During the normal ripening process, increased ethylene production beginning in the mature green fruit stimulates the physiological and biochemical changes required for ripening.

Quality is determined, in large part, by the occasionally conflicting demands, needs, and expectations of producers, shippers, marketers, and consumers. The maturity of tomato fruit at harvest is an important determinant of the composition and quality of tomatoes. Immature green fruit contaminating mature green fruit harvested for fresh market use reduces overall market quality. To avoid this problem, growers may harvest fruit when it just begins to show color (breaker stage). In a typical cherry tomato field, this necessitates an almost daily harvest. However, these cherry tomatoes (considered vine-ripened) have a greatly reduced market life. Reduced temperatures and controlled atmospheres used to retard or slow ripening of fresh cherry tomatoes can have an adverse effect on quality attributes, such as color and flavor, and may cause chilling injury. Ripened and overripened fresh market cherry tomatoes or processing cherry tomato fruit are subject to ripe-rot diseases caused by bacterial and fungal pathogens. Introduction of the DR trait could enable cherry tomatoes to be harvested less frequently at a more mature stage than they might be otherwise, and have an extended shelf life. The DR trait is also expected to delay overripening of processing cherry tomatoes. This may provide growers with more flexibility in harvest dates and potentially reduce losses caused by ripe-rot diseases.

The cherry tomato line for which Agritope is requesting this determination, line 35-1-N, contains a version of the *sam-k* gene modified in the 5' region of the gene with a Kozak consensus sequence. This construct encodes a functional SAMase protein. Since SAM plays

a central role in numerous biosynthetic pathways in plants, expression of *sam-k* gene is under the control of an organ specific (fruit) and temporally regulated (postclimacteric) promoter. The efficacy of this strategy is demonstrated by the fact that the organ specific and temporal expression pattern of ethylene biosynthesis precisely matches the *sam-k* expression kinetics (ethylene synthesis is inversely correlated to *sam-k* expression) and provides an explanation of the observed modified ripening phenotype.

Line 35-1-N was also genetically engineered to express a selectable marker gene (*nptII*), encoding the enzyme neomycin phosphotransferase II (NPTII), that confers resistance of transformed tissue to the antibiotic kanamycin. The introduced DNA also has regulatory sequences that modulate expression of the introduced genes.

A. Introduced Sequences and Their Products

The E8 promoter (Good et al., 1994) from cherry tomato was used to express the modified *sam-k* gene to obtain line 35-1-N. A selectable marker gene (*nptII*), encoding the enzyme NPTII, that confers resistance to the antibiotic kanamycin and expressed from a *nos* (Depicker et al., 1985) promoter and *nos 3'* (Barker et al.,1983) termination sequence was also used. The *nos* promoter and the *nos* terminator are from *Agrobacterium tumefaciens* (Barker et al.,1983). The *nptII* gene was isolated from *E.coli* (Beck et al., 1982). It has been previously reviewed by U.S. regulatory agencies and determined to be safe (USDA, 1992; FDA, 1994).

The *sam-k* gene was derived from *E. coli* bacteriophage T3. T3 is in the T7 group of coliphages. T3 is commonly found in sewage and can be isolated from the human intestinal tract (Furuse et al., 1983). T3 is neither a human pathogen nor a plant pest (42 CFR 72; 7 CFR 340). Since it is a coliphage, it is likely to have been associated with humans and other vertebrates for eons. Coliform bacteria as well as bacteriophages are also present in low levels in drinking water so that it can be reasonably concluded that humans regularly consume bacteriophages that contain *sam-k* genes (Goyal et al., 1980). When a bacteriophage infection of the intestinal *E. coli* occurs, T3 proteins are present in the infected bacteria. Thus, we conclude that both the *sam-k* gene and the SAMase enzyme are commonly found within the intestinal microflora of humans and other vertebrate animals.

SAMase is the first bacteriophage T3 protein produced upon phage infection of *E. coli*. SAMase inhibits the host bacterium's restriction endonuclease system both by binding the type 1 restriction endonuclease and hydrolyzing SAM, an essential co-enzyme. This protects the remaining phage DNA from host mediated degradation (Studier and Movva, 1976; Spoerel et al., 1979). The coliphage BA14 (Mertens and Hausmann, 1982) and klebsiella phage K11 (Dietz et al., 1985) also contain SAMase coding genes, though each is poorly characterized.

The introduced gene sequences also have accompanying nontranslated DNA regulatory sequences, such as promoters and 3' terminator sequences. Specifically, the DNA regulatory sequences associated with the *sam-k* gene. Although some of these regulatory sequences were derived from plant pathogens, the regulatory sequences cannot cause plant disease by themselves or in conjunction with the genes that they regulate.

The *sam-k* transgene was derived from a previously reported M13 clone (Hughes et al., 1987) modified to contain a consensus eukaryotic translation initiation site by altering the nucleotide sequence surrounding the ATG start codon and fused to a modified E8 promoter (Good et al., 1994) isolated from cherry tomato and a *nos 3'* termination sequence from *Agrobacterium tumefaciens*. The resulting was cloned in plasmid pAG 5420 that was used to transform the Large Red Cherry parental line.

The vector system used to transfer pAG 5420 to the Large Red Cherry parental line is based upon the Ti plasmid from *A. tumefaciens*. This system utilizes a "disarmed" Ti plasmid (i.e., all genes responsible for crown gall disease have been removed from binary vector system (Hoekema et al., 1983; An et al., 1988). In the case of line 35-1-N, *Agrobacterium* strain EHA 101 (Hood et al., 1986) was used to transform the plant. This strain, EHA 101; does not contain any DNA sequences responsible for plant pathogenesis (Hood et al., 1986) and it has been demonstrated with this strain that the *vir* genes are not transferred to the plant and therefore the transferred DNA sequences will not be remobilized within the plant.

Following transformation, *Agrobacterium* are killed with the antibiotic carbenicillin or cefotaxine (Fillatti et al., 1987) so no subsequent transformation or infection can occur. After transformation, the plants themselves are grown to fruit, analyzed and selected transformants used for seed production and advancement in the product development process.

Cherry tomato fruit expressing SAMase have been field tested since 1992 in the principal cherry tomato growing regions of the United States. These tests were carried out under field release permits and/or notifications granted by APHIS (USDA permits 92-085-01, 93-49-01N, 93-050-01, 93-176-01N, 93-340-02N, 93-361-01-N, 940048-01N, 94-143-03-N, 94-353-01N, 95-121-03N, 95-121-04N). Further tests are currently being conducted in additional locations in Mexico (permission granted by Sanidad Vegetal, May 4, 1995 and December 4, 1995) Data collected from these trials as well as from laboratory analyses and literature references presented in this petition demonstrate that SAMase expressing cherry tomato line 35-1-N exhibits no plant pest properties, is no more likely to become a weed than the nontransgenic parental variety, is unlikely to increase the weediness potential of any other cultivated plant or native wild species, does not damage or cause to be damaged processed agricultural commodities and finally is unlikely to harm other organisms that are beneficial to agriculture.

Since cherry tomato itself is not considered a weed pest the only possible weediness problems would be the result of outcrossing to weed pest relatives. The only wild species sexually compatible with cultivated cherry tomato are members of the "*esculentum* complex" (Rick, 1976) whose normal geographic range is limited to South America and these are not considered weed pests. In addition, only through specific controlled crosses is hybridization to these relatives possible (Stevens, et al., 1976, De Verna et al., 1990). *Solanum nigrum*, is the only major weed pest related to cherry tomato and is, in the wild, sexually incompatible with *Lycopersicon* species. Because cherry tomato has no weed pest relatives, there is no possibility of a cross between a SAMase cherry tomato and a wild pest relative that would be capable of creating or enhancing the competitiveness of a weed pest.

In cherry tomato, genes can move via pollination from one individual to another within the species *L. esculentum*. When field grown, tomato is predominantly self pollinated due to its self compatibility and floral structure. Rates of cross pollination on the order of 4% have been reported (Rick, 1978), but present commercial varieties have a floral structure (i.e., inserted stigma) which creates a crop which is exclusively inbreeding (Taylor, 1986). As a result of this and the ability to maintain pure seed stocks using standard practices including selection and isolation, there is little if any potential for outcrossing of SAMase cherry tomatoes to commercial stocks.

In APHIS' judgement, the components and processing characteristics of line 35-1-N reveal no differences in any component that could have an indirect plant pest effect on any processed plant commodity. Line 35-1-N exhibits no plant pest characteristics.

B. Weediness

1. Potential Weediness of Cherry Tomato Line 35-1-N

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."

Most definitions of weediness stress the undesirable nature of weeds from the point of view of humans; but from this core, individual definitions differ (Baker, 1965; de Wet and Harlan, 1975; Muenscher, 1980). Baker (1965) defines a plant as a weed if, in any specified geographical area, its populations grow entirely or predominantly in situations markedly disturbed by man (without being deliberately cultivated). Baker also described many ideal characteristics of weeds. Keeler (1989) and Tiedje et al. (1989) have adapted and analyzed these characteristics to develop guidelines to address the weediness potential of transgenic plants. Both authors emphasize the importance of the parent plant and the nature of the specific genetic changes.

Tomato has been grown for centuries throughout the world without any report that it is a serious weed pest. Specifically, cherry tomato is not classified as a serious, principal, or common weed pest in the United States (Holm et al., 1979), and it is not listed under the Federal Noxious Weed Act (7 CFR Part 360). Although volunteers of all types of tomatoes are common, they are easily controlled using herbicides or by mechanical means. Cherry tomato does not possess a significant number of the characteristics of plants that are notably successful weeds (Keeler, 1989). Cherry tomato is considered a highly domesticated, well-characterized crop plant that is not persistent in the environment without human intervention. Line 35-1-N and varieties developed from it will most likely be grown in commercial production areas as discussed above.

Ethylene is known to have many effects on plant growth and development, including effects on seed germination and dormancy, seedling growth, flowering, and fruit ripening (Taiz and Zeiger, 1991). Since ethylene synthesis is reduced significantly in line 35-1-N

compared to control line , it is not unreasonable to expect some changes in these plant growth and development characteristics in line 35-1-N.

The fruit ripening characteristics of other DR tomato lines have been described (Klee et al., 1991 and Klee, 1993). In detached mature fruit of DR tomato lines, ethylene is limited, and the fruit exhibit delayed ripening. But when fruit are allowed to ripen on the vine, ethylene concentration in the fruit is sufficient to allow fruit ripening to the fully red stage in DR tomato lines to proceed at approximately the same rate as in the control nontransgenic parental line. DR fruit, however, do not overripe and they stay firmer longer. These characteristics are qualitatively similar in DR cherry tomato line 35-1-N.

APHIS concludes that the phenotypic differences observed in line 35-1-N are insignificant and will not increase the weediness potential of line 35-1-N, relative to other commercial tomato cultivars, in unmanaged ecosystems.

2. Impact of Interbreeding on Weediness and Biodiversity

Cultivated cherry tomato (*Lycopersicon esculentum* var *cerasiforme)* 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 cherry tomato is not wind-pollinated and insect pollination is limited (Rick, 1976). Agritope, reported no differences in flower morphology between line 35-1-N and the nonmodified recipient; therefore, outcrossing in line 35-1-N is naturally limited by its reproductive biology.

Cherry tomato does not cross-pollinate with other plant species in the United States without human intervention. *Lycopersicon* is a genus of the large and diverse nightshade family (Solanaceae), which also includes many crops (potatoes, peppers, tobacco, and eggplant) and weed species. The genus has been divided into two subgenera based on their ability to be crossed to commercial tomato; the *esculentum*-complex contains those species that are easily crossed, and the *peruvianum*-complex contains those species that are crossed with considerable difficulty (Stevens and Rick, 1986; Taylor, 1986).

The cherry tomato, *L. esculentum* var. *cerasiforme* of the *esculentum* complex, is the direct ancestor of the modern cultivated tomato and is the *only* sexually compatible relative of *L. esculentum* var. *esculentum* found in the United States. *L. esculentum* var. *cerasiforme* is used for human consumption and has been transported around the world. While it can occur as a weed in tropical America, southern Texas, Florida (Rick, 1973), Mexico, Africa, and parts of Southeast Asia (Taylor, 1986; Rick, 1976; Rick and Fobes, 1975), like other tomatoes it can be easily controlled using standard horticultural practices.

Cherry tomato, however, is not considered a serious or principal weed pest (Holm et al, 1979). Although *L. esculentum* var. *esculentum* and var. *cerasiforme* can cross with either plant as male or female parent (Rick, 1979), the probability of tomato line 35-1-N naturally introgressing into var. *cerasiforme* in the United States is extremely low due to the low rate of outcrossing in var. *esculentum* (Rick, 1949). Introgression into cherry tomato is further limited by the fact that cultivation of *L. esculentum* requires maintenance

of genetic purity as a standard breeding practice and for many commercial purposes. Regulations specifying procedures for the maintenance of genetic purity have been codified (See 7 CFR Part 201). Many other members of the nightshade family are found as weeds in tomato fields, but *L. esculentum* is not naturally sexually compatible with any of these weedy relatives occurring in the United States (Rick, 1979).

Because commercial tomatoes are virtually exclusively self-pollinating and standard breeding practices in the United States ensure genetic purity, and because tomato has no relatives with which it can naturally cross in the United States, there is little possibility that unintended gene introgression from line 35-1-N into another plant will occur in the United States.

Although limited by high self-pollination rates, outcrossing of line 35-1-N to primitive tomato cultivars and wild or weedy relatives (particularly those in the *esculentum*-complex) is possible in Mexico, Central America, and northwestern South America. *Lycopersicon* species are native to the Andean region of Ecuador, Peru, and Chile; and one species *L. cheesmanii* is found exclusively on the Galapagos Islands. But most evidence suggests that the site of domestication of *L. esculentum* is Mexico (Taylor, 1986). Five species, in addition to *L. esculentum*, comprise the *esculentum*-complex, and two species comprise the *peruvianum*-complex (Taylor, 1986).

L. pimpinellifolium is the only other *Lycopersicon* species for which there is good evidence for natural hybridization with cultivated tomato (Rick, 1958). This species can be found as a weed in commercial tomato fields and is occasionally harvested from the wild for human consumption. Its range is restricted to certain regions of Latin America (predominantly Peru and Ecuador). In addition to *Lycopersicon* species, two *Solanum* species, *S. lycopersicoides* and *S. rickii*, found only in restricted habitats of Peru and Chile, can be crossed with commercial tomato under specific, controlled conditions. But they do not naturally cross with *L. esculentum*, and the hybrids are generally highly sterile (Stevens and Rick, 1986; De Verna et al., 1990).

Even if an outcrossing event involving pollen from tomato line 35-1-N did occur, there is no reason to believe that the progeny would be any more weedy than progeny from crosses resulting from pollination by the nonmodified parental tomato line. As discussed above, the minor phenotypic differences observed in line 35-1-N and backcross progeny derived from this line will not confer a selective advantage that would increase their weediness potential relative to other commercial tomato cultivars; nor would they be expected to increase the weediness potential if introgressed into other sexually compatible plants.

Gene introgression from line 35-1-N into other plants is also unlikely to adversely impact biodiversity. There is already considerable cultivation throughout the centers of diversity for tomato of improved tomato varieties produced through breeding. The impact of cultivation of line 35-1-N on the genetic diversity of wild relatives or primitive cultivars of tomato is likely to be comparable to that from these other nontransgenic improved varieties.

We note also that any international traffic in DR tomato line 35-1-N would be fully subject to national and regional phytosanitary standards promulgated under the International Plant Protection Convention (IPPC). The IPPC has set a standard for the reciprocal acceptance of phytosanitary certification among the nations that have signed or acceded to the Convention (101 countries as of September 1994). The treaty, administered through the United Nations Food and Agriculture Organization, came into force on April 3, 1952. It establishes standards to facilitate the safe movement of plant materials across international boundaries.

Plant biotechnology products are fully subject to national legislation and regulations, or regional standards and guidelines promulgated under the IPPC. The vast majority of IPPC signatories have promulgated, and are now administering, such legislation or guidelines. Mexico in particular has in place a regulatory process that would require a full evaluation of the transgenic tomatoes before they could be introduced into their environment. Our decision in no way prejudices regulatory action in any country. The IPPC has also led to the creation of regional plant protection organizations such as the North American Plant Protection Organization (NAPPO) whose member countries are the U.S., Canada, and Mexico. Our trading partners are kept informed of our regulatory decisions through NAPPO, and other fora. It should also be noted that all the existing national and international regulatory authorities and phytosanitary regimes that currently apply to introductions of new tomato varieties internationally apply equally to the transgenic tomatoes covered by this analysis.

Nonsexual, horizontal transfer of transgenes from genetically engineered plants into other organisms is not well documented and is difficult to measure. Horizontal gene transfer of transgenes from higher transgenic plants via the soil to a soil microorganism (the filamentous fungus *Aspergillus niger*), however, has been reported (Hoffmann et al., 1994). The efficiency, the occurrence in natural habitats, and the gene transfer mechanism associated with such horizontal gene transfer remain unclear though all indications are that it is extremely rare. Because SAMase activity and kanamycin resistance have already been characterized in many soil microorganisms (Tran and Kretzmer, 1993; Henschke and Schmidt, 1989), horizontal gene transfer into soil microorganisms of the plant transgenes conferring these traits should pose no greater plant pest risk or risk to biodiversity than would direct or possible horizontal gene transfer among soil microorganisms.

C. Impact on Endangered and Beneficial Organisms

There is no reason to believe that deleterious effects on beneficial organisms could result specifically from the cultivation of DR tomato line 35-1-N. Some *Lycopersicon* species contain concentrations of specific glycoalkaloids, particularly tomatine, that are sufficient to produce toxic effects on certain foliar-feeding insect pests. But Agritope, Inc. analysis of biochemical components of line 35-1-N tomato fruit identified no toxic components present in concentrations significantly different from those in nontransgenic tomatoes. Any expression of SAMase in pollen of line 35-1-N should not be harmful to pollinating insects or other organisms for two reasons: (1) SAMase has a high substrate specificity, and (2) tomatoes are self-pollinated and do not produce copious amounts of pollen. SAMase released from decomposing line 35-1-N plant tissue should not have an adverse effect on soil- and detritus-dwelling organisms because they are already exposed to the coliphages that are the donor of

sam-k gene. The biosafety of kanamycin-resistant transgenic plants has been reviewed (Nap et al., 1992). There is also no reason to believe that the NPTII protein conferring kanamycin resistance in line 35-1-N as a selectable marker for transformation would have deleterious effects or significant impacts on nontarget organisms, including beneficial organism. There have been no reports of toxic effects on such organisms in the many field trials conducted with many different plants expressing this selectable marker. No direct pathogenic properties, nor any hypothetical mechanisms for pathogenesis towards beneficial organisms such as bees and earthworms, were identified by Agritope, Inc. for line 35-1-N. APHIS also cannot envision any plausible mechanisms for any hypothetical pathogenic effect.

D. Impact on Agricultural Commodities

There is no reason to believe that fruit derived from line 35-1-N would cause disease, damage, or injury to raw or processed agricultural commodities. The delayed ripening trait could increase fruit quality because fruit could be harvested at increased maturity for fresh market use, thereby avoiding contamination by green immature fruit that reduces flavor quality. Physical injury and/or chilling injury during harvest and storage can make fruits more susceptible to postharvest decay by such fungal plant pathogens as *Alternaria alternata* (black mould), *A. alternata* f. *lycopersici*, *Botrytis cinerea* (grey mould), *Rhizopus stolonifer* (*Rhizopus* rot), *Rhizoctonia solani* (soil rot) and *Geotrichum candida* and by bacterial soft rot pathogens (Grierson and Kader, 1986). Because line 35-1-N fruit harvested for fresh market will have a longer shelf life and can be shipped and stored at higher temperatures, the chance for physical and chilling injury and associated losses due to plant pathogens could be reduced. The delay in overripening of tomatoes harvested for both fresh market and processing should also reduce the incidence or severity of ripe-rot diseases, thereby improving quality.

SAMase is not expected to cause disease, injury, or damage to agricultural commodities because it not associated with plant pathogenicity. The enzymatic reaction it catalyzes and the products of that reaction are not expected to cause damage or injury to tomato fruit. APHIS has previously determined that expression of NPTII in genetically engineered tomatoes does not have an adverse impact on agricultural commodities (see APHIS EA and determination documents for petitions numbered 92-196-01p and 94-290-01p). The FDA has examined the animal and human food safety of other DR tomato line and concluded that it was not altered significantly when compared to other tomato varieties with a history of safe use (FDA memorandum of conference, September 19, 1994). Therefore, APHIS concludes that line 35-1-N should not have a direct or indirect plant pest effect on any raw or processed plant commodity.

APHIS believes that the analysis of the properties of line 35-1-N already field tested will apply equally well to new tomato lines derived from crosses between line 35-1-N and traditional tomato varieties, and that the data provided by Agritope, Inc. justify the conclusion that such new lines derived from line 35-1-N will not present a plant pest risk. The agronomic characteristics observed in line 35-1-N do not differ significantly from those seen in commercial cultivars of tomato that have never been considered regulated articles. APHIS has also determined that another delayed ripening tomato line, similarly developed by genetic engineering to express lower levels of ACC and ethylene during fruit ripening, does not

represent a plant pest risk (see APHIS EA and Determination for petition number 94–228–01p). Therefore, there is no reason to believe that progeny of line 35–1–N will express plant pest properties.

V. Conclusion

APHIS has determined that the DR tomato line 35–1–N, and any progeny derived from crosses involving line 35–1–N and other nonregulated articles, will no longer be considered regulated articles under 7 CFR Part 340. Cultivation, importation, or interstate movement of line 35–1–N or its progeny will no longer be subject to permit or notification requirements stipulated by those regulations. Importation of line 35–1–N and its progeny (including nursery stock or seeds capable of propagation) will, however, remain subject to the restrictions found in foreign quarantine notices in 7 CFR Part 319. This determination has been made based on an analysis of data collected from field trials, laboratory analyses, and literature references included herein that demonstrate that line 35–1–N:

1. exhibits no plant pathogenic properties
2. is no more likely to become a weed than other cherry tomato cultivars developed by traditional breeding techniques
3. is unlikely to increase the weediness potential or adversely impact the biodiversity of other cultivated or wild species with which it can interbreed
4. Will not harm endangered species and other organisms, such as bees, that are beneficial to agriculture
5. should not cause damage to raw or processed agricultural commodities

APHIS has also determined that new progeny derived from crosses between line 35–1–N and other nonregulated articles, or varieties bred from these lines, will not exhibit new plant pest properties.

John H. Payne

MAR 27 1996

JOHN H. PAYNE
ACTING DIRECTOR
BIOTECHNOLOGY, BIOLOGICS, AND
ENVIRONMENTAL PROTECTION
ANIMAL AND PLANT HEALTH INSPECTION SERVICE

DATE

Appendix B:

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