Southern Gardens Citrus Nursery, LLC Permit to Release Genetically Engineered *Citrus tristeza virus*

Draft Environmental Impact Statement

May 2018

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Executive Summary

The U.S. Department of Agriculture (USDA), Animal and Plant Health Inspection Service (APHIS) received a permit application (APHIS Permit Number 17-044-101r) from Southern Gardens Citrus Nursery, LLC (Southern Gardens) seeking a permit to release genetically engineered (GE) *Citrus tristeza virus* (CTV) expressing defensin proteins from spinach (CTV-SoD) as an approach to manage citrus greening disease throughout the state of Florida. The regulations in 7 CFR part 340.4(a) provide that any person may submit an application to APHIS for a permit for the introduction of a regulated article. When APHIS receives a permit application for a regulated article as described in 7 CFR part 340.2, the Agency is required to make a decision. As a Federal agency, APHIS must also comply with applicable U.S. environmental laws and regulations because a decision on a permit, whether positive or negative, is a final Agency action that might cause environmental impacts.

Regulatory Authority

The Plant Protection Act of 2000 (PPA), as amended (7 U.S.C. §§ 7701-7772), provides the legal authorization for the APHIS plant protection mission. It authorizes the Agency to regulate the introduction of potential plant pests into the territorial boundaries of the United States, and their interstate movement within U.S. boundaries by establishing quarantine, eradication and control programs. Implementing rules, regulations and guidelines for this enabling legislation (PPA) are codified in Title 7 of the U.S. Code of Federal Regulations (CFR). Rules that implement this authority specific to GE organisms have been published in 7 CFR part 340.

Under the current regulations, a GE organism is considered to be a regulated article if the donor organism, recipient organism, vector, or vector agent is a plant pest or if the Administrator has reason to believe the GE organism is a plant pest. A plant pest is defined in § 340.1 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." The regulations in 7 CFR part 340.4(a) also provide a process to submit an application to APHIS for a permit for the introduction of a regulated article. Paragraph (b) of § 340.4 describes the form that an application for a permit for the environmental release of a regulated article must take and the information that must be included in the application.

Two other agencies, the Federal Drug Administration (FDA) and the Environmental Protection Agency (EPA), are involved in regulating GE organisms. The regulatory roles of USDA-APHIS, the FDA, and the EPA are described by the "Coordinated Framework," a 1986 policy statement from the Office of Science and Technology Policy that describes the comprehensive Federal policy for ensuring the safety of biotechnology research and products.

The FDA regulates GE organisms under the authority of the Federal Food, Drug, and Cosmetic Act (FFDCA) (21 U.S.C. 301 *et seq.*). The FDA implements a voluntary consultation process to ensure that human food and animal feed safety issues or other regulatory issues, such as labeling, are resolved before commercial distribution of food derived from GE products.

The EPA is responsible for regulating the sale, distribution, and use of pesticides, including pesticides that are produced by an organism through techniques of biotechnology. The EPA regulates plant incorporated protectants (PIPs) and microorganisms used as pesticides, e.g. bacteria, fungi, viruses, bacteriophages; both naturally occurring and genetically engineered under the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) (7 U.S.C. 136 *et seq.*) and certain microorganisms under the Toxic Substances Control Act (15 U.S.C. 53 *et seq.*).

Under FIFRA (7 U.S.C. 136 *et seq.*), the EPA regulates the use of pesticides (requiring registration of a pesticide for a specific use prior to distribution or sale of the pesticide for a proposed use pattern). The EPA examines the ingredients of the pesticide; the particular site or crop on which it is to be used; the amount, frequency, and timing of its use; and storage and disposal practices. Prior to registration of a new pesticide or a new use for a previously registered pesticide, the EPA must determine that the pesticide will not cause unreasonable adverse effects on the environment and a reasonable certainty of no harm to humans when used in accordance with label instructions. The EPA reevaluates all pesticides every fifteen years (or shorter) to ensure they meet current standards for continued safe use (7 U.S.C. 136a(g)(1)(A)(iv)).

The EPA also sets tolerances for residues of pesticides on and in food and animal feed, or establishes an exemption from the requirement for a tolerance, under the FFDCA. The EPA is required, before establishing pesticide tolerance, to reach a safety determination based on a finding of reasonable certainty of no harm under the FFDCA, as amended by the Food Quality Protection Act (FQPA). The FDA enforces the pesticide tolerances set by the EPA.

Purpose and Need for Agency Action

Under the authority of the plant pest provisions of the PPA and 7 CFR part 340, APHIS regulates the safe development and use of GE organisms. The regulations in 7 CFR part 340.4(a) provide that any person may submit an application to APHIS for a permit for the introduction of a regulated article. When APHIS receives a permit application for a regulated article as described in 7 CFR part 340.2, the Agency is required to make a decision. In February 2017, APHIS received a permit application from Southern Gardens seeking a permit for the environmental release of genetically engineered CTV. The virus has been genetically engineered to express defensin proteins from spinach (CTV-SoD) as an approach to manage citrus greening disease throughout the State of Florida. APHIS considers this CTV-SoD to be a biological control agent since it is a biological organism intended to help manage citrus greening disease. APHIS has decided to prepare an EIS to better understand the potential for environmental impacts associated with the issuance of a permit.

Consistent with the Council of Environmental Quality's National Environmental Policy Act (NEPA) regulations and the USDA and APHIS NEPA implementing regulations and procedures (40 CFR parts 1500-1508, 7 CFR part 1b, and 7 CFR part 372), APHIS has prepared this draft Environmental Impact Statement (EIS) to consider the potential environmental impacts that may result from the potential approval of the permit application. Specifically, this draft EIS has been prepared in order to evaluate the impacts on the quality of the human environment[1] that may result from approval of the permit application for release of CTV-SoD.

Public Involvement

As part of its scoping process to identify issues to address in this EIS, APHIS published a Notice of Intent (NOI) to prepare an EIS and sought public input during a 30 day comment period (April 10 to May 10, 2017). Comments were submitted by individuals, academic researchers, non-government organizations, state departments of agriculture, and industry representatives. The majority of the 94 comments submitted were opposed to approving the permit for release of GE CTV.

Alternatives Analyzed

In this EIS, APHIS considered two alternatives for its response to the Southern Gardens permit application. The two alternatives are: 1) take no action, APHIS would not approve the permit request (No Action Alternative) and 2) approve the permit request (Preferred Alternative). These alternatives are further described in Chapter 2.

Affected Environment

The action proposed in the permit under consideration is to release throughout the state of Florida, CTV-SoD as a biological means to manage citrus greening disease. Decisions on where the CTV-SoD would be deployed would be determined by Southern Gardens in agreements with growers, and deployment would be monitored by APHIS. For this reason, APHIS considered the affected environment to be limited to the state of Florida.

Potential Environmental Consequences of Alternatives

Environmental issues are assessed individually in Chapter 4 (Environmental Consequences). The scope of this EIS analyzes the potential for direct and indirect impacts that might result from approving the permit request. APHIS prepared an EIS to better understand the potential for environmental impacts associated with issuance of the permit.

APHIS determined that approving the permit for release of CTV-SoD under the Preferred Alternative would have no direct impacts to the physical environment because CTV-SoD are unlikely to be transmissible by aphids and so are confined to the phloem of the citrus plants in which they are inoculated. Additionally APHIS determined that approving the permit for release of CTV-SoD would have no impacts on the biological environment or human health because organisms, including humans, that may be exposed to CTV-SoD would likely be unaffected since both CTV and spinach defensins are naturally occurring parts of the environment and are already part of the food chain, and neither has been noted as having adverse effects associated with exposure or consumption.

[1] Under NEPA regulations, the “human environment” includes “the natural and physical environment and the relationship of people with that environment” (40 CFR §1508.14).

The majority of Florida citrus is already infected with wild-type T30 and T36 CTV strains (Harper *et al.*, 2015c) (the parent strains used to create the CTV-SoD), and so superinfection exclusion/cross protection (Folimonova, 2012; Atallah *et al.*, 2016; Bergua *et al.*, 2016) will prevent the spread of CTV-SoD to the majority of commercial trees in Florida that are already infected with the naturally-occurring, endemic CTV strains (Harper *et al.*, 2015a; 2015b). CTV-SoD would not increase the plant pest risk that is already present due to the ubiquity of CTV in the state of Florida or create a new plant pest risk due to the CTV-SoD being released into the environment (USDA-APHIS, 2018).

Currently, citrus greening disease is having devastating impacts on the citrus industry in Florida (USDA-NIFA, 2016), reducing orange production by over 40 percent in the last five years (USDA-FAS, 2017b; 2017a) with no currently effective management or cure for citrus greening disease. If the losses from citrus greening disease continue it could jeopardize the existence of the citrus industry in Florida. If the use of CTV-SoD proves to be an effective management tool to control citrus greening disease in the state, it could potentially help mitigate the reduction in orange production in Florida through decreased tree mortality and increased yields on healthy trees. Additionally, because citrus greening disease not only reduces yield, fruit size and quality, but also increases the costs of production as growers have to adjust their inputs to try and maintain yields (USDA-APHIS, 2010; Singerman and Useche, 2016), growers could see lower costs of production through reductions in the additional pesticide applications and additional nutritional inputs.

More detailed descriptions and analyses of the potential environmental consequences can be found in Chapter 4.

Potential Cumulative Impacts

Chapter 5 of this EIS includes an environmental analysis of potential cumulative impacts, focusing on the incremental impacts of the Preferred Alternative taken in consideration with related activities including past, present, and reasonably foreseeable future actions. The approval of the permit for release of CTV-SoD is not expected to have any negative cumulative impacts from approving the permit, because it will be restricted to the trees in which it is grafted. Effective control of citrus greening disease would have beneficial effects for citrus growers, and may result in a long-term method to assist in the management of citrus greening disease. CTV-SoD would not increase the plant pest risk that is already present due to the ubiquity of CTV in the state of Florida or create a new plant pest risk due to the CTV-SoD being released into the environment (USDA-APHIS, 2018).

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ACRONYMS AND ABBREVIATIONS

APHIS	Animal and Plant Health Inspection Service
CAA	Clean Air Act
CFR	Code of Federal Regulations (United States)
CTV	*Citrus tristeza virus*
CTV-SoD	Genetically engineered CTV clones expressing spinach defensins
DNA	deoxyribonucleic acid
EA	environmental assessment
EIS	environmental impact statement
EO	Executive Order
EPA	U.S. Environmental Protection Agency
ESA	Endangered Species Act of 1973
FDA	U.S. Food and Drug Administration
FFDCA	Federal Food, Drug, and Cosmetic Act
FIFRA	Federal Insecticide, Fungicide, and Rodenticide Act
GE	genetically engineered
HLB	huanglongbing
NEPA	National Environmental Policy Act of 1969 and subsequent amendments
NHPA	National Historic Preservation Act
NMFS	National Marine Fisheries Service
PIP	plant incorporated protectants
PRA	Pest Risk Assessment
PPA	Plant Protection Act
T&E	threatened and endangered
USDA	U.S. Department of Agriculture
USDA-NASS	U.S. Department of Agriculture-National Agricultural Statistics Service
USC	United States Code
USFWS	U.S. Fish & Wildlife Service
WPS	Worker Protection Standard for Agricultural Pesticides

1 PURPOSE AND NEED

This document is intended to ensure compliance with the National Environmental Policy Act (NEPA). NEPA requires agencies to prepare an environmental impact statement (EIS) to be included in "every recommendation or report on proposals for legislation and other major Federal actions significantly affecting the quality of the human environment" (42 U.S.C. §4332(2)(C)).

The United States Department of Agriculture (USDA) Animal and Plant Health Inspection Service (APHIS) is currently engaged in decisionmaking on whether to approve a permit request from Southern Gardens Citrus, LLC (Southern Gardens). The Agency has determined that there are possible environmental impacts, as described in Chapter 4, associated with whatever regulatory decision it renders. Therefore, APHIS has decided to prepare an EIS to better understand the potential for environmental impacts associated with the issuance of a permit.

In February 2017, APHIS received a permit application from Southern Gardens seeking a permit for the environmental release of genetically engineered (GE) *Citrus tristeza virus* (CTV). The virus has been genetically engineered to express defensin proteins from spinach (*Spinacia oleracea*) (CTV-SoD) as an approach to manage citrus greening disease throughout the State of Florida. APHIS considers CTV-SoD to be a biological control agent since it is a biological organism intended to help manage citrus greening disease.

1.1 APHIS Regulatory Authority

Summarized as "Protecting American Agriculture," the mission of USDA APHIS[2] is: "To protect the health and value of American agriculture and natural resources." To implement its plant protection mission, the Agency establishes policies and measures to prevent the introduction of plant pests into the United States. It also promotes management of those plants, animals, and microorganisms that currently occur within the United States and cause economic losses to U.S. agriculture, including commercial and non-commercial production of crops and ornamental plants. Its mission encompasses all practices and technologies that have the potential to impact plant pest risks.

The Plant Protection Act of 2000 (PPA), as amended (7 U.S.C. §§ 7701-7772), provides the legal authorization for the APHIS plant protection mission. It authorizes the Agency to regulate the introduction of potential plant pests into the territorial boundaries of the United States, and their interstate movement within U.S. boundaries by establishing quarantine, eradication and control programs. Implementing rules, regulations, and guidelines for this enabling legislation (PPA) are codified in Title 7 of the U.S. Code of Federal Regulations (CFR). Rules that implement this authority specific to GE organisms have been published in 7 CFR part 340.

Under the authority of the plant pest provisions of the PPA, as amended (7 U.S.C. 7701 et seq.), the regulations in 7 CFR part 340, "Introduction of Organisms and Products Altered or Produced Through Genetic Engineering Which Are Plant Pests or Which There Is Reason to Believe Are

[2] For more details about the APHIS mission, visit http://www.aphis.usda.gov/about_aphis/

Plant Pests," regulate, among other things, the introduction (importation, interstate movement, or release into the environment) of organisms and products altered or produced through genetic engineering that are plant pests or that there is reason to believe are plant pests. 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 taxonomic groups listed in the regulation and is also a plant pest, or if there is a reason to believe it is a plant pest. *Citrus tristeza virus* is the recipient organism and is a plant pest.

1.2 Requirement for this Document

The regulations in § 340.4(a) provide that any person may submit an application for a permit for the introduction of a regulated article to APHIS. When APHIS receives a permit application, the Agency is required to make a decision to either grant or deny the permit after review of the application and any data submitted with the application. As a Federal agency, APHIS must also comply with applicable U.S. environmental laws and regulations because a decision on a permit application, whether positive or negative, is an Agency action that might cause environmental impact(s).

This document addresses both of these requirements relevant to decisionmaking for a permit application submitted by Southern Gardens of Clewiston, FL, APHIS Permit Number 17-044-101r, to release CTV-SoD as a biological means to manage citrus greening disease in Florida. The EIS will evaluate any potential environmental impacts associated with this action throughout the State of Florida.

APHIS has issued nine permits for field trials of CTV genetically engineered to express antimicrobial peptides to control citrus greening disease in three counties in Florida since June 2010 under confined conditions that restrict the virus to the site of the field test. APHIS has provided significant oversight of these confined field trials and has not detected any negative impacts on the environment, including those on threatened and endangered species. Data gathered during these field trials as well as data submitted with the permit application, have shown that while aphid transmission of CTV9R is possible, it is poorly transmitted at low rates (0.6%) and no movement of CTV9R by aphids was detected in the field trials (data submitted with 17-044-101r). Additionally, these data showed that CTVT30 was not transmissible by aphids and that CTV9R/T30 is also not expected to be aphid vectored (data submitted with 17-044-101r). Therefore, CTV-SoD is expected to remain within the inoculated trees. Detailed discussion of the strains used and transmissibility can be found in the Pest Risk Assessment (PRA) completed for this permit application (USDA-APHIS, 2018).Permitted field trials are planned and in progress in a number of regions in Florida to determine efficacy of phenotypic expression of spinach defensins in CTV.

1.3 Purpose of Product

Southern Gardens has genetically engineered CTV to express defensin proteins from spinach (CTV-SoD) as an approach to manage citrus greening disease throughout the State of Florida. APHIS considers CTV-SoD to be a biological control agent since it is a biological organism intended to help manage citrus greening disease. Citrus greening disease, also called huanglongbing (HLB), was first detected in the United States in 2005 in Florida, and has since become a devastating disease of citrus in Florida. There is no known cure for citrus greening disease. CTV is a plant pest present throughout the state of Florida and is prevalent in most of the citrus trees in Florida. Southern Gardens has submitted a permit application to release CTV-SoD as a biological control agent to manage citrus greening disease in Florida. CTV-SoD would be applied to citrus trees by grafting. The release of CTV-SoD does not involve genetically engineering citrus trees, and use of CTV-SoD will have no impact on the genetics of the trees. Southern Gardens research yielded several strains of genetically engineered CTV. Table 1 below lists those strains and defines the naming standards that will be used throughout this document.

Table 1. Descriptions of CTV and derivatives.

Designation	Composition[1]
CTV	*Citrus tristeza virus*
CTV clone or GE CTV	A DNA copy of a specific CTV virus isolate
CTV9R	A specific DNA clone derived from isolates of strain T36
CTVT30	A specific DNA clone derived from isolates of strain T30
CTV9R/T30	A specific hybrid DNA clone derived from isolates of strain T36 and T30
CTV-SoD	General term for genetically engineered CTV clones containing genetic material encoding one or more spinach defensins
CTV9R-SoD, CTVT30-SoD, CTV9R/T30-SoD	Specific CTV clones containing genetic material encoding one or more spinach defensins

[1]CTV RNA when extracted from CTV-infected plants may be composed of multiple isolates. Isolates are grouped into strains based on sequence similarity.

1.4 Coordinated Framework Review and Regulatory Review

In 1986, the Office of Science and Technology Policy (OSTP) issued the Coordinated Framework for the Regulation of Biotechnology (Coordinated Framework), which describes the comprehensive Federal regulatory policy for ensuring the safety of biotechnology products. The Coordinated Framework sought to achieve a balance between regulation adequate to ensure the protection of health and the environment while maintaining sufficient regulatory flexibility to avoid impeding innovation. In 1992, OSTP issued an update to the Coordinated Framework that sets forth a risk-based, scientifically sound basis for the oversight of activities that introduce biotechnology products into the environment. The update affirmed that Federal oversight should focus on the characteristics of the product and the environment into which it is being introduced, rather than the process by which the product is created (51 FR 23302, 1986; 57 FR 22984, 1992).

The Coordinated Framework explains the regulatory roles and authorities for the three major agencies involved in regulating GE organisms: USDA-APHIS, the U.S. Environmental Protection Agency (EPA), and the U.S. Food and Drug Administration (FDA). A summary of each role follows.

1.4.1 USDA-APHIS

APHIS administers regulations in 7 CFR part 340, "Introduction of Organisms and Products Altered or Produced Through Genetic Engineering Which are Plant Pests or Which There is Reason to Believe are Plant Pests" (referred to below as the regulations). The current regulations govern the introduction (importation, interstate movement, or release into the environment) of certain GE organisms that are considered “regulated articles.” Under the current regulations, a GE organism is considered to be a regulated article if the donor organism, recipient organism, vector, or vector agent used in engineering the organism belongs to one of the taxonomic groups listed in § 340.2 and is a plant pest or if the Administrator has reason to believe the GE organism is a plant pest. *Citrus tristeza virus* is the recipient organism and is a plant pest.

1.4.2 Environmental Protection Agency

The EPA is responsible for regulating the sale, distribution, and use of pesticides, including pesticides that are produced by an organism through techniques of biotechnology. The EPA regulates plant incorporated protectants (PIPs) and microorganisms used as pesticides, e.g. bacteria, fungi, viruses, bacteriophages; both naturally occurring and genetically engineered under the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) (7 U.S.C. 136 *et seq.*) and certain microorganisms under the Toxic Substances Control Act (15 U.S.C. 53 *et seq.*). CTV-SoD is considered a microbial pesticide by the EPA. Southern Gardens and associated researchers have been working since 2009 to ensure compliance with the EPA regulations. Southern Gardens has been conducting studies required for the EPA registration, including experimental studies that have required permits from APHIS and Biotechnology Notifications and Experimental Use Permits (EUPs) (and associated temporary tolerances) from the EPA in its development of CTV-SoD. The studies Southern Gardens is conducting will ultimately support a Section 3 registration application for these products.

Under FIFRA (7 U.S.C. 136 *et seq.*), the EPA regulates the use of pesticides (requiring registration of a pesticide for a specific use prior to distribution or sale of the pesticide for a proposed use pattern). The EPA examines the ingredients of the pesticide; the particular site or crop on which it is to be used; the amount, frequency, and timing of its use; and storage and disposal practices. Prior to registration of a new pesticide or a new use for a previously registered pesticide, the EPA must determine that the pesticide will not cause unreasonable adverse effects on the environment and a reasonable certainty of no harm to humans when used in accordance with label instructions. The EPA must also approve the language used on the pesticide label in accordance with 40 CFR part 158. Once registered, a pesticide may not legally be used unless the use is consistent with the approved directions for use on the pesticide's label. The overall intent of the label is to provide clear directions for effective product performance while minimizing risks to human health and the environment. Under FIFRA the EPA has a standard of reviewing pesticide registrations every 15 years (US-EPA, 2011). The Food Quality Protection Act (FQPA) of 1996 amended FIFRA, and set a standard to reassess, over a 10-year period, all pesticide tolerances that were in place when the FQPA was signed, make a safety finding when setting tolerances that the pesticide can be used with “a reasonable certainty of no harm,” take into consideration aggregate and cumulative effects/risks in assessing human health, and emphasize risks to special sub-populations such as infants and children (US-EPA, 2015d).

The EPA also sets tolerances for residues of pesticides on and in food and animal feed, or establishes an exemption from the requirement for a tolerance, under the Federal Food, Drug, and Cosmetic Act (FFDCA). The EPA is required, before establishing pesticide tolerance, to reach a safety determination based on a finding of reasonable certainty of no harm under the FFDCA, as amended by the FQPA. The FDA enforces the pesticide tolerances set by the EPA.

1.4.3 Food and Drug Administration

The FDA regulates GE organisms under the authority of the FFDCA (21 U.S.C. 301 *et seq.*). The FDA published its policy statement concerning regulation of products derived from new plant varieties, including those derived from genetic engineering, in the *Federal Register* on May 29, 1992 (57 FR 22984). Under this policy, the FDA implements a voluntary consultation process to ensure that human food and animal feed safety issues or other regulatory issues, such as labeling, are resolved before commercial distribution of GE food. This voluntary consultation process provides a way for developers to receive assistance from the FDA in complying with their obligations under Federal food safety laws prior to marketing.

More recently, in June 2006, the FDA published recommendations in "Guidance for Industry: Recommendations for the Early Food Safety Evaluation of New Non-Pesticidal Proteins Produced by New Plant Varieties Intended for Food Use" (US-FDA, 2006) for establishing voluntary food safety evaluations for new non-pesticidal proteins produced by new plant varieties intended to be used as food, including GE plants. Early food safety evaluations help make sure that potential food safety issues related to a new protein in a new plant variety are addressed early in development. These evaluations are not intended as a replacement for a biotechnology consultation with the FDA, but the information may be used later in the biotechnology consultation.

The CTV-SoD products being developed by Southern Gardens are considered to be microbial pesticides, the primary responsibilities for food and feed safety under the FFDCA will be administered by the EPA. The FDA will not be directly involved in CTV-SoD approvals.

1.5 Purpose and Need for APHIS Action

Under the authority of the plant pest provisions of the PPA and 7 CFR part 340, APHIS regulates the safe development and use of GE organisms. The regulations in § 340.4(a) provide that any person may submit an application for a permit for the introduction of a regulated article to APHIS. Under APHIS regulations, the receipt of a permit application to introduce a GE organism requires a response from the Administrator. After receipt and review by APHIS of the application and the data submitted, including any additional information requested by APHIS, a permit shall be granted or denied. In February 2017, APHIS received a permit application from Southern Gardens requesting a release permit to use CTV-SoD as a biological means to manage citrus greening disease in Florida.

Consistent with the Council of Environmental Quality's NEPA regulations and the USDA and APHIS NEPA implementing regulations and procedures (40 CFR parts 1500-1508, 7 CFR part 1b, and 7 CFR part 372), APHIS has prepared this draft EIS to consider the potential environmental impacts of issuing a permit. Specifically, this draft EIS has been prepared in order to evaluate the impacts on the quality of the human environment[3] that may result from issuing a permit for the environmental release of CTV-SoD as an approach to manage citrus greening disease.

1.6 Public Involvement

When the Agency decides to prepare an EIS as part of its decisionmaking process for a permit application, prior to preparation, it seeks public comments as part of its advance scoping process. Details about the public involvement process for the permit application that is the subject of this document follows.

1.6.1 Public Scoping for this draft EIS

As part of its scoping process to identify issues to address in this draft EIS, APHIS published a Notice of Intent (NOI) to prepare the EIS and sought public input during a 30 day comment period (April 10 to May 10, 2017). The docket received a total of 94 public comments. Issues most frequently cited in public comments on the NOI included:

- the potential for the GE CTV to change over time and the potential for recombination
- impacts to non-target species
- the potential for defensin proteins to be found in other areas other than the phloem of the plant
- the potential for the GE CTV to become more transmissible or transmissible by aphids
- the impacts to organic citrus growers
- health and safety concerns

The issues discussed in this draft EIS were developed by considering the public input, including public comment received from the *Federal Register* notice announcing the NOI, as well as issues raised in public comments submitted for other NEPA documents of GE organisms, issues raised in lawsuits, and other issues raised by various stakeholders. APHIS evaluated these issues to analyze the potential environmental impacts of CTV-SoD and included a discussion of these issues in this EIS.

[3] Under NEPA regulations, the “human environment” includes “the natural and physical environment and the relationship of people with that environment” (40 CFR §1508.14).

2 ALTERNATIVES

This document analyzes the potential environmental consequences of APHIS' response to a permit application (APHIS Number 17-044-101r) received from Southern Gardens to allow the environmental release of CTV-SoD within the state of Florida. The purpose of the environmental release is to use the CTV-SoD expressing defensin proteins from spinach as an approach to manage citrus greening disease throughout the State of Florida.

Under APHIS regulations, the Administrator must either deny or grant permits properly submitted under 7 CFR part 340. Based upon the permit application submitted by the applicant, two alternatives are evaluated in this draft EIS: (1) No Action: APHIS would not approve the permit request and (2) Preferred Alternative: approve the permit application request from Southern Gardens and issue the APHIS permit.

2.1 No Action Alternative: Deny the Permit Request

Under the No Action Alternative, APHIS would deny the permit application (17-044-101r) submitted by Southern Gardens. The applicant would not be authorized to release CTV-SoD. Current methods to manage citrus greening disease in Florida would continue under the No Action Alternative. These methods of control are further described in Section 3.1.2.

2.2 Preferred Alternative: Issue the APHIS Permit

Under the Preferred Alternative, APHIS would issue an environmental release permit to Southern Gardens in accordance with 7 CFR part 340 to allow the release of CTV-SoD within the state of Florida. APHIS may choose this alternative if there is sufficient evidence to demonstrate that CTV-SoD would not increase the plant pest risk that is already present due to the ubiquity of CTV in the state of Florida or create a new plant pest risk due to CTV-SoD being released into the environment. If APHIS chooses this alternative, then the permit will be subject to the conditions described in 7 CFR part 340.4[4] and the Permit Conditions described in the permit.

Under this alternative, the permit would be valid for a three-year period. The permit will need to be renewed by the applicant and subsequently approved by APHIS to allow any additional release of CTV-SoD beyond the three-year period specified in the permit application.

2.3 Comparison of Alternatives

Table 2 presents a summary of the potential impacts associated with selection of either of the alternatives evaluated in this EIS. The impact assessment is presented in Chapter 4 of this EIS.

[4] http://www.gpo.gov/fdsys/granule/CFR-2012-title7-vol5/CFR-2012-title7-vol5-sec340-4/content-detail.html Last accessed May, 2014

Table 2. Summary of Issues of Potential Impacts and Consequences of Alternatives.

Attribute/Measure	Alternative A: No Action	Alternative B: Approve Permit Request
Unlikely to pose an increased pest risk	Satisfied through not authorizing release.	Satisfied—risk assessment. There is sufficient evidence to demonstrate that CTV-SoD would not increase the plant pest risk that is already present due to the ubiquity of CTV in the state of Florida or create a new plant pest risk due to CTV-SoD being released into the environment (USDA-APHIS, 2018).
Physical Environment	Without CTV-SoD growers will continue to manage HLB using currently available methods including removal of dead or diseased trees and replanting with certified disease-free seedlings, increased use of pesticides to target the insect vector, and increased nutritional inputs aimed at prolonging the productive life of the trees. These practices may impact the physical environment.	Approving the permit for release of CTV-SoD under the Preferred Alternative would have no direct impacts to the physical environment because CTV-SoD are either not transmissible or have extremely low transmissibility by aphids and so are expected to be confined to the phloem of the citrus plants in which they are inoculated and therefore there would be no exposure to the physical environment. If CTV-SoD proves to be an effective HLB management tool it could potentially reduce the need for additional pesticide applications and additional nutritional inputs used to combat HLB, potentially resulting in indirect beneficial impacts to the physical environment. However these impacts will likely be minimal since growers are required to use pesticides according to the label instructions which are designed to ensure that the pesticide does not cause

Attribute/Measure	Alternative A: No Action	Alternative B: Approve Permit Request
		unreasonable adverse effects on the environment. Growers will continue to use pesticides and added nutrients as needed to maintain the health of their trees as CTV-SoD will be only one of many tools that growers could use to manage HLB. If CTV-SoD is not an effective HLB management tool growers will continue to use currently available methods and have the same impact on the physical environment as the No Action Alternative.
Biological Environment	While HLB does not itself pose a threat to species other than citrus, some of the methods currently use to mitigate the effects of HLB including the control of the insect vector and increasing nutritional inputs aimed at prolonging the productivity of trees and groves, may impact the biological environment.	Approving the permit for release of CTV-SoD under the Preferred Alternative is expected to have no direct impacts to the biological environment because CTV is not known to be transmitted by pollen, seed, or fruit (Moreno *et al.*, 2008; EFSA, 2014; Jeger *et al.*, 2017) and CTV-SoD are either not transmissible or have extremely low transmissibility by aphids, making exposure to other plants or organisms outside of the trees that have been inoculated highly unlikely. Organisms that may be exposed to CTV-SoD in FL are not expected to be affected because both CTV and spinach defensins are naturally occurring parts of the environment and already part of the food chain, and neither has been noted as

Attribute/Measure	Alternative A: No Action	Alternative B: Approve Permit Request
		having adverse effects associated with consumption. If CTV-SoD proves to be an effective HLB management tool it could potentially reduce the need for additional pesticide applications and additional nutritional inputs used to combat HLB, resulting in potential beneficial indirect impacts to the biological environment. However, growers will continue to use pesticides and added nutrients as needed to maintain the health of their trees as CTV-SoD will be only one of many tools that growers could use to manage HLB. Potential impacts will likely be minimal since growers are required to use pesticides according to the label instructions which are designed to ensure that the pesticide does not cause unreasonable adverse effects to human health, non-target organisms, and the environment. If CTV-SoD is not an effective HLB management tool growers will continue to use currently available methods and have the same impact on the biological environment as the No Action Alternative.
Human Health	While HLB causes misshapen fruit and gives the fruit and juices derived from it a bitter and unpalatable flavor, it does not affect human health. However, the mitigation measures used by growers including the control of	Plant viruses are not pathogenic to humans and since virtually all of the citrus produced in Florida is infected to some extent with CTV (Harper *et al.*, 2015c), it is likely that CTV is consumed on a regular basis, and there are no

Attribute/Measure	Alternative A: No Action	Alternative B: Approve Permit Request
	the insect vector and increasing nutritional inputs aimed at prolonging the productivity of trees and groves, have the potential to impact human health.	reports of adverse effects from CTV exposures by any route of administration. Additionally, several different defensins have been found naturally occurring in spinach (Segura *et al.*, 1998) and are also already a part of the food chain. There is a long history of mammalian consumption of the entire spinach plant (both raw and cooked) as food, without causing any known deleterious human health effects or any evidence of toxicity. If CTV-SoD proves to be an effective HLB management tool it could potentially reduce the need for additional pesticide applications and additional nutritional inputs used to combat HLB. These reductions could have potential beneficial impacts by reducing human exposure to pesticides and nutritional inputs, however these will likely be minimal since growers are required to use pesticides according to the label directions minimizing harmful exposure and the EPA WPS will continue to provide the same level of protection as is currently available. If CTV-SoD is not an effective HLB management tool growers will continue to use currently available methods and have the same impact on human health as the No Action Alternative.

Attribute/Measure	**Alternative A: No Action**	**Alternative B: Approve Permit Request**
Impacts on the Citrus Industry	Orange production in the United States has declined by over 40 percent in the last five years due to HLB (USDA-FAS, 2017b; 2017a). HLB not only reduces yield, fruit size and quality, but also increases tree mortality and the costs of production as growers have to adjust their inputs to try and maintain yields. Under the No Action Alternative, the most likely scenario is that citrus acreage and production in Florida will continue to decline due to HLB until it is no longer profitable.	If the use of CTV-SoD proves to be an effective HLB management tool it could potentially help mitigate the reduction in orange production in Florida through decreased tree mortality and increased yields on healthy trees. Additionally, because HLB increases the costs of production as growers have to adjust their inputs to try and maintain yields (USDA-APHIS, 2010; Singerman and Useche, 2016), growers could potentially see a lower costs of production through reductions in the need for additional pesticide applications and additional nutritional inputs. If CTV-SoD is not an effective HLB management tool growers will continue to use currently available methods and citrus acreage and production in Florida will likely continue to decline as under the No Action Alternative.
Other Regulatory Approvals		
U.S.	CTV-SoD is considered a pesticide and is subject to EPA regulation. Southern Gardens has been conducting studies required for EPA registration under Experimental Use Permits to support a Section 3 registration application for CTV-SoD.	CTV-SoD is considered a pesticide and is subject to EPA regulation. Southern Gardens has been conducting studies required for EPA registration under Experimental Use Permits to support a Section 3 registration application for CTV-SoD.
Compliance with Other Laws		
CWA, CAA, EOs	Fully compliant	Fully compliant

3 AFFECTED ENVIRONMENT

The Affected Environment Section describes the action area and provides a discussion of the current conditions of those aspects of the human environment potentially impacted by issuing a permit for the environmental release of CTV-SoD as an approach to manage citrus greening disease. The permit application from Southern Gardens describes the release sites as totaling 513,500 acres covering all 67 counties in Florida. Therefore, the action area for this EIS is the state of Florida and the analysis will be limited to the state of Florida.

Because CTV-SoD is a GE biocontrol agent this EIS follows a different format than others traditionally done by APHIS for GE row crops. The EIS follows a format similar to those written for non-GE biocontrol agents. Further analysis can be found in Chapter 4.

3.1 Citrus Greening Disease

Citrus greening disease, also called huanglongbing (HLB), is a disease caused by a bacterium (*Candidatus* Liberibacter asiaticus) and transmitted by the Asian citrus psyllid (ACP). The ACP was discovered in Florida in 1998, and HLB was first detected in 2005 and it has since become a devastating disease of citrus in Florida (National Research Council, 2010). Since its discovery in Miami-Dade County, FL, in 2005 HLB has since been detected throughout the state of Florida as well as in California, Georgia, Louisiana, South Carolina, and Texas (Hilf *et al.*, 2007; USDA-APHIS, 2014a). HLB affects the vascular systems of the trees, preventing the tree from effectively transporting water, nutrients and minerals between the roots in the ground and the leaves and fruit at the top, causing decline in fruit production in the tree. HLB causes a reduction in the fibrous roots within a few months after infection and before foliar symptoms develop. Fibrous roots are responsible for the bulk of nutrient uptake; their decline likely explains the deficiency symptoms that develop in the canopy (Morgan and Hamido, 2016). The disease causes misshapen or small green fruit rendering the fruit unmarketable both aesthetically and by producing compounds that give the fruit and juices a bitter and unpalatable flavor. In normal fruits, the orange color develops first at the stylar end, at a time when the peduncular end is still green. On HLB-affected fruit, the orange color starts first at the peduncular end, at a time when the stylar end is still green (Figure 1). Fruits affected by HLB also exhibit orange-stained vascular bundles, are lopsided and smaller than normal fruits, and have aborted seeds (National Research Council, 2010).

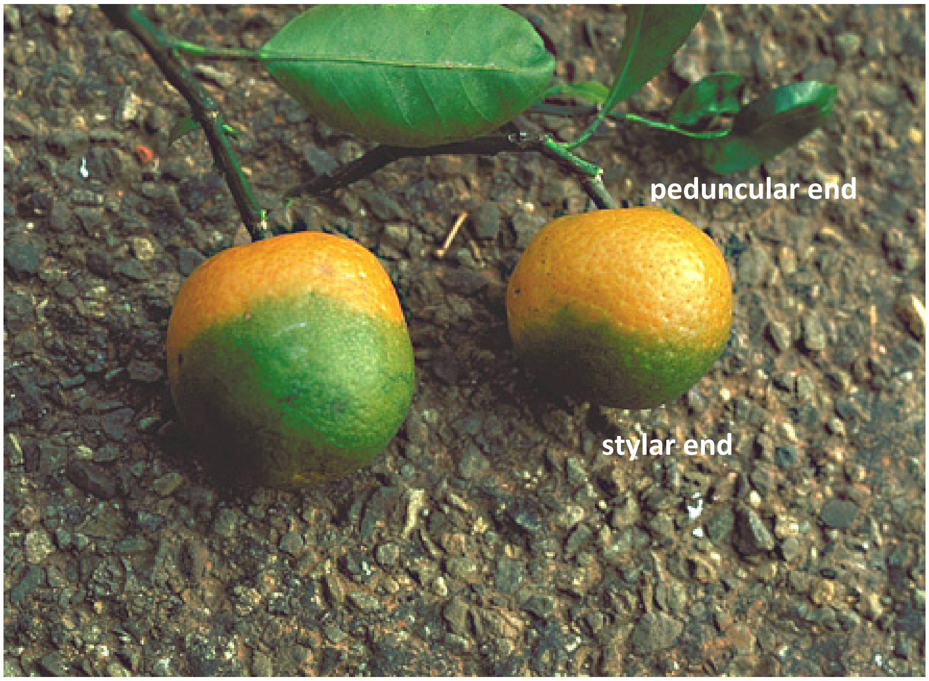


Figure 1. Citrus fruit displaying stylar end greening characteristic of HLB (Gottwald *et al.*, 2007).

Figure 2. Sweet orange tree showing 2 to 3 years of defoliation and dieback due to HLB (Gottwald *et al.*, 2007).

First symptoms of HLB may appear within 6 to18 months after infection. Severe symptoms may appear as early as 6 months post infection in young trees but more typically at 1 to 5 years, especially for mature trees. The disease progress in the orchard can be relatively fast, reaching an incidence of more than 95 percent in 3 to 13 years after onset of the first symptoms (National Research Council, 2010). However, symptoms of HLB may not appear on leaves for months to years after initial infection (Lee *et al.*, 2015). As the disease severity increases, the yield is reduced and makes the orchard production uneconomical in 7 to 10 years after planting (National Research Council, 2010). The average lifespan of a healthy citrus tree is approximately 50 years with some living as long as 100 years. Trees typically begin producing fruits sometime between the second and fifth growing season and continue to produce fruit throughout their lifespan. The yield of affected trees is not only reduced considerably by continuous fruit drop, dieback, and tree stunting, but also by the poor quality of fruits that remain on the trees (National Research Council, 2010). There is no known cure for citrus greening disease (Lee *et al.*, 2015).

<u>Impacts to citrus industry</u>

HLB has the potential to put America's entire citrus crop at risk. Since the initial detection of HLB in Florida in 2005, the disease has affected the vast majority of Florida's citrus-producing areas. In addition to Florida, HLB has been detected in Georgia, Louisiana, and South Carolina, as well as Puerto Rico and the U.S. Virgin Islands. In 2012, it was detected in small areas in Texas and in residential citrus trees in California (USDA-APHIS, 2014b).

As noted above, both fruit yield and quality are affected as disease severity increases in the trees. The fruit yield is reduced, mainly by the early drop of fruits from affected branches. The yield reduction can reach 30 to 100 percent, depending on proportion of affected canopy and makes the orchard uneconomical in 7 to 10 years after planting (Gottwald *et al.*, 2007). Additionally, fruit quality is affected due to them being smaller, lighter, and very bitter which affects the juice quality and marketability (Gottwald *et al.*, 2007).

Since its detection, HLB is having an adverse economic impact on citrus production in Florida (Hodges and Spreen, 2012). Orange acreage in Florida has decreased by 26 percent and yield has decreased by 43 percent (Singerman and Useche, 2016) since 2005. Orange production dropped from 242 million before 2005 to 104.6 million boxes in 2014 (Singerman and Useche, 2016). U.S. orange production continues to decline due to reduced area in Florida and fewer oranges for processing due to HLB (USDA-FAS, 2017b). According to University of Florida research estimates from the 2006/07 to 2010/11 production seasons, HLB has cost the state of Florida more than $4.5 billion in lost citrus production and led to more than 8,200 lost jobs (Hodges and Spreen, 2012). Costs associated with HLB also include costs associated with more intensive management such as more frequent pesticide and nutrient applications as well as costs associated with removal of infected trees and replanting (CISR, 2017).

3.1.1 Range

In Florida HLB was first discovered in August 2005 by a Florida Department of Agriculture and Consumer Services scientist during a routine survey in a pummelo tree in a commercial nursery in Florida City and then in residential areas in Pinecrest and Coral City (Gottwald *et al.*, 2007).

HLB has now been identified in commercial and residential sites in all counties with commercial citrus throughout Florida, as well as in other states in the U.S. such as Alabama, California, Georgia, Louisiana, South Carolina, and Texas (USDA-APHIS, 2014a; Dewdney *et al.*, 2017; USDA, 2017).

3.1.2 Current HLB Control Measures

Currently, there is no cure for HLB, but some growers attempt to suppress the disease through removal of the infected trees while others are targeting the insects (Asian citrus psyllid) that carry the disease. Additionally, some growers are using enhanced nutritional programs to sustain individual HLB-affected trees and to maintain grove yields.

<u>Tree removal</u>

The effectiveness of removing diseased trees, as a control option is directly related to the latency of infection. The first occurrence of visual symptoms can be dramatic in some trees yet subtle in others. It is recommended that diseased trees be removed to ensure that they will not serve as a source of the bacteria for psyllid acquisition and transmission, but recognized that multiple asymptomatic but potentially infected trees, or trees with subclinical symptoms, probably exist in the vicinity (Gottwald *et al.*, 2007; Dewdney *et al.*, 2017). Replanting with certified disease-free planting materials is essential to minimize further spread (Dewdney *et al.*, 2017).

<u>Enhanced nutritional programs</u>

HLB causes fibrous roots to decline within a few months after infection and before foliar symptoms develop. Fibrous roots are responsible for the bulk of nutrient uptake; their decline likely explains the deficiency symptoms that develop in the canopy (Morgan and Hamido, 2016). Enhanced nutritional programs constitute a strategy being employed by citrus growers to mitigate HLB-induced deficiencies by supplying the trees with additional nutrients aimed at increasing production longevity (CRDF, 2013; Stansly *et al.*, 2014). A continuous flow of nutrients available to the tree ensures the tree is getting the necessary nutrients. The roots of trees affected by HLB are smaller than trees without HLB, so having a constant supply of nutrients available for the roots allow the nutrients to be taken in at the pace allowed by the size of the root system (Schreckengost, 2017).

Research has demonstrated that HLB symptoms can be reduced by foliar applications of micronutrients, especially Mg, Mn, Zn and boron, allowing continued production of existing citrus groves affected by HLB (Stansly *et al.*, 2014; Morgan and Hamido, 2016). HLB-infected trees can be economically sustained with good horticultural practices based on a comprehensive ground fertilization program and only minor foliar nutrient additions (Spann and Schumann, 2012). Increasing nutritional inputs may prolong the life of the tree and increase productivity of the tree, but will not prevent infection with HLB or the spread of the disease (Stansly *et al.*, 2014).

<u>Insect control</u>

Perhaps the easiest HLB control strategy to accomplish is chemical control of HLB psyllid vectors. To keep psyllid populations in check, insecticides must be applied frequently. This raises environmental concerns, reduces the effectiveness of biological control, and increases the potential for the psyllid to develop resistance to different classes of compounds (National Research Council, 2010).

Insecticide usage to control ACP, with the goal of preventing single or multiple inoculations, has increased significantly in Florida and other citrus-producing States since the detection of HLB (Grafton-Cardwell *et al.*, 2013; USDA-APHIS, 2014a). In Florida, 8 to 12 treatments per year have been commonly used (Grafton-Cardwell *et al.*, 2013). Prior to this, ACP was considered a minor pest. However, growers must apply both systemic soil drench treatments along with multiple foliar sprays, especially to protect newly planted and young (4 to 8-year old) trees that are more vulnerable to ACP attack because they produce new growth more frequently (USDA-APHIS, 2014a).

The types of chemicals used for controlling ACP consist of horticultural oils and other products derived from natural sources and/or organophosphates. Studies of ACP response to insecticides indicate sensitivity to a number of different insecticide classes, including pyrethroids, organophosphates, carbamates, neonicotinoids, some insect growth regulators (IGRs), horticultural oil, the lipid synthesis inhibitor spirotetramat, spinetoram, abamectin, and sucrose octanoate (Grafton-Cardwell *et al.*, 2013). Broad-spectrum insecticides in the pyrethroid, organophosphate, and neonicotinoid classes have a greater efficacy against ACP (especially adults) than do many of the other classes. Oils and IGRs are more effective against eggs and nymphs than adults. The level of suppression and residual action against ACP varies among rates and types of insecticides, insect stages, and may influence the choice and timing of treatments (Grafton-Cardwell *et al.*, 2013).

Systemic soil-applied insecticides are an important part of psyllid control because they provide a longer period (months) of protection compared with foliar insecticides (weeks) (Grafton-Cardwell *et al.*, 2013; Dewdney *et al.*, 2017). However, systemic insecticides require one to three weeks for uptake into citrus trees, and the effective dose of insecticide varies depending on tree size, irrigation, soil type, and other factors. Systemic insecticides are especially important for young trees that flush nearly continuously and thus require constant protection (Grafton-Cardwell *et al.*, 2013). For ACP management programs, soil-applied systemic insecticides are primarily, if not exclusively, neonicotinoids and therefore best combined with foliar insecticides employing different modes of action to reduce selection for resistance (Grafton-Cardwell *et al.*, 2013).

There is growing concern over the risk to bee populations from the use of neonicotinoid insecticides. Foraging bees are exposed to harmful levels of pesticide residues in pollen and nectar in treated fields and contaminated areas nearby, as well as in dust created when treated seeds are planted (Butler, 2018). This is of particular concern for citrus because bees are often placed in orchards for honey production. The EFSA conducted an assessment of more than 1,500 studies and found that most uses of neonicotinoid pesticides represent a risk to wild bees and honeybees (EFSA, 2018a; 2018b; 2018c). Due to the risk to bee populations the EPA has instituted label language designed to reduce exposure and risk to these types of pollinators.

Research indicates that the incidence of the HLB bacterium in psyllids is highest in the spring and in the late fall. Thus, applying pesticides during these times is most effective and avoiding summer sprays may allow recovery of biological control agents for psyllids and other pests (National Research Council, 2010). Treatments targeting overwintering ACP adults have the greatest impact on populations because reproduction is severely reduced during this time. Another advantage of these so-called dormant sprays is their minimal effect on psyllid predators due to the predators being largely absent or in protected stages when sprays are applied (Grafton-Cardwell *et al.*, 2013).

While it is important to control psyllids on an area-wide basis so that psyllid populations cannot build up between applications and move between groves readily (National Research Council, 2010), the necessity of additional sprays is both costly and depending upon the economics of individual producers may not be feasible (Gottwald *et al.*, 2007). Although there is information available on the effect of chemical control of various insecticides and programs on psyllid vectors, the effect of this practice to control HLB increase and spread remains largely anecdotal and undocumented (Gottwald *et al.*, 2007). While systemic insecticides used for vector suppression reduced disease spread, they did not fully prevent the spread of HLB (Grafton-Cardwell *et al.*, 2013).

Biological control of HLB vectors

In addition to chemical control of HLB psyllid vectors there are a number of biological control agents that target the psyllids. The nymphs of both psyllid species (ACP and the African citrus psyllid, *Tryoza erytreae*) that can transmit HLB are parasitized by hymenopterous ectoparasites, *Tamarixia dryi* Waterston and *T. radiata* Waterston (Gottwald *et al.*, 2007; Grafton-Cardwell *et al.*, 2013). The use of these parasitoids have successfully controlled the vector populations on Reunion Island and have been used to attempt to reduce populations elsewhere. On Reunion Island, the parasitoids significantly reduced the psyllid populations sufficiently to mitigate the impact of HLB. Elsewhere, biocontrol via introduction of parasites has also been attempted but has had very limited success (Gottwald *et al.*, 2007).

Field observation in Florida where *T. radiata* was introduced, indicate that the effect of biological control can range broadly from 4 to 70% reduction in ACP populations. Another internal parasitoid, *Diaphorencyrtus aligarhensis*, has also been found to attack ACP (Gottwald *et al.*, 2007; Grafton-Cardwell *et al.*, 2013). Unfortunately, *D. aligarhensis* has yet to be successfully introduced in Florida, where multiple attempts have been unsuccessful (Grafton-Cardwell *et al.*, 2013). Additionally there are several major predators of ACP and these include lady beetles, lacewings, syrphids, and spiders. However, the relative importance of each group toward the control of ACP is less certain due in part to the difficulty of evaluating their individual contributions to mortality (Grafton-Cardwell *et al.*, 2013).

The use of insecticides to reduce psyllid populations also has a strong detrimental effect on biocontrol populations. What is not clearly understood so far, is the proportion of the psyllid population that must be controlled to have a significant effect on the rate of HLB increase and spread (Gottwald *et al.*, 2007). To date the amount of psyllid control provided by introduced parasitoids has been insufficient to slow the disease spread (Dewdney *et al.*, 2017)

Interplanting

Another control strategy to manage HLB is to interplant citrus with guava, with the intention of controlling ACP. Control could be either direct via attracting the ACP to the guava and killing it when it feeds on some toxin, or indirectly by repelling or confusing the ACP by some released volatile, but the exact effect is currently unknown (Gottwald *et al.*, 2007). Interplanting crops or planting mixed crops to deter insect pests and diseases is not a new strategy, but is rarely used in western agriculture. Interplanting of any crop with citrus presents many problems in terms of harvesting practices, equipment movement, potential competition and shading, and may require modification of irrigation and fertilization practices (Spann *et al.*, 2007). While the use of interplanting with guava has shown some success in Vietnam at reducing psyllid populations and delaying infection with HLB (Gottwald *et al.*, 2010; Gottwald *et al.*, 2014), guava is considered an invasive species in Florida and so is not recommended at this time for use (Spann *et al.*, 2007).

3.2 Citrus Tristeza Virus

The purpose of the proposed permit is to use a plant virus, *Citrus tristeza virus* (CTV), as a biological control organism to express spinach defensin proteins as a means to control citrus greening disease in Florida. Insertion of foreign sequences in CTV allows for transient expression in citrus plants without actually inserting DNA sequences into the genome of citrus plants. Previous field trials authorized under APHIS permits have demonstrated the efficacy of the transgenic CTV technique, leading to testing this strategy at a large scale. A thorough discussion of CTV taxonomy, distribution, genetics, strains, and diseases can be found in the PRA completed for this permit application (USDA-APHIS, 2018).

CTV is a member of the virus family *Closteroviridae,* a genetically and biologically diverse family of viruses that can cause stunting, slow or quick decline, stem pitting, or no symptoms depending on the virus strain, citrus cultivar, rootstock, time of infection, and environmental conditions (Powell *et al.*, 1999; Ng *et al.*, 2016). Some strains of CTV are mild and produce no noticeable symptoms while other strains are severe causing decline and death of the tree or deep pits in the trunk and stem. In Florida, two general types of CTV strains are prevalent: those that cause no detectable symptoms and those that cause stunting and/or decline of citrus on sour orange rootstock (Powell *et al.*, 1999).

CTV is transmitted by aphids in a semi-persistent manner. The aphid can acquire the virus within minutes of feeding on an infected plant and transmit it to healthy plants shortly after acquisition of the virus (Roberts *et al.*, 2016). In Florida, CTV became a serious threat in the late 1990s when the brown citrus aphid, considered the most efficient vector of CTV, was introduced in the United States (Grafton-Cardwell *et al.*, 2006; Hilf *et al.*, 2007; Harper *et al.*, 2016; Roberts *et al.*, 2016).

Since 1999, at least 80,000 acres of citrus trees using sour orange as the rootstock were lost, and estimates suggest that an additional 100,000 acres were affected before 1999 (Stover and Castle, 2002). Only trees on sour orange rootstock are affected by tristeza decline (Roberts *et al.*, 2016). CTV infection, unlike HLB, is not necessarily a death sentence to the tree. The onset of quick decline is dependent on three factors: the scion species or cultivar infected, the rootstock species on which the scion is grafted, and the infecting strain or isolate(s) of CTV (Harper *et al.*, 2015a). Outbreaks of CTV have led to changes in agricultural practices, including the wide-scale abandonment of sour orange as a rootstock for more CTV-tolerant rootstocks (National Research Council, 2010). Trees on tolerant rootstocks are now infected but show no disease symptoms (Ng *et al.*, 2016; Roberts *et al.*, 2016).

3.2.1 Range

CTV is an almost ubiquitous pathogen of citrus, both in the U.S. and globally (Bar-Joseph *et al.*, 1989; Hilf *et al.*, 2005; Nolasco, 2009; Albiach-Marti, 2012; Bar-Joseph and Mawassi, 2013; Silva and Nolasco, 2013). CTV is endemic in many countries of Asia, Africa, North and South America, Australia, as well as in some Mediterranean countries, and citrus trees grown in these regions are tolerant to the virus. Problems arise from CTV primarily where citrus is grown on sour orange rootstock, which is now avoided in Florida citrus orchards (National Research Council, 2010). Mild isolates have been widespread in Florida for many years and these have been widely disseminated by aphids and in budwood. However, these isolates do not usually cause decline symptoms (Roberts *et al.*, 2016).

Using currently available molecular tools, researchers have identified and determined the incidence of major strains of CTV present in Florida citrus groves (Harper *et al.*, 2015a; Harper *et al.*, 2015c; Bergua *et al.*, 2016; Harper and Cowell, 2016). Nearly 70 percent and 95 percent of trees tested were positive for the T36 and T30 strains, respectively (Harper *et al.*, 2015c). Additionally, over 95 percent of the trees tested were infected with one or more strains of CTV (Harper *et al.*, 2015c). In 2015, an additional study evaluated historical samples and found that three major CTV strains, T36, T30, and VT, had been present in Florida for approximately 40 years (Harper *et al.*, 2015a).

3.2.2 Current Control Measures

The primary CTV control strategies in Florida have focused on use of tolerant rootstocks to protect against endemic decline strains and on budwood certification to avoid distribution of any stem pitting strains (Powell *et al.*, 1999). Diseased trees should be replaced with certified trees on tolerant rootstocks as the yields of affected trees decline to uneconomical levels (Roberts *et al.*, 2016). Use of insecticides to control aphids is unlikely to reduce the spread of the virus in commercial groves, since aphids can usually acquire and move the virus before it is killed by an insecticide. However, rigorous aphid control in nurseries and on budwood source trees could reduce infection rates (Roberts *et al.*, 2016).

Another method to mitigate the effects from CTV is to protect trees using cross protection. Cross protection is the practice of inoculating trees with mild virus strains to protect them from a second infection by severe strains (Atallah *et al.*, 2016). It is also referred to as “superinfection exclusion” because the primary virus infection is able to completely exclude secondary infection with the same or a similar virus (Folimonova, 2013). Cross protection against decline causing isolates of CTV have been effectively used in South Africa and Australia in reducing losses in grapefruit due to stem-pitting, and against losses in sweet orange in Brazil. Cross protection against CTV decline on sour orange rootstock has not yet been developed as an effective control measure (Powell *et al.*, 1999; Roberts *et al.*, 2016).

3.3 Citrus Production

Since the permit application from Southern Gardens describes the release sites as within the 67 counties in Florida, the main focus of this section will be on citrus production in the state of Florida.

Citrus is an important crop grown in more than 140 countries and traded worldwide as a fresh and processed product (Davies and Jackson, 2009). Citrus grows in moderate to tropical climates from about 40°N to 40°S (Davies and Jackson, 2009). The main species of citrus include sweet orange (*C. sinensis*), grapefruit (*C. paradisi*), mandarin (*C. reticulata*), lemon (*C. limon*), and lime (*C. aurantifolia*) (Jackson, 1999). Production of citrus in the United States includes oranges, tangelos, tangerines, grapefruit, lemons, and limes mainly in the States of Florida, California, Texas, and Arizona.

Citrus trees are evergreen perennials which may live for more than 200 years under commercial conditions in some regions. The average lifespan of a healthy citrus tree is 50 years and the oldest trees in Florida are about 100 years old (Davies and Jackson, 2009). Trees typically begin producing fruits sometime between the second and fifth growing season and continue to produce fruit throughout their lifespan. Freeze, pest, disease, and land use change over time are the primary causes of decreased tree longevity (Davies and Jackson, 2009). Citrus trees were originally grown from seeds, but such plants had numerous disadvantages including, delayed fruit production, a vigorous and upright growth habit, thorniness of the branches, and a general lack of adaptations to local conditions (Jackson, 1999). These problems have led to the production of citrus on grafted trees consisting of the scion, the above the ground portion of the tree that bears fruit, and the rootstock, the below ground portion comprising the root system (Jackson, 1999; Davies and Jackson, 2009).

3.3.1 Citrus Acreage

National

Sweet orange is the leading citrus crop produced and consumed in the world today. In 2016/2017, the world production of oranges was estimated to be 49.6 million metric tons (USDA-FAS, 2017b; 2017a). In 2016/17, the United States was the fourth largest producer of oranges in the world behind Brazil, China, and the European Union (USDA-FAS, 2017b; 2017a). In 2016/17, the United States is estimated to have produced 9.9 percent of the world's production of fresh oranges (USDA-FAS, 2017b; 2017a). However, orange production in the United States has declined by over 40 percent in the last five years (USDA-FAS, 2017b; 2017a). U.S. orange production is forecast down 470,000 tons to 4.9 million metric tons in 2016/17 continuing a multiyear downward trend, as citrus greening continues to reduce area in Florida (USDA-FAS, 2017b; 2017a). Exports are down slightly while consumption is forecast 8 percent higher. Fruit for processing is down due to the smaller crop in Florida (USDA-FAS, 2017b).

The major citrus producing states in the United States include Florida, California, Texas, and Arizona (Figure 3). Of these states, California produced 51 percent of the total U.S. citrus crop in 2016, Florida produced 45 percent, and Texas and Arizona combined produced the remaining 4 percent (USDA-NASS, 2017c). Florida grows citrus on nearly twice as much acreage as California, but accounts for less production because HLB is reducing production so significantly in Florida. Most orange production in Florida is used for processing whereas California produces mainly for the fresh market (USDA-FAS, 2017b). Florida is the largest producer of oranges, accounting for about 57 percent of total U.S. production, and of grapefruit, producing nearly 47 percent of total production (USDA-NASS, 2017c). California is the largest producer of lemons, producing more than 92 percent of production, and of tangerines, accounting for about 94 percent of production (USDA-NASS, 2017c).

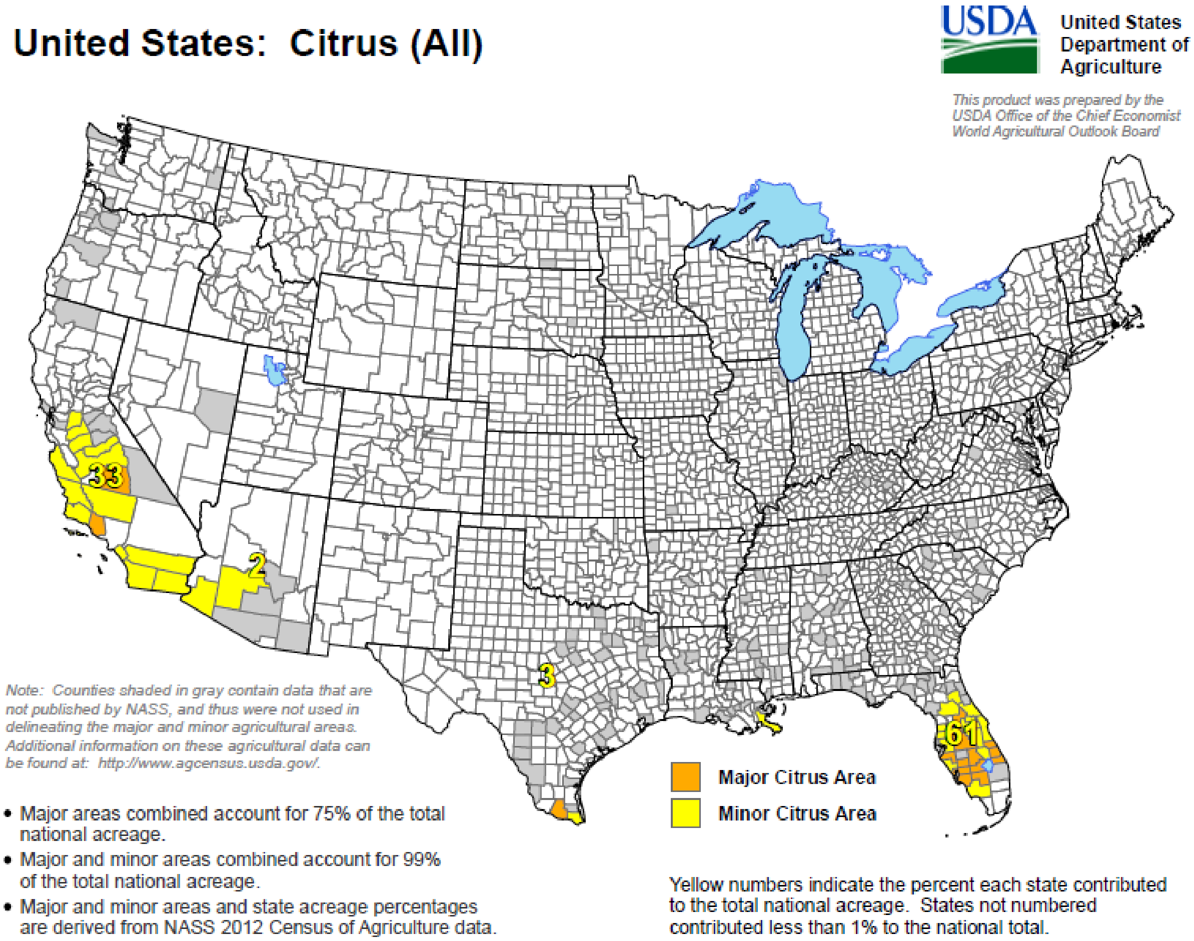
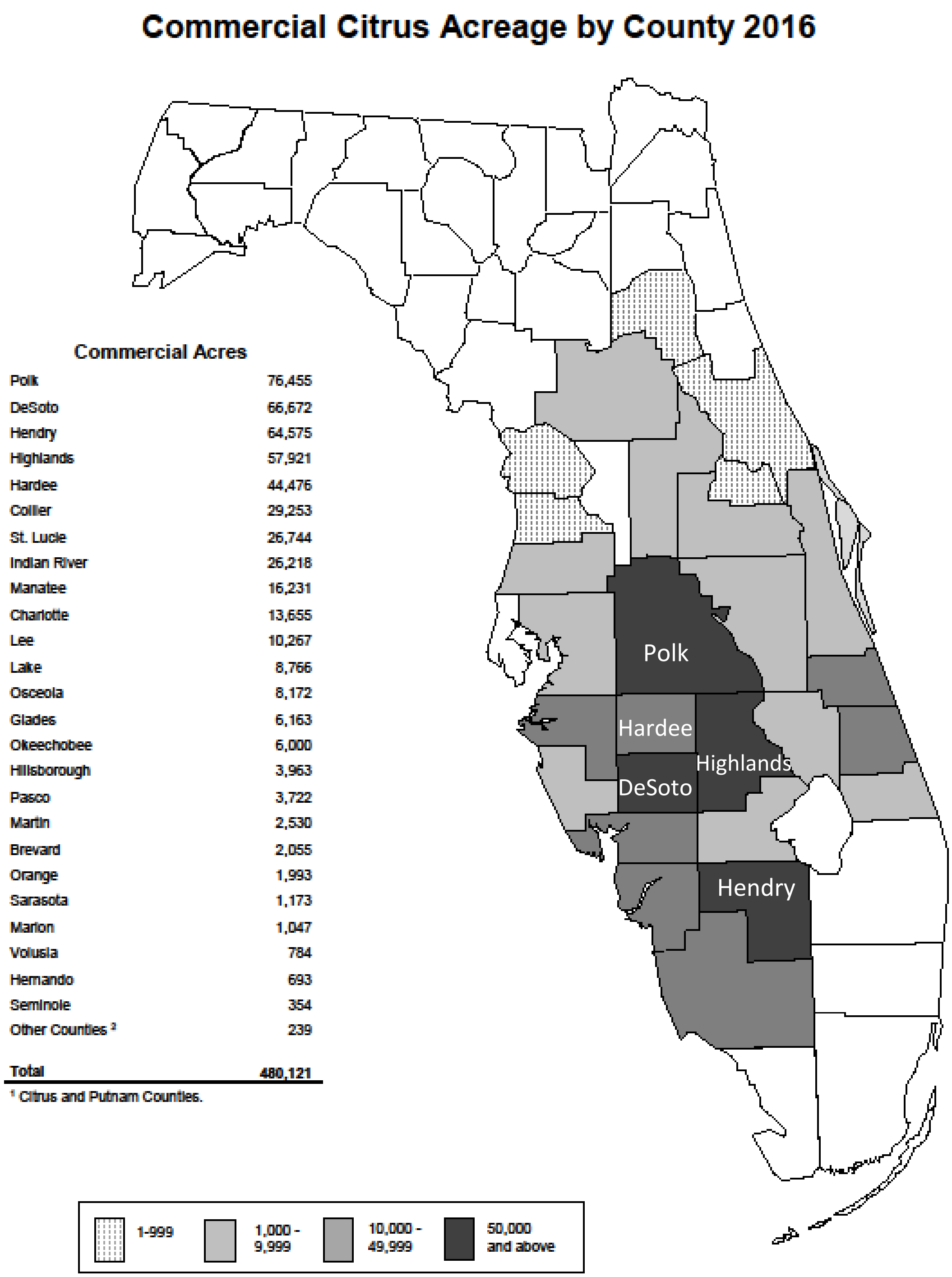


Figure 3. Areas of citrus production in the United States (USDA-OCE, 2012).

Florida

While citrus is grown in all of Florida, the top 5 citrus producing counties are Hendry, De Soto, Polk, Highlands, and Hardee (Figure 4). Together they account for 61 percent of the state's total citrus production (FDACS, 2017). Sweet oranges dominate Florida's citrus production (88 percent) followed by grapefruit (10 percent) and specialty fruit consisting of mostly tangerines and tangelos. Florida produces very few lemons (USDA-NASS, 2017b). More than 97 percent of Florida's orange production is processed into juice, accounting for the majority of the U.S. orange juice production (USDA-NASS, 2017c).



County	Commercial Acres
Polk	76,455
DeSoto	66,672
Hendry	64,575
Highlands	57,921
Hardee	44,476
Collier	29,253
St. Lucie	26,744
Indian River	26,218
Manatee	16,231
Charlotte	13,655
Lee	10,267
Lake	8,766
Osceola	8,172
Glades	6,163
Okeechobee	6,000
Hillsborough	3,963
Pasco	3,722
Martin	2,530
Brevard	2,055
Orange	1,993
Sarasota	1,173
Marion	1,047
Volusia	784
Hernando	693
Seminole	354
Other Counties [1]	239
Total	480,121

[1] Citrus and Putnam Counties.

Figure 4. Citrus acreage by county. (FDACS, 2017)

Florida's orange production, at 68.8 million boxes, is down 16 percent from the previous season as citrus greening continues to impact trees in Florida (USDA-FAS, 2017b; USDA-NASS, 2017c; 2017b). Grapefruit production in Florida, at 7.76 million boxes, is down 28 percent from last season. Florida's total citrus production decreased 17 percent from the previous season to 4.2 million tons continuing a multiyear downward trend (Figure 5). Bearing citrus acreage, at 410,700 acres, is 24,600 acres below the 2015-16 season (USDA-NASS, 2017c; 2017b).

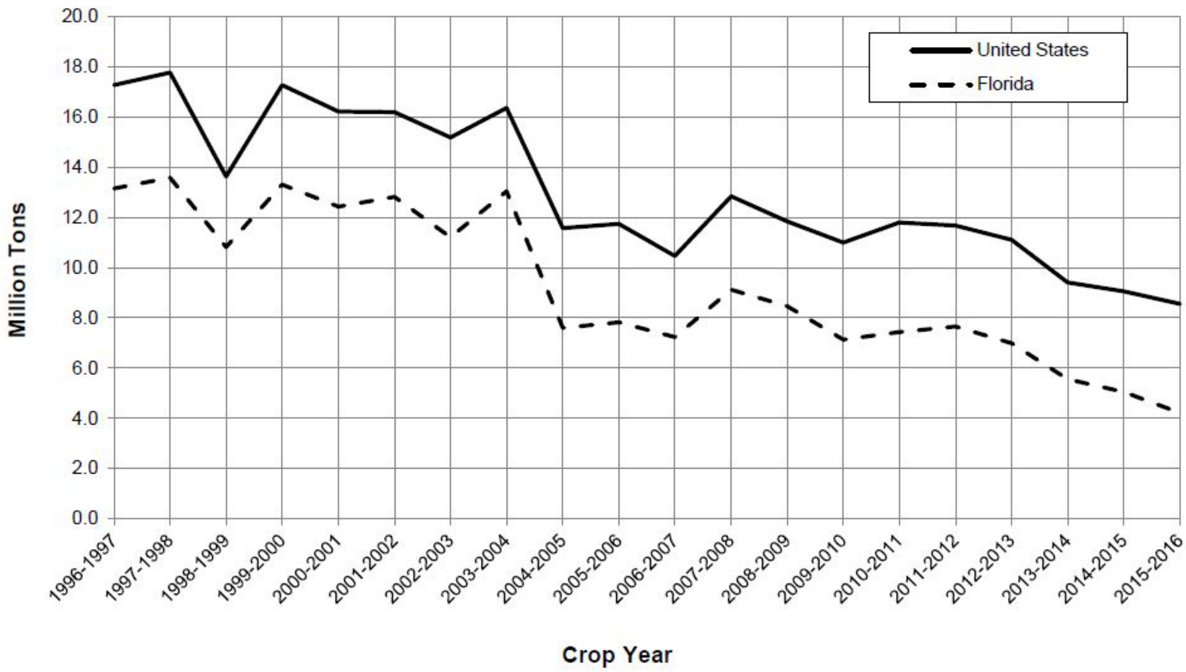


Figure 5. Citrus Production – United States and Florida: Crop Years 1996-1997 through 2015-2016. (FDACS, 2017)

3.3.2 Socioeconomics of Citrus

Market Overview

As noted above in Section 3.3.1, orange production in the United States has declined by over 40 percent in the last five years due to HLB in Florida (USDA-FAS, 2017b; 2017a). U.S. orange juice production is forecast down 20 percent as a result of fewer oranges for processing from Florida due to HLB. Imports of orange juice are forecast up slightly while exports are lower due to reduced supplies (USDA-ERS, 2017; USDA-FAS, 2017a).

The citrus industries in Florida and California contribute more than $10 billion total to their states' economies. Since its introduction, HLB has had a devastating impact on Florida's citrus industry (USDA-NIFA, 2016). Despite reduced overall volume, the 2016/17 U.S. citrus crop was valued at $3.4 billion, relatively unchanged from the previous season (Figure 6). The relative stability in the value of the total U.S. citrus crop is due to higher crop values for lemons and tangerines/mandarins from California offsetting lower returns for oranges and grapefruit from Florida (USDA-ERS, 2017; USDA-NASS, 2017c).

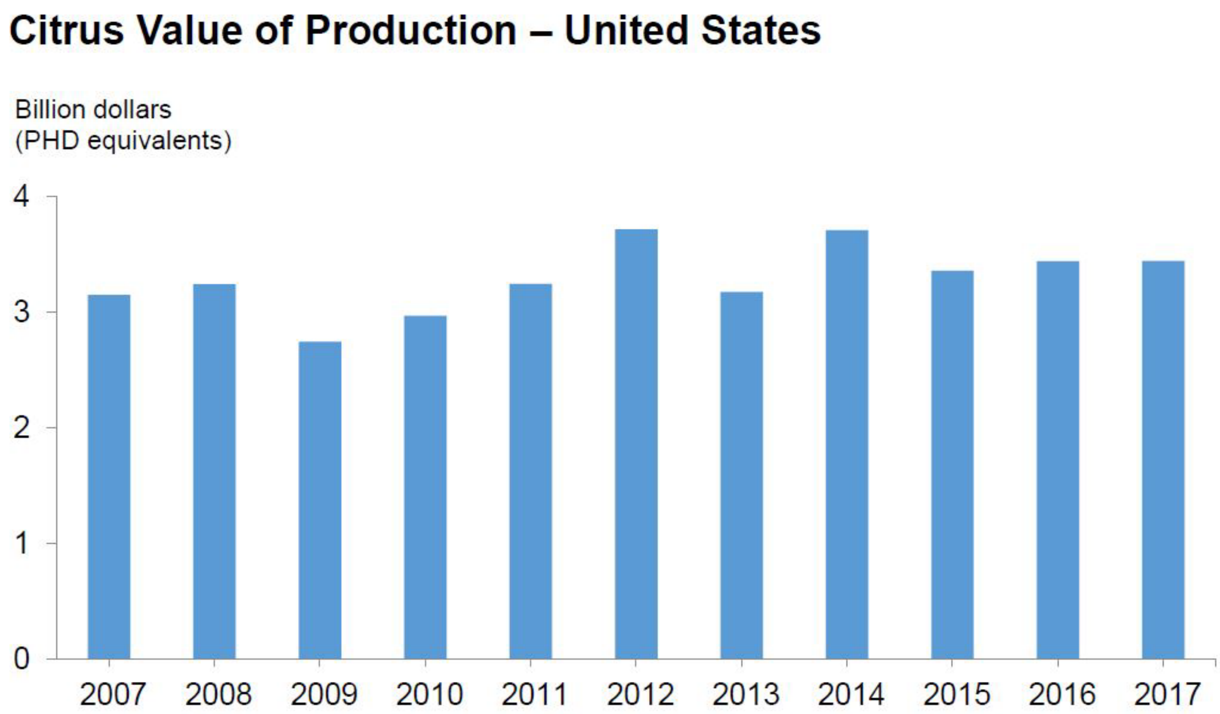


Figure 6. Citrus Value of Production. (USDA-NASS, 2017c)

Florida

As noted above, Florida produced 45 percent of the total U.S. citrus crop (USDA-NASS, 2017c). More than 97 percent of orange production in Florida is used for processing into juice, accounting for the majority of the U.S. orange juice production (USDA-FAS, 2017b; USDA-NASS, 2017c). Florida is the largest producer of oranges, accounting for about 57 percent of total U.S. production, and of grapefruit, producing nearly 47 percent of total production (USDA-NASS, 2017c). The state of Florida is the second largest producer of orange juice in the world, behind Brazil (Hodges and Spreen, 2012).

The Florida citrus industry represents an important part of the Florida agricultural economy, with Florida citrus fruits alone valued at $1.03 billion for the 2016/17 growing season (USDA-NASS, 2017c). Orange production alone in Florida is valued at over $886 million (USDA-NASS, 2017c). Organic citrus is grown on 964 acres in Florida, which accounts for approximately 0.2 percent of the citrus acres grown in Florida and is valued at approximately $2.3 million (USDA-NASS, 2017a; 2017c). The Florida citrus industry generated at least 75,800 jobs, based on over 203 million boxes of citrus fruit produced in the 2007/08 season (Hodges and Spreen, 2012). Current levels of production and citrus acreage are sharply lower than the levels of the mid-1990s through 2004. The number of juice processors has also declined sharply. There were 37 processors in Florida in 2001, but only 15 remained in 2008 (National Research Council, 2010).

HLB not only reduces yield, fruit size and quality, but also increases tree mortality and the costs of production as growers have to adjust their inputs to try and maintain yields (Singerman and Useche, 2016).

Since 2005, total costs and, specifically, spray costs have increased in Florida due to increased costs of dealing with HLB. The 2003/04 costs per acre for producing processed oranges in southwest Florida without tree replacement was $936.87; foliar sprays and fertilization accounted for $185.63 and $207.69 per acre, respectively. In 2014/15, the cost per acre in that region was $1,651.34, and the cost of foliar sprays and fertilization were $666.00 and $486.96 per acre, respectively. Thus, since 2003/04, the cost of the foliar spray program alone increased by almost 260 percent, and that of fertilizer increased by 134 percent (Singerman and Burani-Arouca, 2017). The rate of tree loss also increased with the introduction of HLB. In 2003/04, the average number of trees replaced in orchards in southwest Florida was 4 trees per acre. The tree replacement program at that time totaled $145.98 per acre, or $36.49 per tree. In 2014/15, the number of trees replaced in orchards was 9 trees per acre, and growers spent, on average, $346.77 per acre, or $38.53 per tree. Thus, the increase in the tree replacement program is mostly driven by the higher mortality rate (Singerman and Burani-Arouca, 2017). It is very difficult to quantify the cost of declines in yield and loss of entire groves that are likely to occur in Florida over the next few years (National Research Council, 2010). The agricultural alternatives for most citrus land in Florida are cattle pasture or pine forests, neither of which is highly profitable, so growers have few options (National Research Council, 2010).

4 ENVIRONMENTAL CONSEQUENCES

This analysis of potential environmental consequences addresses the potential impact to the human environment from the alternatives analyzed in this EIS: the No Action Alternative and the Preferred Alternative, issuing a permit for the field release of CTV-SoD. Potential environmental impacts from the No Action Alternative and the Preferred Alternative for issuing the permit are described in detail throughout this section. A cumulative impact analysis is presented for each potentially affected environmental concern in Chapter 5.

4.1 Scope of Analysis

Potential environmental impacts from the No Action Alternative and the Preferred Alternative for issuing a permit for release of CTV-SoD are described in detail throughout this section. An impact would be any change, positive or negative, from the existing (baseline) conditions of the affected environment (described for each resource area in Chapter 3); the baseline conditions would be those as they exist today. Impacts may be categorized as direct, indirect, or cumulative. A direct impact is an effect that results solely from a proposed action without intermediate steps or processes. An indirect impact may be an effect that is related to but removed from a proposed action by an intermediate step or process.

A cumulative impacts analysis is also included for each environmental issue and is discussed fully in Chapter 5. A cumulative impact may be an effect on the environment which results from the incremental impact of the proposed action when added to other past, present, and reasonably foreseeable future actions. If there are no direct or indirect impacts identified for a resource area, then there can be no cumulative impacts.

Where it is not possible to quantify impacts, APHIS provides a qualitative assessment of potential impacts. Certain aspects of this product may be no different between the alternatives.

4.2 No Action

Under the No Action Alternative, APHIS would deny the permit application (17-044-101r) submitted by Southern Gardens. The applicant would not be authorized to release the CTV-SoD. Current methods to manage citrus greening disease in Florida would continue under the No Action Alternative.

4.2.1 Impacts of current HLB Control Measures

Currently, there is no cure for HLB, but growers attempt to suppress the disease through removal of the infected trees and targeting the insects (Asian citrus psyllid) that carry the disease. Growers also use additional nutritional supplements to prolong the life and productivity of trees.

APHIS prepared an EA for the Asian Citrus Psyllid Control Program in the Continental United States and Puerto Rico that resulted in a finding of no significant impact (USDA-APHIS, 2010). That document and its findings is incorporated by reference into this EIS. It describes the environmental and human health impacts of many of the insecticides used for control of ACP.

Physical Environment

As noted above in Section 3.1.2, there are several methods that growers currently use to mitigate the effects of HLB. These typically include removal of dead or diseased trees and replanting with certified disease-free seedlings and control of the insect vector. Additionally, citrus producers are increasing nutritional inputs aimed at prolonging the productivity of trees and groves.

Insecticide usage to control ACP has increased significantly in Florida and other citrus producing States since the introduction of HLB (Grafton-Cardwell *et al.*, 2013; USDA-APHIS, 2014a). Before 2004, average annual pest control consisted of two sprays for processed juice fruit and six sprays for fresh market grapefruit (Singerman and Burani-Arouca, 2017). With the discovery of HLB in Florida, annual pest control increased to 11 sprays for processed oranges and 14 sprays for fresh market grapefruit (Singerman and Burani-Arouca, 2017). In Florida, it is not uncommon for 8 to 12 treatments per year to be used (Grafton-Cardwell *et al.*, 2013).

To keep psyllid populations in check, insecticides must be applied frequently. Insecticides used for ACP control must be registered by EPA under FIFRA. Impacts to the physical environment from many of the insecticides used for control of ACP have been previously evaluated by APHIS (USDA-APHIS, 2010) and will be summarized here. Detailed information on the individual insecticides and their impacts can be found in the Asian Citrus Psyllid Control Program in the Continental United States and Puerto Rico EA (USDA-APHIS, 2010). As noted in that document, all insecticides that are used for ACP control are currently registered by EPA for use on citrus. As long as they are used in accordance with their labels, its applicable directions, and all restrictions and precautions, the effects to the environment are not expected to be substantial (USDA-APHIS, 2010).

Two application strategies are typically used for chemical control of ACP: foliar sprays for immediate reduction of ACP populations to target the adult life stage, and soil applications to provide longer lasting, systemic ACP control and to target the immature life stages, such as eggs and nymphs (USDA-APHIS, 2010). Systemic insecticides include imidacloprid, aldicarb, and thiamethoxam. Foliar sprays are applied to the leaves and include insecticides such as fenpropathrin, zeta-cypermthrin, chlorpyrifos, chlorantraniliprole, spirotetramat, abamectin, and carbaryl. Organic insecticide options are available to control ACP including Neem oil, pyrethrin, and narrow range oil (USDA-APHIS, 2010; Grafton-Cardwell *et al.*, 2013; USDA-APHIS, 2014a).

Use of pesticides in sandy soils, typical of those found in Florida, can raise water quality concerns due to mobility and persistence of pesticides. For example, the soil-applied insecticide aldicarb has been shown to provide control of psyllids on trees, but studies have shown that aldicarb can be found in wells and groundwater when applied to sandy soils (National Research Council, 2010). Under Florida conditions, aldicarb degrades relatively rapidly, but has been found in some shallow water wells. As a result, aldicarb is limited to applications only in the dry season (November–April) and cannot be applied within 1000 ft of a drinking water well to minimize risks to human health and the general environment (National Research Council, 2010). Due to the mobility and persistence of certain pesticides, there is the potential for surface and ground water residues to occur in areas that are vulnerable to runoff and leaching. Exposure and risk associated with these pesticides will be minimized by adherence to label requirements regarding applications near water.

Similar to impacts from pesticides, enhanced nutritional programs could add nutrients to the environment beyond what is typical of commercial citrus. The goal of enhanced nutritional programs is to increase the longevity and continued production in groves affected by HLB by supplying trees with additional nutrients. When nutrients applied exceed plant needs they can wash into aquatic ecosystems. There they can cause major environmental damage as well as serious health problems in people and animals (US-EPA, 2005). To combat nutrient losses, growers can apply fertilizers in the proper amount, at the right time of year and with the right method to reduce the potential for runoff (US-EPA, 2005). The state of Florida has programs in place to monitor and develop area wide water quality management plans to abate water pollution from point and nonpoint sources (FDEP, 2015). Florida takes a functional approach to address the issue of non-point source pollutants contributed by agriculture, including regulation, regulatory incentives, technical and financial assistance, public education, and compliance monitoring (FDEP, 2015). Reducing agricultural non-point source pollution is primarily accomplished through developing cost-effective, research-based best management practices that, prevent nutrient levels in ground water from exceeding ground water standards (FDEP, 2015).

<u>Biological Environment</u>

While HLB does not itself pose a threat to species other than citrus, some of the methods currently used to mitigate the effects of HLB including the control of the insect vector and increasing nutritional inputs aimed at prolonging the productivity of trees and groves, may impact the biological environment.

For all insecticide applications in citrus groves, there is the potential for indirect impacts to wild mammals and birds from the loss of available invertebrate prey that would occur after treatment. Those birds and mammals that can forage outside of the groves treated would be less likely to be impacted. Recovery of most invertebrate populations within the citrus groves after treatment will ensure that any impacts to mammals and birds are short-term in nature. The extent and time for recovery would be based on how persistent and broad spectrum a selected insecticide may be in its non-target effects (USDA-APHIS, 2010).

An additional environmental consequence of the use of insecticides for control of ACP is a reduction in occurrence of natural predators such as lady beetles, syrphids, and spiders. Many of the insecticides used to control ACP are broad-spectrum insecticides which disrupt not only native predators of ACP but also of other citrus pests (USDA-APHIS, 2014a). The use of insecticides to reduce psyllid populations would also potentially reduce biocontrol populations.

All of the pesticides used to control APC are registered for use on citrus by the EPA. The EPA considers the direct and indirect impacts from pesticide use on non-target organisms as part of their regulatory decision. Citrus producers are legally required to use pesticides in accordance with the pesticide label. Provided that persons applying the pesticide treatments follow the pesticide label, its applicable directions, and all restrictions and precautions, the pesticide will not cause unreasonable adverse effects on non-target species. Impacts to non-target species from the pesticides used to control ACP were previously analyzed by APHIS in Asian Citrus Psyllid Control Program in the Continental United States and Puerto Rico - Environmental Assessment, where APHIS found that the effects to non-target organisms are not expected to be substantial (USDA-APHIS, 2010).

Enhanced nutritional programs could add nutrients to the environment beyond what is typical of commercial citrus. Excess nutrients, specifically nitrogen and phosphorus, have been shown to impact biological communities. Excess nitrogen and phosphorus in the water causes algae to grow faster than ecosystems can handle. Significant increases in algae harm water quality, food resources and habitats, and decrease the oxygen that fish and other aquatic life need to survive. Algal blooms can severely reduce or eliminate oxygen in the water, leading to illnesses and death of fish and other aquatic organisms (US-EPA, 2018). Some algal blooms produce toxins that can kill fish and other animals. After being consumed by small fish and shellfish, these toxins move up the food chain and impact larger animals like sea lions, turtles, dolphins, birds, manatees, and larger fish (US-EPA, 2018). Finding elevated nitrogen and phosphorus residues in ground water, surface water, and drinking water in various areas throughout Florida at levels in excess of established water quality standards led Florida to develop monitoring and water quality management plans (FDEP, 2015). As described above, the state of Florida has programs in place to reduce agricultural non-point source pollution and prevent nutrient levels in ground and surface waters from exceeding water quality standards (FDEP, 2015).

Human Health

While HLB causes misshapen fruit and gives the fruit and juices derived from it a bitter and unpalatable flavor, it does not affect human health. However, the mitigation measures used by growers including the control of the insect vector and increasing nutritional inputs aimed at prolonging the productivity of trees and groves, have the potential to impact human health.

As noted above, insecticide usage to control ACP has increased significantly in Florida and other citrus producing States since the introduction of HLB (Grafton-Cardwell *et al.*, 2013; USDA-APHIS, 2014a). Insecticides used for ACP control must be registered by EPA under FIFRA for use on citrus. The impacts to human health from the insecticides used for control of ACP were previously analyzed by APHIS (USDA-APHIS, 2010) and will be summarized here. Detailed information on the individual impacts from each herbicide can be found in the Asian Citrus Psyllid Control Program in the Continental United States and Puerto Rico EA (USDA-APHIS, 2010).

Human health impacts related to exposure during pesticide applications to control ACP would likely be restricted to workers and applicators at the time of application. Pesticide application represents the primary exposure route to pesticides for farm workers. The EPA pesticide registration process involves the design of use restrictions that if followed have been determined to be protective of worker health. The EPA's Worker Protection Standard (WPS) (40 CFR Part170) was published in 1992 to require actions to reduce the risk of pesticide poisonings and injuries among agricultural workers and pesticide handlers (US-EPA, 2015a).

The WPS contains requirements on pesticide safety training, notification of pesticide applications, use of personal protective equipment, restricted entry intervals following pesticide application, decontamination supplies, and emergency medical assistance. Under the WPS, the EPA requires the pesticide label to specify personal protective equipment and restricted entry intervals that will provide an appropriate level of protection, based on the properties of the product. Furthermore, the Occupational Safety and Health Administration (OSHA) requires all employers to protect their employees from hazards associated with pesticides.

On November 2, 2015, the EPA announced changes to the agricultural WPS to increase protections from pesticide exposure for agricultural workers and their families[5]. The changes to the WPS requirements, specifically improved training on reducing pesticide residues brought from the treated area to the home on workers and handlers' clothing and bodies and establishing a minimum age for handlers and early entry workers, other than those covered by the immediate family exemption, mitigate the potential for children to be exposed to pesticides directly and indirectly. The EPA expects the revisions to prevent unreasonable adverse effects from exposure to pesticides among agricultural workers and pesticide handlers; vulnerable groups, such as minority and low-income populations, child farmworkers, and farmworker families; and the general public. The revised WPS requirements became effective January 2018.

All pesticides labeled for use on crops in the United States must be evaluated for safety and registered by the EPA. Worker safety precautions and use restrictions are clearly noted on pesticide registration labels. Growers are required to use pesticides consistent with the application instructions provided on the EPA-approved pesticide labels. These restrictions provide instructions as to the appropriate levels of personal protection required for agricultural workers to use pesticides. These may include instructions on personal protective equipment, specific handling requirements, and field reentry procedures. These label restrictions carry the weight of law and are enforced by the EPA and the states (FIFRA 7 U.S.C. 136j (a)(2)(G) Unlawful Acts); therefore, it is expected that pesticide use would be consistent with the EPA-approved labels.

Before a pesticide can be used on a food or feed crop, the EPA, pursuant to the FFDCA, and FQPA, establishes tolerance limits, which is the amount of pesticide residue allowed to remain in or on each treated food commodity (21 U.S. Code § 346a - Tolerances and exemptions for pesticide chemical residues). Pesticide tolerance limits established by the EPA are to ensure the safety of foods and feed for human and animal consumption (US-EPA, 2015c). Applications made in accordance with the label directions, including harvest and reentry intervals, and washing and disinfection of fruit at packinghouses reduces public exposure so that it is below tolerance limits and is unlikely to be harmful to the public (USDA-APHIS, 2010).

Enhanced nutritional programs could add nutrients to the environment beyond what is typical of commercial citrus. As noted above, excess nutrients in water cause algal blooms which impact water quality, harm food resources and habitats, and decrease the oxygen that fish and other aquatic life need to survive. Some algal blooms produce toxins and promote bacterial growth that can cause human health concerns if people come into contact with polluted water, consume

[5] For the changes to the WPS see: http://www.epa.gov/oppfead1/safety/workers/proposed/index.html

tainted fish or shellfish, or drink contaminated water (US-EPA, 2018). Nutrient pollution in ground water, often used as a drinking water source, can be harmful, even at low levels. Infants are vulnerable to a nitrogen-based compound called nitrates in drinking water and can develop symptoms including shortness of breath and blue-tinted skin, a condition known as blue baby syndrome (US-EPA, 2018). Applying fertilizers in the proper amount, at the right time of year and with the right method can significantly reduce the potential for pollution (US-EPA, 2018). Although there is some potential for increased nutrient pollution due to the enhanced nutritional programs, it is unlikely to be significant due to best management practices utilized by growers and regulations including drinking water standards aimed at preventing excess nutrients from ending up in drinking water.

4.2.2 Impacts of HLB on the Citrus Industry

As described above in Sections 3.3.1 and 3.3.2, the citrus industry in Florida represents an important part of the Florida agricultural economy, with Florida citrus valued at $1.03 billion for the 2016/17 growing season (USDA-NASS, 2017c). Since its introduction, HLB has had a devastating impact on Florida's citrus industry (USDA-NIFA, 2016). Orange production in the United States has declined by over 40 percent in the last five years due to HLB (USDA-FAS, 2017b; 2017a). HLB not only reduces yield, fruit size and quality, but also increases tree mortality and the costs of production as growers have to adjust their inputs to try and maintain yields (USDA-APHIS, 2010; Singerman and Useche, 2016). Since the discovery of HLB, total costs and, specifically, spray costs have increased in Florida due to increased costs of dealing with HLB. The cost of foliar sprays increased by almost 260 percent, and fertilizer increased by 134 percent (Singerman and Burani-Arouca, 2017).

It is very difficult to quantify the cost of declines in yield and loss of entire groves that are likely to occur in Florida over the next few years (National Research Council, 2010). The agricultural alternatives for most citrus land in Florida are cattle pasture or pine forests, neither of which is highly profitable, so growers have few options (National Research Council, 2010). Economic losses are directly related to the life span of the citrus trees. The longer the trees live, the greater the return to farmers (USDA-APHIS, 2010). As levels of production and citrus acreage continue to decline the number of juice processors has also declined resulting in fewer citrus industry jobs (National Research Council, 2010).

Under the No Action Alternative, the most likely scenario is that citrus acreage and production in Florida will continue to decline due to HLB. Growers will have to endure the increased costs from HLB management including increased pesticides and enhanced nutritional programs until they are no longer profitable. Options for managing HLB in organic citrus orchards is limited due to restrictions on the use of most pesticides in organic standards. Growers are limited to the use of biological control organisms to control ACP and enhanced nutritional programs to manage the health of their trees (USDA-APHIS, 2010; Schreckengost, 2017). Impacts to the economic environment associated with citrus are likely to remain the same or become more severe as the citrus industry continues to decline due to HLB.

4.3 Preferred Alternative

Under the Preferred Alternative, APHIS would issue an environmental release permit to Southern Gardens in accordance with 7 CFR part 340 to allow the release of CTV-SoD within the state of Florida. If APHIS chooses this alternative, then the permit will be subject to the conditions described in 7 CFR part 340.4[6] and the Permit Conditions described in the permit. Under this alternative, the permit would be valid of a three-year period. The permit will need to be renewed by the applicant and subsequently approved by APHIS to allow any additional release of CTV-SoD beyond the three-year period specified in the permit application.

Southern Gardens has genetically engineered two CTV parental strains (T30 and T36) to express defensin proteins from spinach (CTV-SoD) as an approach to manage HLB in the State of Florida. The spinach defensin proteins expressed by the CTV-SoD, individually or in combination, are designed to target the bacterium *Candidatus* Liberibacter asiaticus, the bacterium that causes HLB.

Under the permit application Southern Gardens is proposing to release CTV-SoD expressing spinach defensin proteins in citrus by inoculating citrus trees with CTV-SoD containing one or more spinach defensin genes. Specifically, Southern Gardens proposes to plant and grow trees of various citrus varieties/species (sweet orange, grapefruit, lemon, etc.) that have been inoculated via grafting with one or more strains of CTV-SoD. The genetic material encoding defensins is not inserted into the citrus chromosome, but transiently expressed for a limited period of time in the inoculated trees by the CTV-SoD vector. Data gathered during previous field trials have shown that foreign sequences have been maintained in a portion of the infected trees for 5 years so far. However, all foreign sequences eventually are deleted such that the vector sequences return to the wild-type virus sequence. A more detailed discussion of the strains used to develop CTV-SoD, the defensins produced, and stability of CTV-SoD can be found in the PRA (USDA-APHIS, 2018).

4.3.1 Environmental Impacts of the Proposed Release of CTV-SoD

The CTV strains (T30 and T36) that were engineered are endemic and widespread throughout the state of Florida (Harper *et al.*, 2015a; Harper *et al.*, 2015c; Harper and Cowell, 2016). Researchers have identified and determined the incidence of major strains of CTV present in Florida citrus groves (Harper *et al.*, 2015a; Bergua *et al.*, 2016; Harper and Cowell, 2016). Nearly 70% and 95% of trees tested (Harper *et al.*, 2015c) were positive for the T36 and T30 strains of CTV, respectively.

The CTV9R[7] strain is poorly transmitted at low rates (0.6%) by aphids and no movement of CTV9R by aphids was detected in previous field trials. Additionally, CTV9R only causes disease symptoms on trees planted using a sour orange rootstock. The CTVT30 strain does not cause disease symptoms on any known commercial citrus cultivars/species and is considered not readily transmissible by aphids. Southern Gardens will also be incorporating a chimeric CTV9R/T30 construct in the plantings under the permit. The CTV9R/T30 construct is not

[6] http://www.gpo.gov/fdsys/granule/CFR-2012-title7-vol5/CFR-2012-title7-vol5-sec340-4/content-detail.html

[7] See Table 1 for the names used in this document for the different genetically engineered CTV strains.

expected to be transmissible by aphids and has reduced disease symptoms on citrus similar to CTVT30. CTV-SoD is expected to remain within the inoculated trees. A more detailed discussion of the strains used and transmissibility can be found in the PRA (USDA-APHIS, 2018).

Spinach defensins are naturally occurring plant defensins derived from spinach. Defensins exist in plants, animals, and insects with varying activity against bacteria and fungi depending on the origin of the defensin (Broekaert *et al.*, 1995; Thomma *et al.*, 2002; Stotz *et al.*, 2009; Gachomo *et al.*, 2012; van der Weerden and Anderson, 2013; Lacerda *et al.*, 2014). Typically, they are small, stable, cysteine-rich peptides involved in a plant's immune system, primarily exhibiting antimicrobial activity against fungi, while some including some spinach defensins are antibacterial (Segura *et al.*, 1998; Stotz *et al.*, 2009).

Under the Southern Gardens permit, CTV-SoD will be grafted into citrus plants and then those trees will be planted in orchards. Once in the phloem of the plant, the CTV-SoD will express the spinach defensin proteins designed to target the *Candidatus* Liberibacter asiaticus bacterium and provide resistance to HLB.

Physical Environment

CTV replicates in the cytoplasm of cells and is present only in the phloem (EFSA, 2014). It can be transmitted by purposely grafting and by some aphid vectors, but cannot exist or multiply in the air, soil, water or other environments that do not contain a living-host plant. Additionally, CTV is not known to be transmitted by seed, pollen, or fruit in any of its hosts (EFSA, 2014; Jeger *et al.*, 2017). As described above and in the PRA (USDA-APHIS, 2018), the CTV-SoD proposed for release under this permit are either not transmissible or have extremely low transmissibility by aphids and so are expected to remain within the citrus plant in which they are inoculated. Therefore, there would be no exposure of CTV-SoD to the physical environment and so there could be no known or conceivable direct effects of CTV-SoD on the physical environment.

In laboratory and confined field studies conducted by Southern Gardens under APHIS permits, CTV-SoD has been shown to control the bacterium responsible for causing HLB. If CTV-SoD were to become an integral part of any IPM strategy to control HLB in the state, it could potentially reduce the need for additional pesticide applications targeting the insect vector, reducing the overall pesticide inputs in groves. Similarly, many growers currently use additional nutritional inputs to try to improve tree health to combat HLB, and those inputs could also potentially be reduced. However, the release of CTV-SoD under this permit would likely not result in the need to change existing grove management techniques in ways that would impact the physical environment because CTV-SoD will be one of the tools that growers could use to manage HLB in addition to those practices they currently use. Growers will continue to need to use pesticides and added nutrients to maintain the health of trees in their groves including those not treated with CTV-SoD.

If CTV-SoD is not an effective HLB management tool growers will continue to use currently available methods and have the same impact on the physical environment as the No Action Alternative.

Biological Environment

The Southern Gardens permit application is for release in the state of Florida of CTV-SoD expressing spinach defensin proteins as an approach to manage HLB. The spinach defensin proteins expressed by the CTV-SoD, individually or in combination, are designed to target the bacterium *Candidatus* Liberibacter asiaticus, the bacterium that causes HLB. Defensins including spinach defensins are present in many plants including food plants (Thomma *et al.*, 2002; Aerts *et al.*, 2008). Defensins provide the plants with a defense response to biotic (including bacterial and fungal infection) and abiotic factors and in plant growth and development (Segura *et al.*, 1998; Aerts *et al.*, 2008). More information describing how defensins function can be found in the PRA (USDA-APHIS, 2018). Previous small-scale confined field trials conducted by Southern Gardens under APHIS permits have demonstrated efficacy of CTV-SoD as a potential HLB management tool.

Since CTV is not known to be transmitted by pollen, seed, or the fruit (Moreno *et al.*, 2008; EFSA, 2014; Jeger *et al.*, 2017), and the CTV-SoD that is the subject of this permit application is either not transmissible or has extremely low transmissibility by aphids, making exposure to other plants or organisms outside of the trees that have been inoculated highly unlikely. Therefore, no impacts to the biological environment are expected. The intended impact of the release is to prevent, treat, or mitigate infection of citrus trees by HLB (Dawson *et al.*, 2015; Kress, 2015). While CTV-SoD is intended to negatively impact the bacteria that causes HLB within citrus trees, the small amounts of spinach defensin peptides found in the treated citrus trees would be unlikely to negatively impact beneficial bacteria and other microorganisms typically found in the citrus grove environment. Spinach defensins are expected to be readily degraded once the associated plant tissues die and are exposed to sun, soil, water, and associated dilution and degradation effects. This could lead to increased longevity of individual trees as well as increased productivity of groves when compared to trees and groves impacted by HLB.

As noted above, the majority of Florida citrus is already infected with wild-type T30 and T36 CTV strains (Harper *et al.*, 2015c) and therefore the virus is already in the environment and food chain. Several different defensins have been found naturally occurring in spinach (Segura *et al.*, 1998) that have activity against various fungal and bacterial plant pathogens. Since these defensins are naturally occurring in spinach, they are already a part of the environment and food chain and have not been noted as having adverse effects associated with consumption. Plant defensins inhibit the growth of a broad range of fungi but seem nontoxic to either mammalian or plant cells (Thomma *et al.*, 2002). Animal communities are unlikely to be affected by either the presence or consumption of CTV-SoD or the spinach defensins that might be found in the phloem of citrus trees containing CTV-SoD.

The CTV-SoD being developed by Southern Gardens is being considered a microbial pesticide by the EPA. To ultimately support a Section 3 registration application for CTV-SoD, Southern Gardens has conducted field trials under Experimental Use Permit (EUP) from the EPA to gather data and evaluate spinach defensin proteins in plant tissues, compositional and nutritional data in raw and processed citrus commodities, assess impacts to non-target organisms, test the efficacy and specificity of the transgenes, evaluate agronomic performance and optimization, produce sample material for environmental risk assessment and compositional studies, and to test the viability of the CTV-SoD. Southern Gardens submitted data to the EPA on spinach defensins for allergenicity and toxicity as part of tolerance exemption and Experimental Use Permit (EUP) application and the data was analyzed by the EPA in a Risk Assessment (US-EPA, 2016b; 2016c). No significant sequence matches were found between either spinach defensin 2 or 7 and any allergens, and the EPA concluded, "there are no potential safety concerns related to allergenicity that would require further testing" (US-EPA, 2015b). Spinach defensins were found to be highly digestible and to have no toxic or adverse effects. The EPA has authorized EUPs under FIFRA for field trials of CTV-SoD containing spinach defensin proteins applied to EPA Crop Group 10 Citrus[8] with the intent of preventing, destroying, repelling, or mitigating any pest responsible for citrus greening disease (80 FR 52270-52271; 81 FR 59499-59503). Pursuant to section 408(d)(1) of FFDCA, the EPA has established a temporary tolerance exemption from the requirement of a tolerance for residues of the CTV expressing spinach defensin proteins 2, 7, and 8 alone or in various combinations on citrus that expires on August 31, 2020 (US-EPA, 2016a).

Both CTV and the spinach defensin proteins are expected to be naturally present in the environment and no adverse effects to non-targets are expected. CTV-SoD has been released under small scale confined field trials since 2010 with nine APHIS permits issued for 3 counties in Florida. As required in the permit conditions for those permits, Southern Gardens was required to report any detectable novel or negative effects. No adverse effects to non-targets have been reported. While CTV-SoD is intended to negatively impact the bacteria that causes HLB within citrus trees, the small amounts of spinach defensin peptides found in the treated citrus trees would be unlikely to negatively impact beneficial bacteria and other microorganisms typically found in the citrus grove environment. Spinach defensins are expected to be readily degraded once the associated plant tissues die and are exposed to sun, soil, water, and associated dilution and degradation effects. Therefore, control of HLB using CTV-SoD should have no negative impacts on other species.

As described in section 3.2.2, superinfection exclusion/cross protection is a phenomenon in which a primary viral infection restricts a secondary infection with the same or closely related virus (Folimonova, 2012; Atallah *et al.*, 2016; Bergua *et al.*, 2016). Trees infected with one strain of wild-type CTV resisted infection from another strain of the same genotype. CTV-SoD is expected to exhibit the same characteristics as wild-type CTV, meaning that superinfection exclusion/cross protection will prevent the spread of CTV-SoD to the majority of commercial trees in Florida that are already infected with the naturally-occurring, endemic CTV strains (Harper *et al.*, 2015a; 2015b). Therefore, CTV-SoD would not increase the plant pest risk that is

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[8]40 CFR § 180.41 Crop Group 10: Calamondin (*Citrus mitis Citrofortunella mitis*), Citrus citron (*Citrus medica*), Citrus hybrids (*Citrus* spp.) (includes chironja, tangelo, tangor), Grapefruit (*Citrus paradisi*), Kumquat (*Fortunella* spp.), Lemon (*Citrus jambhiri, Citrus limon*), Lime (*Citrus aurantiifolia*), Mandarin (tangerine) (*Citrus reticulata*), Orange, sour (*Citrus aurantium*), Orange, sweet (*Citrus sinensis*), Pummelo (*Citrus grandis, Citrus maxima*), Satsuma mandarin (*Citrus unshiu*)

already present due to the ubiquity of CTV in the state of Florida or create a new plant pest risk due to CTV-SoD being released into the environment (USDA-APHIS, 2018).

Southern Gardens has shown that CTV-SoD can control the bacterium responsible for causing HLB, through laboratory and confined field studies conducted under APHIS permits. If CTV-SoD were to become part of an IPM strategy to control HLB in the state, it could potentially reduce the need for additional pesticide applications targeting the insect vector, reducing the overall pesticide inputs in groves. Similarly, additional nutritional inputs, used to try to improve tree health to combat HLB, could also potentially be reduced. The size of these reductions is hard to quantify and dependent on the success of CTV-SoD against HLB. Because CTV-SoD will be just one of the tools that growers could use to manage HLB and growers will continue to need to use pesticides and added nutrients to maintain the health of trees in their groves including those not treated with CTV-SoD, the release of CTV-SoD under this permit would likely not result in the need to change existing grove management techniques in ways that would impact the biological environment.

If CTV-SoD is not an effective HLB management tool growers will continue to use currently available methods and have the same impact on the biological environment as the No Action Alternative.

Human Health

Human exposure to a variety of natural plant viruses and plant viral proteins is common. Plant viruses are not pathogenic to humans and since virtually all of the citrus produced in Florida is infected to some extent with CTV (Harper *et al.*, 2015c), it is likely that CTV is consumed on a regular basis. Zhang *et al.* (2006) have demonstrated that the human gastrointestinal tract contains a wide variety of plant viruses within the intestines. Human consumption of citrus products is common, there are no reports of adverse effects from CTV exposures by any route of administration. The lack of human pathogenicity coupled with the long-standing, safe consumption of citrus products suggest no human health impacts from consumption of CTV-infected citrus.

Several different defensins have been found naturally occurring in spinach (Segura *et al.*, 1998) and are also already a part of the food chain. There is a long history of mammalian consumption of the entire spinach plant (both raw and cooked) as food, without causing any known deleterious human health effects or any evidence of toxicity (US-EPA, 2015b). Spinach plant leaves have long been part of the human diet and there have been no findings that indicate toxicity or allergenicity of spinach proteins (US-EPA, 2015b). As part of its review of the EUP permit for CTV-SoD, the EPA conducted relevant literature and database searches on the potential allergenicity of spinach defensins. No significant sequence matches were found between either spinach defensin 2 or 7 and any allergens, and the EPA concluded, “there are no potential safety concerns related to allergenicity that would require further testing” (US-EPA, 2015b). Spinach defensin 8 was also included in the EUP for temporary tolerance exemption because EPA acknowledged it to be similar to spinach defensin 2 and 7 (US-EPA, 2015b). Southern Gardens plans to submit additional toxicity data to the EPA in support of their Section 3 registration application with information on additional spinach defensins used. Thus, the spinach defensin proteins expressed by CTV-SoD are highly unlikely to produce an allergic response, or be toxic to mammals given the similarity to those already reviewed by the EPA.

Available studies demonstrate that spinach defensin 2 and spinach defensin 7 proteins have very low oral toxicity. In an acute oral toxicity study in mice, conducted with a single dose of 5,000 milligram/kilogram of microbial-produced spinach defensin 2 protein, no evidence of toxic or adverse effects was observed. Due to the high similarity between spinach defensin 2 and spinach defensin 7, the toxicity assessment is applicable to both proteins (US-EPA, 2015b). In an *in vitro* study, microbial-produced defensin 2 and spinach defensin 7 proteins were rapidly and extensively hydrolyzed in simulated gastric and intestinal conditions in the presence of pepsin (at pH 1.2) and pancreatin, respectively. Both microbial-produced spinach defensin proteins demonstrated half-lives of approximately five minutes when subjected to pepsin digest, and both proteins were completely proteolyzed to amino acids and small peptide fragments in less than 1 minute in the presence of 0.15 mg/mL pancreatin. These results indicate that both spinach defensin proteins are highly susceptible to degradation in the human digestive tract (US-EPA, 2015b). Due to no evidence of toxic or adverse effects from spinach defensins and high digestibility of the defensin proteins the potential for adverse health effects from exposure is to spinach defensin proteins expressed by CTV-SoD is unlikely.

Because CTV-SoD is considered a microbial pesticide by the EPA, the EPA evaluates issues relating to the safety to humans during its pesticide registration process. The EPA must determine through specified studies conducted by the applicant that the pesticide does not cause unreasonable adverse effects on humans, the environment, and non-target species, when used in accordance with label instructions. Southern Gardens has been conducting studies to support a Section 3 registration application under Experimental Use Permits from the EPA in its development of CTV-SoD. Pursuant to section 408(d)(1) of FFDCA, the EPA has established a temporary tolerance exemption from the requirement of a tolerance for residues of CTV-SoD expressing spinach defensin proteins 2, 7, and 8 alone or in various combinations on citrus that expires on August 31, 2020 (US-EPA, 2016a). Southern Gardens plans to submit additional toxicity data to the EPA in support of their Section 3 registration application. Consumer health impacts related to the treatment of trees with CTV-SoD or consumption of citrus products from such trees are unlikely to be different from impacts associated with wild type CTV.

Under previous studies conducted by Southern Gardens, CTV-SoD has been shown to control the bacterium responsible for causing HLB. If CTV-SoD were to be used as a strategy to control HLB in the state, it could potentially reduce the need for additional pesticide applications targeting the insect vector, reducing the overall pesticide inputs and exposure to those pesticides by grove workers. Similarly, many growers use additional nutritional inputs to try to improve tree health to combat HLB, and those inputs could also potentially be reduced. The size and timing of these reductions is hard to quantify and dependent on the success of CTV-SoD against HLB. The release of CTV-SoD under this permit would likely not result in the need to change existing grove management techniques in ways that would impact the human exposure to pesticide or nutritional inputs because CTV-SoD will only be one of the tools that growers could use to manage HLB. Growers will continue to need to use pesticides and added nutrients to maintain the health of trees in their groves including those not treated with CTV-SoD.

If CTV-SoD is not an effective HLB management tool growers will continue to use currently available methods and have the same impact on the physical environment as the No Action Alternative.

4.3.2 Impacts of the Release of CTV-SoD on the Citrus Industry

As described previously in this document, HLB is having devastating impacts on the citrus industry in Florida (USDA-NIFA, 2016), reducing orange production by over 40 percent in the last five years (USDA-FAS, 2017b; 2017a). It is expected that citrus acreage and production in Florida will continue to decline due to HLB because there is no cure or effective management for HLB, potentially jeopardizing the existence of the citrus industry in Florida.

Southern Gardens has shown CTV-SoD to be effective in controlling the bacterium responsible for causing HLB, through laboratory and confined field studies conducted under APHIS permits, and believes that CTV-SoD can become an integral part of an IPM strategy to control HLB in the state. If CTV-SoD were to become part of an IPM program, it could potentially help mitigate the reduction in orange production in Florida as a result of decreased tree mortality and increased yield from trees not infected with HLB. The rate at which orange production and the citrus industry in Florida could recover depends on the effectiveness of CTV-SoD against HLB.

Because HLB also increases the costs of production as growers have to adjust their inputs to try and maintain yields (USDA-APHIS, 2010; Singerman and Useche, 2016), growers could see a lower costs of production through reductions in the additional pesticide applications and additional nutritional inputs, if CTV-SoD proved effective at controlling HLB. However, the size and timing of these reductions is hard to quantify and dependent on the success of CTV-SoD against HLB. Because CTV-SoD will only be one of the tools that growers could use to manage HLB, growers will continue to need to use pesticides and added nutrients to maintain the health of trees in their groves including those not inoculated with CTV-SoD. The release of CTV-SoD under this permit would likely be as replacement trees and so reductions in the need for additional pesticides and nutrients would not be immediate. The most likely impact to the citrus industry would be through reduced tree mortality and increased yield on healthy trees and not as a result of changes to grove management techniques.

As noted previously, options for managing HLB in organic citrus orchards is limited due to restrictions on the use of most pesticides in organic systems. Growers are limited to the use of biological control organisms to control ACP and enhanced nutritional programs to manage the health of their trees (USDA-APHIS, 2010; Schreckengost, 2017). Because CTV-SoD is confined to the trees in which it is inoculated, it cannot impact organic orchards. Impacts to the organic citrus industry are likely to remain the same as under the No Action Alternative.

If CTV-SoD does not prove to be an effective HLB management tool, growers will continue to use currently available methods and have the same impact on the citrus industry as the No Action Alternative.

5 CUMULATIVE IMPACTS

A cumulative impact may be an effect on the environment which results from the incremental impact of the proposed action when added to other past, present, and reasonably foreseeable future actions. For example, the potential impacts associated with a determination of nonregulated status for a GE crop in combination with the future production of crop seeds with multiple deregulated traits (i.e., “stacked” traits), including drought tolerance, herbicide resistance, and pest resistance, would be considered a cumulative impact.

5.1 Assumptions Used for Cumulative Impacts Analysis

Cumulative impacts have been analyzed for each environmental issue assessed in Chapter 4, Environmental Consequences. In this EIS, the cumulative impacts analysis is focused on the incremental impacts of the Preferred Alternative taken in consideration with related activities including past, present, and reasonably foreseeable future actions. In this analysis, if there are no direct or indirect impacts identified for a resource area, then APHIS assumes there can be no cumulative impacts. Where it is not possible to quantify impacts, APHIS provides a qualitative assessment of potential cumulative impacts.

Under the Southern Gardens permit, CTV-SoD will be inoculated via grafting into citrus plants. Once in the phloem of the plant, the CTV-SoD will express the spinach defensin proteins designed to provide resistance to HLB. The genetic material encoding spinach defensins is not inserted into the citrus chromosome, but expressed by the CTV-SoD vector in the phloem of the plant. Southern Gardens then plans to plant and grow trees of various citrus varieties/species (sweet orange, grapefruit, lemon, etc.) that have been inoculated with one or more strains of CTV-SoD. These plantings will allow Southern Gardens to continue research and data gathering on CTV-SoD as a HLB management tool.

CTV-SoD will not be aphid transmissible and so will be confined to the individual trees it is grafted into. As a result, exposure to other plants or organisms outside the plantings becomes highly unlikely. Those organisms exposed to CTV-SoD would be exposed to strains of CTV already endemic throughout the state of Florida and naturally occurring plant defensins derived from spinach. Both CTV and spinach defensins are naturally occurring parts of the environment and already part of the food chain.

5.2 Cumulative Impacts: Environmental Impacts of CTV-SoD Release – Physical Environment

As discussed in Section 4.3.1, Environmental Impacts of the Proposed Release of CTV-SoD, approving the permit for release of CTV-SoD under the Preferred Alternative would have no direct impacts to the physical environment because the CTV-SoD are either not transmissible or have extremely low transmissibility by aphids and so are confined to the phloem of the citrus plants in which they are inoculated. Therefore, there will be no exposure of CTV-SoD to the physical environment.

If the use of CTV-SoD proves to be an effective HLB management tool and becomes an integral part of an IPM strategy to control HLB in the state, it could potentially reduce the need for additional pesticide applications targeting the insect vector, reducing the overall pesticide inputs in groves. Similarly, many growers currently use additional nutritional inputs to try to improve tree health to combat HLB, and those inputs could also potentially be reduced. These reductions could have potential beneficial impacts to the physical environment by reducing environmental exposure to pesticides and nutritional inputs, however these will likely be minimal since growers are required to use pesticides according to the label instructions which are designed to ensure that the pesticide does not cause unreasonable adverse effects on the environment.

In the long term, the cumulative impacts of the release of CTV-SoD will depend on the effectiveness of CTV-SoD against HLB. While there is potential for beneficial cumulative impacts to the physical environment, current citrus production practices and EPA regulations on pesticide use make it unlikely for those impacts to be significant.

5.3 Cumulative Impacts: Environmental Impacts of CTV-SoD Release – Biological Resources

As discussed in Section 4.3.1, Environmental Impacts of the Proposed Release of CTV-SoD, approving the permit for release of CTV-SoD under the Preferred Alternative would have no direct impacts to the biological environment because CTV is not known to be transmitted by pollen, seed, or fruit (Moreno *et al.*, 2008; EFSA, 2014; Jeger *et al.*, 2017) and the CTV-SoD that is the subject of this permit application is either not transmissible or has very low transmissibility by aphids, making exposure to other plants or organisms outside of the trees that have been inoculated highly unlikely.

Although exposure is highly unlikely, if organisms were exposed to CTV-SoD they would likely be unaffected because both CTV and spinach defensins are naturally occurring parts of the environment and already part of the food chain, and neither has been noted as having adverse effects associated with consumption. While CTV-SoD is intended to negatively impact the bacteria that causes HLB within citrus trees, the small amounts of spinach defensin peptides found in the treated citrus trees would be unlikely to negatively impact beneficial bacteria and other microorganisms typically found in the citrus grove environment. Spinach defensins are expected to be readily degraded once the associated plant tissues die and are exposed to sun, soil, water, and associated dilution and degradation effects.

Additionally, the majority of Florida citrus is already infected with wild-type T30 and T36 CTV strains (Harper *et al.*, 2015c), and so superinfection exclusion/cross protection (Folimonova, 2012; Atallah *et al.*, 2016; Bergua *et al.*, 2016), will prevent the spread of CTV-SoD to the majority of commercial trees in Florida that are already infected with the naturally-occurring, endemic CTV strains (Harper *et al.*, 2015a; 2015b).

If the use of CTV-SoD proves to be an effective HLB management tool and becomes an integral part of an IPM strategy to control HLB in the state, it could lead to increased longevity of individual trees as well as increased productivity of groves when compared to trees and groves impacted by HLB. Additionally, the use CTV-SoD as part of an IPM strategy, could potentially reduce the need for additional pesticide applications targeting the insect vector, reducing the overall pesticide inputs in groves. Similarly, many growers currently use additional nutritional inputs to try to improve tree health to combat HLB, and those inputs could also potentially be reduced. These reductions could have potential beneficial impacts to the biological environment by reducing exposure of non-target organisms to pesticides and nutritional inputs, however these will likely be minimal since growers are required to use pesticides according to the label instructions which are designed to ensure that the pesticide does not cause unreasonable adverse effects on humans, the environment, and non-target species.

In the long term, the cumulative impacts of the release of CTV-SoD will depend on the effectiveness of CTV-SoD against HLB. While there is potential for beneficial cumulative impacts to the biological environment, current citrus production practices and EPA regulations on pesticide use make significant impacts unlikely.

5.4 Cumulative Impacts: Environmental Impacts of CTV-SoD Release – Human Health

GE CTV is not a food and is being considered a microbial pesticide, so the primary responsibilities for food and feed safety under the FFDCA will be administered by the EPA and the FDA will not be directly involved. As discussed in Section 4.3.1, Environmental Impacts of the Proposed Release of CTV-SoD, plant viruses are not pathogenic to humans and since virtually all of the citrus produced in Florida is infected to some extent with CTV (Harper *et al.*, 2015c), it is likely that CTV is consumed on a regular basis, with no reports of adverse effects from CTV exposures by any route of administration. The lack of human pathogenicity coupled with the long-standing, safe consumption of citrus products suggest no human health impacts from consumption of CTV-infected citrus. Consumer health impacts related to the treatment of trees with CTV-SoD or consumption of citrus products from such trees are unlikely to be different from impacts associated with wild type CTV.

Several different defensins have been found naturally occurring in spinach (Segura *et al.*, 1998) and are also already a part of the food chain. There is a long history of mammalian consumption of spinach plants, without causing any known deleterious health effects or any evidence of toxicity. The EPA did not find any toxicity or allergenicity related to spinach proteins (US-EPA, 2015b). Due to no evidence of toxic or adverse effects from spinach defensins and high digestibility of the defensin proteins the potential for adverse health effects from exposure to spinach defensin proteins expressed by CTV-SoD is unlikely.

In the long term, the cumulative impacts of the release of CTV-SoD will depend on the effectiveness of CTV-SoD against HLB. If the use of CTV-SoD proves to be an effective HLB management tool and becomes an integral part of an IPM strategy to control HLB in the state, it could potentially reduce the need for additional pesticide applications targeting the insect vector, reducing the overall pesticide inputs in groves. Similarly, many growers currently use additional nutritional inputs to try to improve tree health to combat HLB, and those inputs could also potentially be reduced. These reductions could have potential beneficial impacts by reducing human exposure to pesticides and nutritional inputs, however these will likely be minimal since growers are required to use pesticides according to the label directions minimizing harmful exposure and the EPA WPS will continue to provide the same level of protection as is currently available.

5.5 Cumulative Impacts: Impacts of Release of CTV-SoD on the Citrus Industry

As described in Section 4.3.2, Impacts of the Release of CTV-SoD on the Citrus Industry, HLB is having devastating impacts on the citrus industry in Florida (USDA-NIFA, 2016), reducing orange production by over 40 percent in the last five years (USDA-FAS, 2017b; 2017a). Currently there is no effective management or cure for HLB. If the losses from HLB continue it could jeopardize the existence of the citrus industry in Florida.

If the use of CTV-SoD proves to be an effective HLB management tool and becomes an integral part of an IPM strategy to control HLB in the state, it could potentially help mitigate the reduction in orange production in Florida through decreased tree mortality and increased yields on healthy trees. Additionally, because HLB not only reduces yield, fruit size and quality, but also increases the costs of production as growers have to adjust their inputs to try and maintain yields (USDA-APHIS, 2010; Singerman and Useche, 2016), growers could see a lower costs of production through reductions in the additional pesticide applications and additional nutritional inputs. While these impacts are possible, they are unlikely to be significant because growers will continue to need to use pesticides and added nutrients to maintain the health of trees in their groves including those not inoculated with CTV-SoD. Growers would most likely deploy CTV-SoD in replacement trees and so reductions in the need for additional pesticides and nutrients would not be immediate. The most likely impact to the citrus industry would be through reduced tree mortality and increased yield on healthy trees and not as a result of changes to grove management techniques.

5.6 Cumulative Impacts Summary

In summary, release of CTV-SoD is not expected to have any negative cumulative impacts from approving the permit, because it will be restricted to the trees in which it is grafted. The cumulative impacts of the release of CTV-SoD will depend on the effectiveness of CTV-SoD against HLB. Effective control of HLB may result in beneficial impacts for citrus growers, and may result in a long-term method to assist in the management of HLB. CTV-SoD would not increase the plant pest risk that is already present due to the ubiquity of CTV in the state of Florida or create a new plant pest risk due to CTV-SoD being released into the environment (USDA-APHIS, 2018).

6 THREATENED AND ENDANGERED SPECIES

The Endangered Species Act (ESA) of 1973, as amended, is a far-reaching wildlife conservation law. The purpose of the ESA is to prevent extinctions of fish, wildlife, and plant species by conserving endangered and threatened species, and the ecosystems on which they depend. To implement the ESA, the U.S. Fish & Wildlife Service (USFWS) works in cooperation with the National Marine Fisheries Service (NMFS), together "the Services," as well as other Federal, State, and local agencies, Tribes, non-governmental organizations, and private citizens. Before a plant or animal species can receive protection under ESA, it must be added to the Federal list of threatened and endangered wildlife and plants. Threatened and endangered (T&E) species are plants and animals at risk of becoming extinct throughout all or part of their geographic range (endangered species) or species likely to become endangered in the foreseeable future throughout all or a significant portion of their ranges (threatened species).

The Services add a species to the list when they determine it is endangered or threatened because of any of the following factors:

- The present or threatened destruction, modification, or curtailment of its habitat or range;
- Overutilization for commercial, recreational, scientific, or educational purposes;
- Disease or predation;
- The inadequacy of existing regulatory mechanisms; or
- The natural or manmade factors affecting its survival.

Once an animal or plant is added to the list, in accordance with the ESA, protective measures apply to the species and its habitat. These measures include protection from adverse effects of Federal activities.

6.1 Requirements for Federal Agencies

Section 7 (a)(2) of the ESA requires that federal agencies, in consultation with USFWS and/or the NMFS, ensure that any action they authorize, fund, or carry out is "not likely to jeopardize the continued existence of a listed species or result in the destruction or adverse modification of designated critical habitat." It is the responsibility of the federal agency taking the action to assess the effects of their action and to consult with the USFWS and NMFS if it is determined that the action "may affect" listed species or designated critical habitat. This is known as a Section 7 Consultation. The request before APHIS is an application for a permit, and the issuance of a permit is considered an agency action whose effects must be assessed.

The APHIS regulatory authority over GE organisms is limited to those GE organisms for which it has reason to believe might be a plant pest or those for which APHIS does not have sufficient information to determine that the GE organism is unlikely to pose a plant pest risk (7 CFR §340.1). In this case, Southern Gardens is requesting a permit for release from the APHIS for CTV-SoD. As part of its review process, APHIS conducted a Pest Risk Assessment and found that CTV-SoD would not increase the plant pest risk that is already present due to the ubiquity of CTV in the state of Florida or create a new plant pest risk due to the CTV-SoD being released into the environment (USDA-APHIS, 2018). As part of its EIS analysis, APHIS analyzed the potential effects of CTV-SoD on the environment, including any potential effects to threatened and endangered (T&E) species and critical habitat. As part of this process, APHIS thoroughly reviews GE product information and data related to the GE organism to inform the ESA effects analysis and, if necessary, the biological assessment. APHIS considered the following potential impacts in its effects analysis:

- the potential for spread of the CTV-SoD beyond the trees in which it is grafted
- the potential for toxicity and allergenicity of CTV-SoD and the spinach defensins it produces
- the potential for CTV-SoD to infect listed plants
- Any other information that may inform the potential for CTV-SoD to impact T&E species

In following this review process, APHIS, as described below, has evaluated the potential effects that a determination to issue the permit for release of CTV-SoD may have, if any, on federally-listed T&E species and species proposed for listing, as well as designated critical habitat and habitat proposed for designation.

6.2 Potential Effects of CTV-SoD on T&E Species

As discussed in Chapter 3, Affected Environment, the permit application from Southern Gardens describes the release sites as totaling 513,500 acres within the 67 counties in Florida. Because CTV-SoD are confined to the phloem of the citrus plant in which they are inoculated, movement of CTV-SoD from the release site is highly unlikely. Therefore, the action area for this EIS is limited to the state of Florida. APHIS assessed potential impacts to species and habitat within the state of Florida. APHIS obtained and reviewed the USFWS list of T&E species (listed and proposed) for the state of Florida from the USFWS Environmental Conservation Online System (USFWS, 2018). The search found 67 animal species and 68 plant species.

Based on the information submitted by the applicant and reviewed by APHIS, CTV-SoD is derived from CTV strains (T30 and T36) that are endemic throughout the state of Florida and are widespread there. Researchers have identified and determined the incidence of major strains of CTV present in Florida citrus groves and determined that nearly 70% and 95% of trees tested were positive for the T36 and T30 strains of CTV, respectively (Harper *et al.*, 2015a; Harper *et al.*, 2015c; Bergua *et al.*, 2016; Harper and Cowell, 2016). Since most citrus in Florida is infected with naturally occurring, wild-type T30 and T36 strains of CTV the virus is already in the environment and food chain.

CTV-SoD has been engineered to express defensin proteins from spinach as an approach to manage HLB in the State of Florida. Spinach defensins are naturally occurring plant defensins derived from spinach. Defensins exist in plants, animals, and insects with varying activity against bacteria and/or fungi depending on the origin of the defensin (Broekaert *et al.*, 1995; Thomma *et al.*, 2002; Stotz *et al.*, 2009; Gachomo *et al.*, 2012; van der Weerden and Anderson, 2013; Lacerda *et al.*, 2014). Since these defensins are naturally occurring in spinach, they are already a part of the environment and food chain and have not been noted as having adverse effects associated with consumption.

Under the Southern Gardens permit, CTV-SoD will be inoculated via grafting into citrus plants. Southern Gardens proposes to plant and grow trees of various citrus varieties/species (sweet orange, grapefruit, lemon, etc.) that have been inoculated via grafting with one or more strains of CTV-SoD. CTV is not known to be transmitted by seed, pollen, or fruit in any of its hosts (Moreno *et al.*, 2008; EFSA, 2014; Jeger *et al.*, 2017), and the CTV-SoD proposed for release under this permit are either not transmissible or have extremely low transmissibility by aphids. As a result, CTV-SoD subject to this permit are confined to the phloem of the citrus plant in which they are inoculated, making exposure to other plants or organisms, including T&E species, highly unlikely.

For its analysis on T&E plants and critical habitat, APHIS focused on the differences between CTV-SoD and CTV strains currently in Florida; the potential for CTV-SoD to move beyond the trees in which it is grafted; and the potential for CTV-SoD to infect any listed plants. For its analysis of effects on T&E animals, APHIS focused on the implications of exposure to CTV-SoD and the spinach defensin proteins it expresses.

6.2.1 Threatened and Endangered Plant Species and Critical Habitat

CTV is a phloem-limited virus (Dawson *et al.*, 2013) whose natural hosts include members of the *Rutaceae*, with the exception of few non-Rutaceous *Passiflora* species, and include species in the genus *Citrus* and *Fortunella*. Virus infection has also been detected in experimentally inoculated relatives of citrus of the genera *Aegle*, *Aeglopsis*, *Afraegle*, *Atalantia*, *Citropsis*, *Clausena*, *Eremocitrus*, *Hesperthusa*, *Merrillia*, *Microcitrus*, *Pamburus*, *Pleiospermium*, *Severinia*, and *Swinglea* (Moreno *et al.*, 2008; Harper, 2013). No genera that are susceptible to CTV were found in the USFWS list of T&E species in the state of Florida (USFWS, 2018).

Southern Gardens is proposing to release CTV-SoD expressing spinach defensins in citrus by inoculating citrus trees with CTV-SoD containing one or more spinach defensin genes and then proposes to plant and grow trees of various citrus varieties/species (sweet orange, grapefruit, lemon, etc.) that have been inoculated with one or more strains of CTV-SoD. Since CTV is not known to be transmitted by pollen, seed, or fruit (Moreno *et al.*, 2008; EFSA, 2014), and the CTV-SoD that is the subject of this permit application are either not transmissible or have extremely low transmissibility by aphids, exposure to other plants or organisms outside of the trees that have been inoculated is highly unlikely and no impacts to other species are expected.

Both CTV and the spinach defensin proteins are expected to be naturally present in the environment and no adverse effects to non-targets are expected. CTV-SoD has been released under small scale confined field trials since 2010 with nine APHIS permits issued for 3 counties in Florida. As required in the permit conditions for those permits, Southern Gardens was required to report any detectable novel or negative effects. No adverse effects to non-targets have been reported. Control of HLB by CTV-SoD is not expected to have negative impacts on other species.

After reviewing the list of threatened and endangered plant species and finding no genera that are susceptible to CTV were found on the USFWS list of T&E species in the state of Florida; that there has been no history of any impacts to non-target organisms from previous small scale confined field trials; and that CTV-SoD is confined to the trees it is inoculated in and therefore exposure of T&E species to CTV-SoD is highly unlikely, APHIS has determined that CTV-SoD will have no effect on threatened or endangered plant species or on critical habitat.

6.2.2 Threatened and Endangered Animal Species

APHIS considered the risks to threatened and endangered animals from exposure to CTV-SoD and the spinach defensin proteins it expresses. Threatened and endangered animal species that may be exposed to the gene products from CTV-SoD would be those T&E species that inhabit orchards where commercial citrus is grown and/or may feed on citrus.

As described in Section 4.3.1, CTV is not known to be transmitted by pollen, seed, or fruit (Moreno *et al.*, 2008; EFSA, 2014; Jeger *et al.*, 2017) and the CTV-SoD that is the subject of this permit application are either not transmissible or have extremely low transmissibility by aphids. Once the CTV-SoD is grafted onto a tree, there is no potential for spread through any mechanism other than deliberate grafting. Exposure to other organisms outside of the trees that have been inoculated is highly unlikely.

As noted in Section 4.3.1, the majority of Florida citrus is already infected with wild-type T30 and T36 CTV strains (Harper *et al.*, 2015c) and therefore the virus is already in the environment and food chain and it is likely that CTV is consumed on a regular basis. There are no reports of adverse effects from CTV exposures. Impacts related to the treatment of trees with CTV-SoD or consumption of citrus products from such trees are unlikely to be different from impacts associated with wild type CTV.

Several different defensins have been found naturally occurring in spinach (Segura *et al.*, 1998) that have activity against various fungal and bacterial plant pathogens. Since these defensins are naturally occurring in spinach, they are already a part of the environment and food chain. There is a long history of mammalian consumption of the entire spinach plant, without causing any known deleterious effects or any evidence of toxicity (US-EPA, 2015b). Animal communities are unlikely to be affected by either the presence or consumption of CTV-SoD or the spinach defensins that might be found in the phloem of citrus trees inoculated with CTV-SoD.

Because CTV-SoD is considered a microbial pesticide by the EPA, the EPA, during the pesticide registration review process, must determine through specified studies conducted by the applicant that the pesticide does not cause unreasonable adverse effects on humans, the environment, and non-target species, when used in accordance with label instructions. Southern Gardens has been conducting studies in support of a Section 3 registration application under Experimental Use Permits from the EPA in its development of CTV-SoD. Pursuant to section 408(d)(1) of FFDCA, the EPA has established a temporary tolerance exemption from the requirement of a tolerance for residues of CTV-SoD expressing spinach defensin proteins 2, 7, and 8 alone or in various combinations on citrus that expires on August 31, 2020 (US-EPA, 2016a). As part of its review of the Experimental Use Permit for CTV-SoD, the EPA conducted relevant literature and database searches on the potential allergenicity of spinach defensins. No significant sequence matches were found between either spinach defensin 2 or 7 and any allergens, and the EPA concluded, “there are no potential safety concerns related to allergenicity that would require further testing" (US-EPA, 2015b). Thus, the spinach defensin proteins expressed by CTV-SoD are highly unlikely to produce an allergic response, or be toxic to mammals.

APHIS considered the possibility that a citrus tree inoculated with CTV-SoD could serve as a host plant. APHIS reviewed the complete T&E species database available on the FWS website (USFWS, 2018) and found no listed or proposed species that depend on citrus plants as a host plant necessary to complete its lifecycle.

After reviewing information on CTV and spinach defensins and finding no reports of adverse effects from exposures to either; that there has been no history of any impacts to non-target organisms from previous small scale confined field trials; that CTV-SoD is confined to the trees it is inoculated in and therefore exposure of T&E species to CTV-SoD is highly unlikely; and that no listed or proposed species would use an inoculated citrus tree as a host plant, APHIS has determined that CTV-SoD will have no effect on threatened or endangered animals.

6.3 Summary of Effects and Determination

After reviewing the possible effects of issuing a permit for release of CTV-SoD, APHIS has not identified any stressor that could affect the reproduction, numbers, or distribution of a listed T&E species or species proposed for listing. Because the CTV-SoD will be confined to the trees in which it is inoculated into, there is no reasonable expectation that T&E species will be exposed. Further, no listed or proposed species would use an inoculated citrus tree as a host plant. APHIS also considered the potential effect of issuing a permit for release of CTV-SoD on designated critical habitat or habitat proposed for designation, and again found that because the CTV-SoD will be confined to the trees in which it is inoculated and will be used in existing citrus groves, there can be no exposure to critical habitat. Consumption of CTV-SoD by any listed species or species proposed for listing will not result in a toxic or allergic reaction (US-EPA, 2015b).

Based on these factors, APHIS has concluded that issuing a permit for release of CTV-SoD, will have no effect on listed species or species proposed for listing, and would not affect designated habitat or habitat proposed for designation. Because of this no-effect determination, consultation under Section 7(a)(2) of the Act or the concurrences of the USFWS or NMFS is not required.

7 CONSIDERATION OF EXECUTIVE ORDERS, STANDARDS, AND TREATIES RELATING TO ENVIRONMENTAL IMPACTS

7.1 Executive Orders Related to Domestic Issues

The following executive orders require consideration of the potential impacts of the Federal action to various segments of the population.

> ***Executive Order (EO) 12898 (US-NARA, 2010), "Federal Actions to Address Environmental Justice in Minority Populations and Low-Income Populations,"*** requires Federal agencies to conduct their programs, policies, and activities that substantially affect human health or the environment in a manner so as not to exclude persons and populations from participation in or benefiting from such programs. It also enforces existing statutes to prevent minority and low-income communities from being subjected to disproportionately high and adverse human health or environmental effects.
>
> ***EO 13045 (US-NARA, 2010), "Protection of Children from Environmental Health Risks and Safety Risks,"*** acknowledges that children may suffer disproportionately from environmental health and safety risks because of their developmental stage, greater metabolic activity levels, and behavior patterns, as compared to adults. The EO (to the extent permitted by law and consistent with the agency's mission) requires each Federal agency to identify, assess, and address environmental health risks and safety risks that may disproportionately affect children.

The No Action and Preferred Alternatives were analyzed with respect to EO 12898 and EO 13045. The environmental and human health impacts are presented in Chapter 4 of this EIS. Neither alternative is expected to have a disproportionate adverse impact on minorities, low-income populations, or children.

As discussed in Section 4.3.1, Environmental Impacts of the Proposed Release of CTV-SoD, human exposure to a variety of natural plant viruses and plant viral proteins is common. Plant viruses are not pathogenic to humans and since virtually all of the citrus produced in Florida is infected to some extent with CTV (Harper *et al.*, 2015c), it is likely that CTV is consumed on a regular basis, and there are no reports of adverse effects from CTV exposures by any route of administration. The lack of human pathogenicity coupled with the long-standing, safe consumption of citrus products suggest no human health impacts from consumption of CTV-infected citrus. Consumer health impacts related to the treatment of trees with CTV-SoD or consumption of citrus products from such trees are unlikely to be different from impacts associated with wild type CTV.

CTV-SoD is not a food and is being considered a microbial pesticide, so the primary responsibilities for food and feed safety under the FFDCA will be administered by the EPA and the FDA will not be directly involved. The EPA must determine through specified studies conducted by the applicant that the pesticide does not cause unreasonable adverse effects on humans, the environment, and non-target species, when used in accordance with label instructions. As part of its review of the EUP permit for CTV-SoD, the EPA conducted relevant literature and database searches on the potential allergenicity of spinach defensins. Several different defensins have been found naturally occurring in spinach (Segura *et al.*, 1998) and are also already a part of the food chain. There is a long history of mammalian consumption of the entire spinach plant (both raw and cooked) as food, without causing any known deleterious human health effects or any evidence of toxicity. Spinach plant leaves have long been part of the human diet and there have been no findings that indicate toxicity or allergenicity of spinach proteins (US-EPA, 2015b). The EPA found no significant sequence matches between either spinach defensin 2 or 7 and any allergens, and the EPA concluded, "there are no potential safety concerns related to allergenicity that would require further testing" (US-EPA, 2015b). Thus, the spinach defensin proteins expressed by CTV-SoD are highly unlikely to produce an allergic response, or be toxic to mammals.

Based on these factors, a determination to issue the permit for CTV-SoD is not expected to have a disproportionate adverse impact on minorities, low-income populations, or children.

The following executive order requires consideration of the potential impacts of the Federal action on tribal lands.

> ***EO 13175 (US-NARA, 2010)***, ***"Consultation and Coordination with Indian Tribal Governments"***, pledges agency communication and collaboration with tribal officials when proposed Federal actions have potential tribal implications.

Consistent with EO 13175, APHIS sent a letter of notification and request for comment and consultation on the proposed action to tribes in Florida and Alabama on April 10, 2017. This letter contained information regarding the Southern Gardens permit request and CTV-SoD. Additionally, this same notification also asked tribal leaders to contact APHIS if they believed that there were potentially significant impacts to tribal lands or resources that should be considered. APHIS will continue to consult and collaborate with tribal officials to ensure that they are well-informed and represented in policy and program decisions that may impact their agricultural interests, in accordance with EO 13175.

Issuing the permit for CTV-SoD will not adversely impact cultural resources on tribal properties.

The No Action and Preferred Alternatives were analyzed with respect to EO 12898, EO 13045, and EO 13175. Neither alternative is expected to have a disproportionate adverse impact on minorities, low-income populations, or children. Nor is any alternative expected to have potential Tribal implications.

The following executive order addresses Federal responsibilities regarding the introduction and effects of invasive species:

> ***EO 1311 (US-NARA, 2010), "Invasive Species,"*** states that Federal agencies take action to prevent the introduction of invasive species, to provide for their control, and to minimize the economic, ecological, and human health impacts that invasive species cause.

CTV is not listed as an invasive species. As discussed in Section 4.3.1, Environmental Impacts of the Proposed Release of CTV-SoD, CTV is not known to be transmitted by pollen, seed, or fruit (Moreno *et al.*, 2008; EFSA, 2014; Jeger *et al.*, 2017) and the CTV-SoD that is the subject of this permit application are either not transmissible or have extremely low transmissibility by aphids and so are expected to remain in the phloem of the citrus plants in which they are inoculated. CTV-SoD would not increase the plant pest risk that is already present due to the ubiquity of CTV in the state of Florida or create a new plant pest risk due to CTV-SoD being released into the environment (USDA-APHIS, 2018).

Because of several citrus diseases, the entire state of Florida is under quarantine (USDA-APHIS, 2017). These quarantine regulations mean that citrus plants or plant parts may not enter or leave Florida without proper Federal and State of Florida Permits. Therefore, it would be highly unlikely for CTV-SoD to move out of the state of Florida.

The following executive order requires the protection of migratory bird populations:

> ***EO 13186 (US-NARA, 2010), "Responsibilities of Federal Agencies to Protect Migratory Birds,"*** states that federal agencies taking actions that have, or are likely to have, a measurable negative effect on migratory bird populations are directed to develop and implement, within two years, a Memorandum of Understanding (MOU) with the Fish and Wildlife Service that shall promote the conservation of migratory bird populations.

Migratory birds may be found in citrus groves where this CTV-SoD will be deployed. As noted in Section 4.3.1, Environmental Impacts of the Proposed Release of CTV-SoD, organisms that may be exposed to CTV-SoD would likely be unaffected because both CTV and spinach defensins are naturally occurring parts of the environment and already part of the food chain, and neither has been noted as having adverse effects associated with consumption. Animal communities are unlikely to be affected by either the presence or consumption of CTV-SoD or the spinach defensins that might be found in the phloem of citrus trees inoculated with CTV-SoD. Based on APHIS' assessment of CTV-SoD, it is unlikely that issuing a permit for release of CTV-SoD would have a negative impact on migratory bird populations.

7.2 Compliance with Clean Water Act and Clean Air Act

As discussed in Section 4.3.1, Environmental Impacts of the Proposed Release of CTV-SoD, approving the permit for release of CTV-SoD under the Preferred Alternative would have no direct impacts to the physical environment because CTV is not known to be transmitted by pollen, seed, or fruit (Moreno *et al.*, 2008; EFSA, 2014; Jeger *et al.*, 2017) and the CTV-SoD that are the subject of this permit application are either not transmissible or have extremely low transmissibility by aphids, making exposure to the environment outside of the trees that have been inoculated highly unlikely. Therefore, no significant negative impacts to water resources or air quality associated with CTV-SoD are expected. Based on these analyses, APHIS concludes that issuing a permit for release of CTV-SoD would comply with the CWA and the CAA.

7.3 Impacts on Unique Characteristics of Geographic Areas

Issuing the permit for CTV-SoD is not expected to impact unique characteristics of geographic areas such as park lands, prime farmlands, wetlands, wild and scenic areas, or ecologically critical areas.

As discussed in Section 4.3.1, Environmental Impacts of the Proposed Release of CTV-SoD, no different agronomic activities within the action area are anticipated as a result of the Preferred Alternative. If the permit is issued, the field release will occur on land already under agricultural management, and is not expected to alter land use patterns within the action area.

There are no proposed major ground disturbances; no new physical destruction or damage to property; no alterations of property, wildlife habitat, or landscapes; and no prescribed sale, lease, or transfer of ownership of any property. This action is limited to issuing a permit for release of CE CTV in the state of Florida. This action would not convert land use to nonagricultural use and, therefore, would have no adverse impact on prime farmland. Standard agricultural practices for land preparation, planting, irrigation, and harvesting of fruit would be used on agricultural lands where CTV-SoD would be deployed under the Preferred Alternative, including the use of EPA-registered pesticides. Standard Operating Procedures (SOPs) submitted as part of the permit application restrict plantings of CTV-SoD containing trees to commercial plantings in Florida.

Based on these findings, including the assumption that label use restrictions are in place to protect unique geographic areas and that those label use restrictions are adhered to, issuing a permit for release of CTV-SoD is not expected to impact unique characteristics of geographic areas such as park lands, prime farm lands, wetlands, wild and scenic areas, or ecologically critical areas.

7.4 National Historic Preservation Act of 1966 as Amended

The National Historic Preservation Act (NHPA) of 1966 and its implementing regulations (36 CFR 800) require Federal agencies to: 1) determine whether activities they propose constitute "undertakings" that have the potential to cause effects on historic properties and 2) if so, to evaluate the effects of such undertakings on such historic resources and consult with the Advisory Council on Historic Preservation (i.e., State Historic Preservation Office, Tribal Historic Preservation Officers), as appropriate.

APHIS' proposed action, issuing a permit for release of CTV-SoD, is not expected to adversely impact cultural resources on tribal properties. Any farming activity that may be taken by farmers on tribal lands would only be conducted at the tribe's request; thus, the tribes would have control over any potential conflict with cultural resources on tribal properties.

APHIS' Preferred Alternative would have no impact on districts, sites, highways, structures, or objects listed in or eligible for listing in the National Register of Historic Places, nor would it likely cause any loss or destruction of significant scientific, cultural, or historical resources. This action is limited to issuing a permit for release of CTV-SoD.

APHIS' proposed action is not an undertaking that may directly or indirectly cause alteration in the character or use of historic properties protected under the NHPA. In general, common agricultural activities conducted under this action do not have the potential to introduce visual, atmospheric, or noise elements to areas in which they are used that could result in effects on the character or use of historic properties. For example, there is potential for increased noise on the use and enjoyment of a historic property during the operation of tractors and other mechanical equipment close to such sites. A built-in mitigating factor for this issue is that virtually all of the methods involved would only have temporary effects on the audible nature of a site and can be ended at any time to restore the audible qualities of such sites to their original condition with no further adverse effects. Additionally, these cultivation practices are already being conducted throughout the citrus production area in Florida. The release of CTV-SoD is not expected to change any agronomic practices that would result in an adverse impact under the NHPA.

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No additional parties requested physical copies of the EIS. In addition to this distribution list APHIS notified all of its stakeholders of the availability of the EIS for review and comment.

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11 INDEX