Southern Gardens Citrus Nursery, LLC Permit 17-044-101r to Release Genetically Engineered *Citrus tristeza virus*

Preliminary Pest Risk Assessment

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A. Introduction

Purpose

Southern Garden Citrus Nursery, LLC (Southern Gardens) submitted a permit application (17-044-101r) to the Animal and Plant Health Inspection Service (APHIS) of the United States Department Agriculture under APHIS' 7 Code of Federal Regulations (CFR) part 340 for confined release various genetically engineered *Citrus tristeza virus* (CTV) clones producing spinach defensins (CTV-SoD)[1] as an approach to control Huanglongbing (HLB, citrus greening) disease. CTV clones derived from CTV isolates of strains T36 and T30 were engineered to produce one or a combination of defensin proteins (SoD2, SoD7, SoD8, SoD9, SoD11, SoD12 and/or SoD13) that are naturally present in spinach to control damage in citrus trees caused by the bacterium (*Candidatus* Liberibacter asiaticus, *C*Las). The purpose of this document is to assess the plant pest risk associated with the actions provided in the permit. As described more fully in the draft Environmental Impact Statement, HLB has spread rapidly since first discovered in Florida in 2005, resulting in a drastic reduction in citrus yield and greatly increasing the cost of citrus production (Halbert and Manjunath 2004; Bove 2006; National Academy of Sciences 2010; Hodges and Spreen 2012; Baldwin et al. 2014; Fagen et al. 2014; Singerman and Burani-Arouca 2017).

In the permit application 17-044-101r Southern Gardens proposes to graft inoculate citrus plant sections containing CTV-SoD onto citrus trees, including trees currently planted in Florida citrus groves and trees before planting in the field. This method of applying SoD allows the spinach defensin to be produced without the genetic material encoding defensins being inserted into the citrus tree chromosome. Southern Gardens has previously carried out small-scale field trials of CTV-SoD contained within citrus trees under APHIS permits that demonstrated efficacy against HLB. This led Southern Gardens to consider employing CTV-SoD as a HLB disease control strategy beyond small-scale research field trials for more extensive use. The large scale permit application is an extension of these previous field trials where CTV-SoD will continue to be regulated under APHIS permits. These actions will be carried out under APHIS mandated controls and continued oversight during use.

Plant viruses are best known as parasites that may damage plants and reduce plant yield. In some situations plant viruses may actually reduce plant damage caused by other viruses, such as when plants infected with a mild strain of a virus become immune to much more damaging strains of the same virus (cross protection). This and other applications of organisms to manage plant diseases and pests are referred to as biological control. The most common uses of microbes such as viruses, bacteria and fungi for biological control of agriculturally important pests are for control of insects. Of these the most widely known is the spray application of the bacterium *Bacillus thuringiensis* expressing Bt-toxins to plant surfaces to control insect pests (Wozniak et al. 2013; Organic Materials Review Institute 2017). Microbial biopesticides also include insecticidal viruses such as *Cydia pomonella* granulovirus used in control of the codling moth larvae in apple, pear, plum, prune and walnut trees (EPA 2011; Organic Materials Review Institute 2017). Other microbial

[1] For definitions of the specific CTV terms used in this document, see Table 1, p.11.

pesticides have been developed in the U.S. that were genetically engineered, such as the bacteria *B. thuringiensis* and *Agrobacterium radiobacter*, and the plant pathogenic fungus *Cryphonectria parasitica* (Wozniak et al. 2013). CTV-SoD is being developed as a genetically engineered microbial pesticide using the virus as a vehicle to deliver the spinach defensin within citrus tissues as a method to control the plant disease caused by the *C*Las bacterium.

As proposed in the 17-044-101r permit application, citrus trees will be treated with CTV-SoD by a graft inoculation technique. The plant virus CTV survives and multiplies in citrus trees where it is highly concentrated in the vascular tissue. The proposed inoculation of citrus trees is carried out by grafting a stem, leaf or bark piece from a citrus tree already infected with CTV-SoD onto a citrus tree where the vascular tissue has been exposed. This method directly introduces the CTV-SoD into the inner vascular tissues of the recipient tree, which is the location where *C*Las bacteria reside. This risk assessment considers the first genetically engineered plant virus for controlling plant disease to be used in the U.S. beyond limited small scale field trials.

Regulatory Authority

APHIS administers 7 CFR part 340 under the authority of the plant pest provisions of the Plant Protection Act (PPA) of 2000 (7 U.S.C. 7701 *et seq*.)[2]. This Preliminary Pest Risk Assessment (PRA) was conducted to assess the plant pest risk associated with the actions provided in the permit. APHIS regulations in 7 CFR part 340 regulate the introduction (importation, interstate movement, or release into the environment) of certain genetically engineered (GE) organisms and products. A GE organism is considered a regulated article under part 340 if the donor organism, recipient organism, or vector, or vector agent used in engineering the organism belongs to any genera or taxa designated in 7 CFR 340.2 and meets the definition of plant pest, or is an unclassified organism and/or an organism whose classification is unknown, or any product which contains such an organism, or any other organism or product altered or produced through genetic engineering which the Administrator determines is a plant pest or has reason to believe is a plant pest[3]. *Citrus tristeza virus* (CTV) is a plant pest as described in 7 CFR 340.1 and 7 CFR 340.2 . Therefore, genetically engineered CTV is considered a regulated article under APHIS regulations at 7 CFR part 340.

The regulations in § 340.4(a) provide that a person may submit an application for a permit for the introduction of a regulated article to the Animal and Plant Health Inspection Service (APHIS). Paragraph (b) of § 340.4 describes the information that must be included in the permit application. In addition, paragraph (b) states that applications must be submitted at least 120 days in advance of the proposed release into the environment in order to allow for APHIS review. However, the 120-day review period would be extended if preparation of an environmental impact statement is necessary.

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[2] Plant Protection Act in 7 U.S.C. 7702 § 403(14) defines plant pest as: "Plant Pest - The term "plant pest" means any living stage of any of the following that can directly or indirectly injure, cause damage to, or cause disease in any plant or plant product: (A) A protozoan. (B) A nonhuman animal. (C) A parasitic plant. (D) A bacterium. (E) A fungus. (F) A virus or viroid. (G) An infectious agent or other pathogen. (H) Any article similar to or allied with any of the articles specified in the preceding subparagraphs."

[3] Limited exclusions or exemptions apply for certain engineered microorganisms and for interstate movement of some organisms, as in 7 CFR 340.1 and 340.2.(b).

On February 13, 2017, APHIS received a permit application from Southern Gardens (APHIS Permit Number 17-044-101r) for widespread environmental release for the duration of the three year permit in Florida of CTV genetically engineered to express defensin proteins from spinach (CTV-SoD). The CTV-SoDs express antimicrobial proteins (SoD2, SoD7, SoD8, SoD9, SoD11, SoD12 and/or SoD13) and are designed to target *Candidatus* Liberibacter asiaticus (*C*Las), which is an uncultureable bacterium associated with HLB currently causing major economic damage to citrus production in Florida. The action proposed in the permit under consideration is the use of CTV-SoD on up to 513,500 acres in 67 counties in Florida to manage HLB disease in Florida. Potential impacts examined in this Preliminary PRA are those that pertain to plant pest risks associated with using CTV-SoD as a biological control organism to help manage the HLB disease.

Confined environmental releases[4] of GE CTV have been carried under APHIS permitted authorizations since 2010 (permits 08-330-101r-a2, 09-121-103r, 11-357-101r, 12-109-102r, 13-039-101r-a2, 14-320-101r, 16-036-101r, 16-067-104r-a1 and 16-308-101r-a1). As required under § 340.4 (b) the permit applications provided information including the developer, genetics of the inserted material, biological properties, amount and location of release, and control measures to prevent dissemination and escape from the confines of the field trials. APHIS reviewed the submitted information and evaluated the proposed confinement measures for maintaining the regulated GE CTV material at the field site, carried out an assessment on potential effects to Threatened and Endangered Species (TES), and determined that the permits were categorically excluded action under section 7 CFR 372.5(c)(ii) because they were considered a confined field trial. Accordingly, permits were issued containing permit conditions for continued APHIS oversight for the duration of the field trials. As described below, because APHIS and EPA share authority over these field trials, APHIS and EPA coordinated oversight regarding confinement for these field trials under EPA Notifications, EPA Experimental Use Permits and APHIS part 340 permits. In addition to evaluation for safe use under part 340, APHIS inspectors visited the field trial locations to ensure compliance with part 340 regulations and the permit conditions. According to part 340 and permit conditions the developer submitted reports on monitoring for spread of the engineered virus to ensure the confinement measures were adequate. As with earlier small scale permits the action proposed under this permit application 17-044-101r will also be carried out under APHIS oversight. This Preliminary PRA and an accompanying Draft EIS will assess plant pest risks that may arise due to the extended nature of the proposed release where the GE CTV is expected to be restricted to the site of the release, but where the release authorized is for up to 513,500 acres in the 67 counties in the State of Florida. As discussed in this Preliminary PRA, the CTV-SoD is expected to remain within the inoculated trees. Regulatory oversight commensurate with plant pest risk will provide for adjusted permit conditions to allow for large scale use (see attached permit conditions). Any potential effects on TES will be addressed in the Draft EIS.

Consistent with the National Environmental Policy Act (NEPA) 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 a Draft Environmental Impact Statement (EIS) to consider the

[4] The terms confined environmental release, field trial or field test in this document are taken to mean restricting the material to the field site by the use of measures such as isolation distance, border rows, genetic alterations in the material being field released that affect confinement, etc.

potential environmental impacts that may result if the permit application is approved. APHIS announced the notice of intent (NOI) to prepare an EIS (82 FR 17179 2017). APHIS received 94 comments and will consider the information provided in the comments in this Preliminary PRA and the Draft EIS to inform decision making relative to the 17-044-101r permit application.

APHIS may also consider information relevant to reviews conducted by other agencies that are part of the Coordinated Framework for the Regulation of Biotechnology (51 FR 23302 1986; 57 FR 22984 1992). Under the Coordinated Framework, the oversight of biotechnology-derived products are subject to regulatory authority administered by APHIS, Food and Drug Administration (FDA), and Environmental Protection Agency (EPA). Depending on its characteristics, certain biotechnology-derived products are subject to review by one or more of these agencies. EPA regulates under the Federal Insecticide, Fungicide and Rodenticide Act (FIFRA) (7 U.S.C. 136 *et seq.*) the distribution, sale, use and testing of pesticidal substances produced in plants and microbes, including those pesticides that are produced by an organism through techniques of modern biotechnology. EPA also sets tolerance limits for residues of pesticides on and in food and animal feed, or establishes an exemption or a time-limited temporary exemption from the requirement for a tolerance, under the Federal Food, Drug and Cosmetic Act (FFDCA) (21 U.S.C. Chapter 9). Prior to registering a new pesticide EPA must determine through testing that the pesticide does not cause unreasonable adverse effects on humans, the environment, and non-target species when used in accordance with label instructions. EPA also approves the language used on the pesticide label according to 40 CFR part 158. Other applicable EPA regulations include 40 CFR part 152 Pesticide Registration and Classification Procedures part 172 - Experimental Use Permits to gather field data for registration.

EPA has authorized Biotechnology Notifications and Experimental Use Permits (EUPs) under FIFRA for field trials of CTV-SoD containing the genetic material to produce defensin proteins grafted to certain citrus[5] with the intent of preventing, destroying, repelling, or mitigating any pest responsible for HLB disease (80 FR 52270-52271; 81 FR 59499-59503). Pursuant to section 408(d)(1) of FFDCA, EPA has established a temporary tolerance exemption from the requirement of a tolerance for residues of CTV clones expressing spinach defensin proteins 2, 7, and 8 alone or in various combinations on citrus that expires on August 31, 2020 (81 FR 59499-59503). EPA will continue to regulate use of CTV-SoD as a microbial pesticide in accordance with applicable laws, regulations and procedures.

Biology of CTV and Use of CTV as disease control vector

Citrus tristeza virus (CTV) may damage citrus plants wherever they are grown (Bar-Joseph et al. 2010; Moreno and Garnsey 2010). While some strains of CTV may cause significant economic damage other strains grow and multiply within citrus without causing either symptoms or yield loss (Dawson et al. 2013). CTV is a well-studied model system to understand the genetic and biochemical mechanisms of virulence and epidemiology (Fishman et al. 1983; Moreno et al. 2008;

[5]Citrus plants provided in 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*) (40 CFR § 180.41).

Bar-Joseph et al. 2010; Atta et al. 2012; Albiach-Marti 2013; Dawson et al. 2013; Gottwald et al. 2013; Dawson et al. 2015a). Taxonomically, CTV is a member of *Closterovirus* genus in the *Closteroviridae* family (Order: Unassigned) with an unusually long single-stranded positive-sense RNA genome (see Fig. 4, p. 10) (Pringle 1996; King et al. 2012).

The species *Citrus tristeza virus* is distinguished by a number of features including being able to be moved from one plant to another by aphids and the requirement for a citrus host plant in the family *Rutaceae* (Bar-Joseph 1989). Isolates of CTV[6] recovered from infected citrus trees may contain a large amount of genetic variability, and the viruses have been classified into strains when they differ by greater than 7.5 percent at the nucleotide level (Fig. 1) (McClean 1974; Albiach-Marti 2013; Harper 2013; Yokomi et al. 2017).

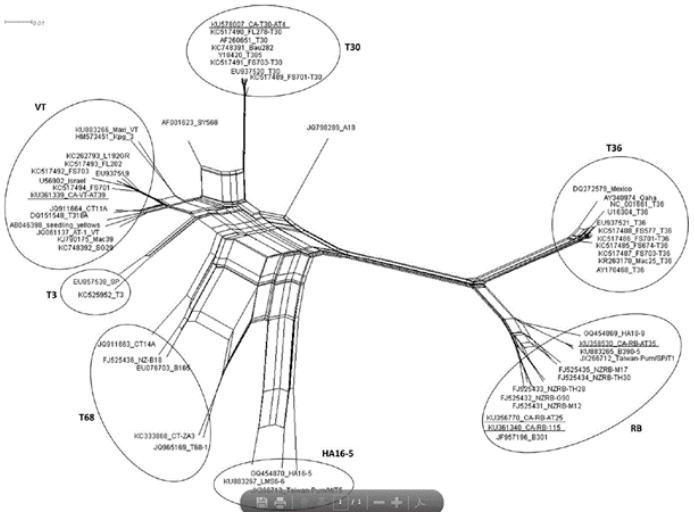

Figure 1. Phylogenetic tree: DNA sequencing of CTV reveals evolutionary relationships of different CTV strains (circles) composed of isolates from (Yokomi et al. 2017).

CTV first evolved along with citrus plants in Southern Asia and is now widely distributed throughout most of the citrus growing regions of the world (Roistacher and Moreno 1991; Garnsey et al. 1999; Bar-Joseph et al. 2010). Dispersal occurs by moving virus-infected plant material from one location to another, by grafting one citrus plant part onto another, and by aphids which transfer the virus from one plant to another while feeding (Bar-Joseph et al. 1979; Bar-Joseph 1989; Moreno et al. 2008; Bar-Joseph et al. 2010; Dawson et al. 2013). The virus is perpetuated by citrus cultivation practices of propagating material from stem sections. This is because for various genetic reasons seeds are not generally used for citrus nursery stock proliferation except for rootstock. Instead, plant increase typically involves transferring a cutting from the above ground portion of the parent plant citrus plant (referred to as the budwood or scion) and grafting it onto a different genetic rootstock for optimum fruit quality and yield (Frost and Soost 1968; Schneider

[6] There is variability in the scientific literature concerning the terminology for isolates of CTV that make up a strain of CTV. The term isolate can be used for a sample taken from a citrus plant that can be composed of CTV individuals from one or more strains or it can mean a genetically distinct individual. In this risk assessment, an isolate of CTV means a single genetically distinct genotype.

1968; Davies and Jackson 2009). Because CTV can remain in citrus without symptoms (latent) this practice unintentionally led to worldwide distribution of the virus in stem and root citrus plant material (Hughes and Lister 1949; Wallace 1956; Bar-Joseph et al. 1979; Bar-Joseph 1989; Roistacher and Moreno 1991; Lee and Rocha-Pena 1992; CABI 2015). No other opportunities for plant dispersal of the virus such as transmission by seed, pollen or fruit are known to occur (McClean 1957; Bar-Joseph 1989; Mink 1993; Nelson et al. 2011; USDA APHIS PPQ 2015; European Food Safety Authority 2017).

CTV can be moved from one citrus plant to another by aphids but not by other insects (Norman and Grant 1956). It is transported passively without replicating in the aphid (Hull 2014b). All growth forms of aphids (nymphs, adult winged and nonwinged forms) have the ability to acquire the virus, but only the winged forms have the ability to transfer the virus from tree to tree (Camp et al. 1953). Once present in an area of citrus cultivation, CTV can spread from tree to tree when winged aphids feed on infected new growth and then move to neighboring citrus trees while foraging (Fig. 2)(Norman and Grant 1956; McClean 1975).

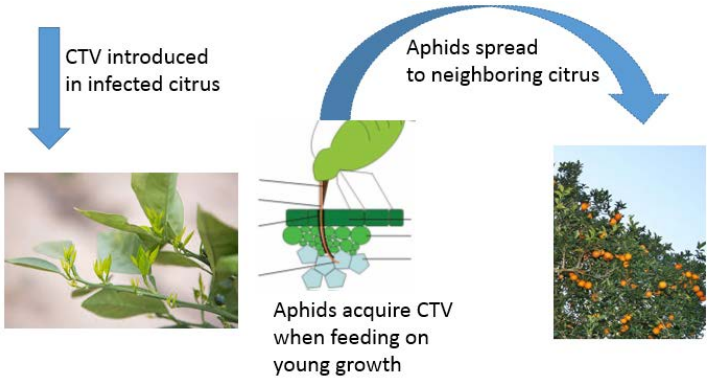

Figure 2. CTV present in infected citrus plant material can be moved from one location to another by people during citrus cultivation. Winged aphids feeding on the phloem[7] vascular tissue containing CTV acquire the virus and spread the virus to neighboring citrus trees. Aphid image from (Guerrieri and Digilio 2008), new growth (Mike Lewis, Center of Invasive Species Research, UC Riverside), citrus tree bearing fruit (Reb Huber, Orlando Sentinel).

CTV infected citrus exhibits a variety of distinct disease symptoms depending on the CTV isolate and citrus cultivar[8] (Webber 1943; Grant and Costa 1948; Grant 1949; Hughes and Lister 1949; Camp et al. 1953; Salibe 1977; Wallace 1978; Garnsey et al. 1987a; Bar-Joseph 1989; Roistacher and Moreno 1991; Garnsey et al. 1996; Gottwald et al. 2002; Garnsey et al. 2005; Bar-Joseph et al. 2010; Dawson et al. 2013; Yokomi et al. 2017). Strains and sometimes isolates within strains may

[7] Phloem is the vascular tissue that transports the products of photosynthesis throughout the plant. It is composed of sieve elements, parenchyma (living cells that transfer material to and from the sieve elements) and fibers (sclerenchyma for structural support).

[8] Horticultural and cultivated form of citrus are derived from various citrus species through crossing and clonal propagation and are identified as cultivars or variety clones of citrus.

exhibit a diverse range of symptoms. A spectrum of disease reactions is seen in citrus groves, including asymptomatic trees (when the virus multiplies without noted effect on growth or yield), unusual development of the food transport system (phloem) leading to stem pitting, stunting and leaf chlorosis referred to as seedling yellows, and degeneration of phloem cells that may result in loss of leaves and wilting generally resulting in tree death (decline). The type of symptoms and amount of damage is the result of an interaction of the strain(s) and isolate(s) of CTV present in the plant, the type of host plant, and the environment.

Introduced Trait: Defensin

This proposed method to control HLB uses genetically engineered CTV as a vehicle to introduce spinach defensins into the citrus tree phloem for distribution throughout the tree (Folimonov et al. 2007) to protect against or as a treatment for HLB caused by the bacterium *Candidatus* Liberibacter asiaticus (*C*Las). CTV is engineered to contain genetic material from spinach (*Spinacia oleracea* L.) to produce defensin proteins SoD2, SoD7, SoD8, SoD10, SoD11, SoD12 and/or SoD13 (Segura et al. 1998; Dohm et al. 2014). In addition to the genetic material encoding for the defensin proteins, CTV-SoD contains regulatory sequences[9] and signal peptides to produce the defensin proteins. Secretion signal peptides are known to transport proteins out of cells into the intercellular space where the protein can be dispersed in the liquids between cells (Folimonov et al. 2007). The regulatory sequences and signal peptides are claimed as confidential business information (CBI) in the 17-044-101r permit application.

Defensins are widely present in plants including a variety of food plants (Broekaert et al. 1995; Thomma et al. 2002; Carvalho and Gomes 2009; Pelegrini et al. 2011; GenBank 2017). They provide host defense response to biotic (including bacterial and fungal infection) and abiotic stressors and participate in plant growth and development (Franco 2011; Cools et al. 2017; Shafee et al. 2017). Spinach defensins share common features of most other plant defensins. They comprise a N-terminal acidic signal peptide and a basic mature peptide of 45-54 amino acids with eight Cysteine residues that form four disulfide bridges which stabilize the three-dimensional structure, one α-helix and three antiparallel β-sheets (Fig. 3) (Carvalho and Gomes 2009; De Coninck et al. 2013; Dias and Franco 2015).

[9] Regulatory sequences control production of the final protein product, but are themselves not expressed as proteins. These regulatory sequences have not been shown to confer any biological activity other than controlling and allowing for the production of proteins from RNA template.

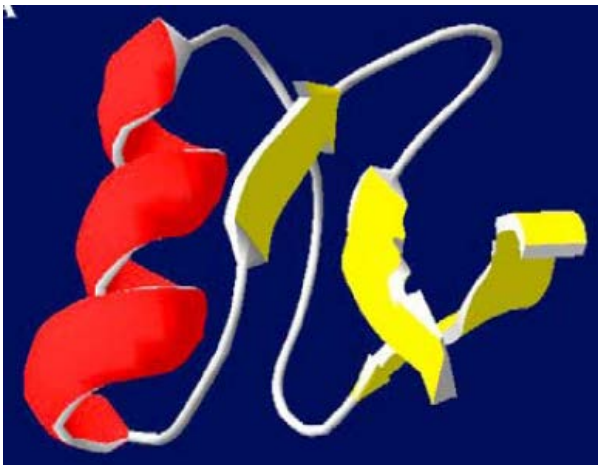

Figure 3. Three dimensional structure of a plant defensin showing the globular form with one α-helix and three antiparallel β-sheets (from Carvalho and Gomes 2009)

Plant defensin action towards microbes often occurs via a variety of multi-step mechanisms with most attributed to plasma membrane interactions (De Coninck et al. 2013; Shafee et al. 2017). Numerous crop plants have been developed containing defensins to control disease where the genetic material is inserted into the plants chromosome and permanently retained (Kaur et al. 2011).

Laboratory studies using protein extracted from spinach plants found that SoD2 and SoD7 have activity against some bacterial and fungal pathogens, with a 50 percent growth inhibition towards the Gram positive bacterium *Clavibacter michiganensis* (at 0.1-1 µM), the Gram negative bacterium *Ralstonia solanacearum* (at 1-2 µM), and the fungal pathogens *F. solani* (at 9-11 µM), *Fusarium culmorum* (at 0.2 µM for SoD2), *Colletotrichum lagenarium* (at 11 µM for SoD2) and *Bipolaris madis* (at 6 µM for SoD2). The proteins did not have activity against either *Parastagonospora nodorum* (*Septoria nodorum*) or the non-pathogenic *Trichoderma viridae* (for SoD2 at concentrations less than 20 µM) (Segura et al. 1998). Other studies with laboratory synthesized defensins found no growth inhibition following treatment with 30 µM SoD2 for 48 hr in the bacterial pathogen *Agrobacterium tumefaciens* and the symbiont *Sinorhizobium meliloti*, which were selected because they are closely related to the uncultureable *C*Las (Stover et al. 2013). The lack of effectiveness of synthesized SoD2 against bacteria may be due the source SoD2 having been synthesized with inaccurate post-translational processing (Segura et al. 1998; Stover et al. 2013). In greenhouse and field trials, SoD2 transgenically expressed in citrus has shown substantial activity against HLB (Erik Mirkov, unpublished data, cited in (Stover et al. 2013)).

Engineered CTV-SoD

Numerous plant viruses expressing recombinant proteins in plants have been developed for applied and basic research and as therapeutics for disease control (Pogue et al. 2002; Dawson 2011; Dawson and Folimonova 2013; Dawson et al. 2015b). The strategy in using CTV is for a virus to survive for prolonged periods of time within the plant while expressing a recombinant protein. In CTV-SoD, the virus has been engineered in such a way that the spinach defensin protein is produced in citrus plants while preserving the ability of the virus to infect, replicate and spread throughout vascular tissues in citrus trees without making genetic changes to the citrus genome

(Folimonov et al. 2007; Dawson 2011; Dawson and Folimonova 2013; Dawson et al. 2015b). To this end the defensin genes are inserted in one or more of three locations at the 3' end of the CTV genome (Karasev et al. 1994; Pappu et al. 1994; Karasev et al. 1995) (Fig. 4).

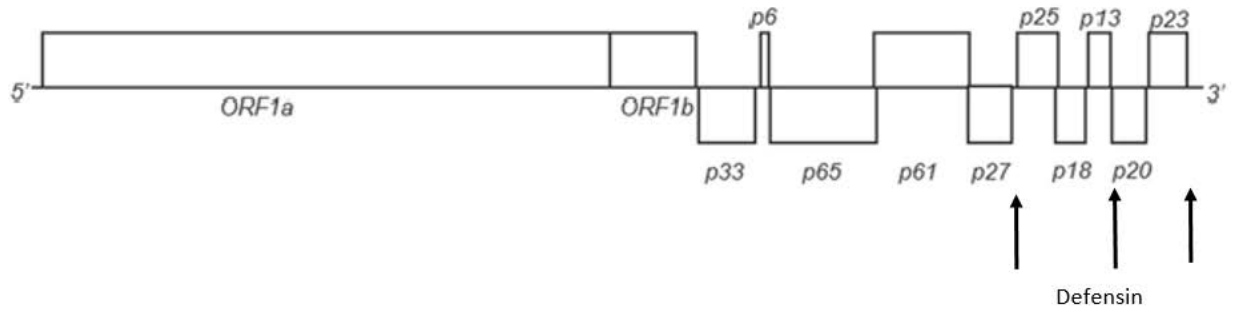

Figure 4. Schematic representation of the CTV 19 kb positive-stranded RNA that forms long flexuous virons (RNA and capsid proteins) and is one of the largest genomes among plant viruses (King et al. 2012). CTV RNA genome organization with the protein (p) products size indicated by molecular weight in kD. The defensin gene insertion site is indicated by arrows between the genes encoding p27 and p25, p13 and p20, or p23 and 3' terminus (17-044-101r permit, diagram adapted from (Harper et al. 2016)).

One of the most important aspects of CTV which enables its use in this novel disease control approach is that CTV localizes within specific plant tissues. Whether the CTV is introduced into the plant by grafting or by aphids, the virus resides in the plant vascular tissue which facilitates movement throughout the plant (Gottwald et al. 2002; Folimonov et al. 2007). Similarly this vascular tissue is where the *C*Las bacterium causing HLB reside (Bove 2006; Folimonova and Achor 2010). This co-localization places the CTV in the same plant tissue as the *C*Las to directly deliver the defensin where the bacterium also grows and multiplies in the citrus plant (Bove 2006).

In earlier studies, a full length DNA clone of CTV was generated and shown to replicate and move systemically throughout the citrus plant with similar symptom phenotype across a range of plant cultivars (Satyanarayana et al. 1999; Satyanarayana et al. 2001). Various forms of CTV are referenced in this Preliminary PRA, as summarized in the following table:

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

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

The CTV-SoD DNA clones in the current permit application are derived from CTV infected citrus originating from Florida. The CTV in a tree is made up of a population of different isolates from one or more strains. Because of the long length (approximately 20,000 nucleotides), CTV genetic material is isolated in sections of approximately 4,000 nucleotides which are then pieced together to form a copy of the complete genome (Satyanarayana et al. 1999). The final clone of a complete genome of CTV can be based on any one of a number of individual CTVs present in the tree. After extraction, the genetic sequence is used to designate the strain from which the clone was derived. Thus the strain is actually identified after it is removed from the tree and sequenced. Isolates of strain T36 used to generate CTV9R were originally collected from a sweet orange scion grown on sour orange rootstock in Orange County, Florida (Garnsey and Jackson 1975). The starting material for CTVT30 was from various citrus trees in Florida infected with strain T30 (data submitted with 17-044-101r). The CTV9R/T30 hybrids were created by replacing the p23 and 3' UTR region of CTV9R with the p23 and 3' UTR region from strain T30 (Albiach-Marti et al. 2010).

Figure 5. CTV9R/T30 hybrid showing sequences in red from CTV9R and green T30 (diagram from (Dawson et al. 2015a).

The genetic makeup of the CTV-SoD clones are similar to wildtype isolates except for the addition of restriction cloning sites, signal peptide sequences, promoters and the gene(s) for spinach defensin.

B. Pest Risk Assessment

In this document APHIS assesses relevant plant pest risks for the proposed use of CTV-SoD including whether CTV-SoD will produce a virus that causes greater disease symptoms and damage than CTV currently present in Florida, infect plants other than those which are already host to CTV, be more readily transferred from one tree to another by aphids and survive in the environment beyond the inoculated tree, and/or change the ability to control CTV. Also considered is whether CTV-SoD may transfer genetic material to viruses other than CTV. The disease symptoms and resulting physical or economic damage caused by different strains of CTV extend from a symptomless infection (most isolates of strain T30) to a complete loss of a citrus tree in certain circumstances. The genetically engineered clones of CTV-SoD were derived from CTV strains T36 and T30, both of which are currently widely present in Florida. Data on disease expression and biological activity obtained in the greenhouse and in the field environment for CTV9R, CTVT30, CTV9R/T30, and some CTV-SoD was used along with the extensive amount of information on CTV to address the pest risk aspects of CTV-SoD deployment.

This analyses incorporates the understanding that field use will be done under strict controls as provided in APHIS's permit conditions and reporting obligations. For the production process, CTV-SoD will be multiplied first in tobacco plants (*Nicotiana benthamiana*) by *Agrobacterium tumefaciens* inoculation followed by a second round of multiplication in citrus trees. Tissue from these CTV-SoD inoculated trees will be used to introduce CTV-SoD into citrus trees by grafting, either in the greenhouse or directly to trees already planted in the field. Because the CTV was genetically engineered at the University of Florida (Dr. William Dawson) and Southern Gardens Citrus Nursery, LLC also located in Florida, the 17-044-10r permit application pertains to CTV-SoD use in the environment beyond laboratory and greenhouse containment. Southern Gardens has submitted Standard Operating Procedures (SOPs) for the production, movement and tracking of the CTV-SoD infected trees. The starting point for this assessment is the current situation in Florida, where the non-engineered the strains of the CTV virus (T30 and T36) are currently widely prevalent.

Potential Changes to Ability to Cause Disease

Strains T36 and T30 used in the construction of the CTV clones are widely distributed in the state of Florida and have been so for decades (Garnsey et al. 1980; Powell and Pelosi 1993; Gottwald et al. 1996; Brown and Davison 1997; Hilf and Garnsey 2002; Sieburth and Nolan 2005; Harper et al. 2015; Harper and Cowell 2016). Citrus growers in Florida have been coping with CTV since the 1950s although the virus may have been present well before molecular and strain indexing methods were available for definitive detection (Grant 1952; Camp et al. 1953; Cohen 1956; University of Florida 1956; Brlansky et al. 1986; Powell and Pelosi 1993; Brown and Davison 1997; Halbert et al. 2004; Harper et al. 2015; Harper and Cowell 2016). Starting in 1953, and mandatory since 1997, the Florida Citrus Nursery Stock Certification Program implemented measures to exclude severe CTV strains, such as strain T36, that cause severe stem pitting, seedling yellows or quick decline on sour orange rootstock (Permar et al. 1990; Sieburth 2000; Florida Administrative Code Rule Chapter 5B-62 2015; Roberts and et al. 2017). Therefore, currently no new isolates of T36 strain are thought to be introduced in newly planted citrus trees. Strain T36 is generally only problematic in citrus planted on sour orange rootstock, which is one of dozens of rootstocks used by citrus growers in Florida (Stover and Castle 2002;

Castle et al. 2016; Florida Department of Agriculture and Consumer Services 2016). However, strain T36 continues to be widespread in Florida because T36-tolerant citrus rootstocks serve as a reservoir (where T36 multiplies without causing plant damage) providing an ongoing supply of T36 strain that can be moved from tree to tree by aphids (Harper et al. 2015; Harper and Cowell 2016).

Since the major economic damage to Florida citrus due to CTV has been successfully managed through a combination of judicious selection of rootstocks and the Nursery Stock Certification Program, since 2005 Florida citrus grower concerns have shifted to controlling citrus canker, HLB and blackspot, all caused by bacterial pathogens (Singerman and Burani-Arouca 2017). The overarching harm being considered in this risk assessment is whether citrus inoculated with CTV-SoD will have increased disease compared to disease caused by the CTV currently present in Florida.

When citrus plants are infected with CTV the plant responds with a variety of distinct disease symptoms depending on the CTV strain and isolate, citrus cultivar[10] and the environment under which the citrus tree is grown (Webber 1943; Grant and Costa 1948; Grant 1949; Hughes and Lister 1949; Camp et al. 1953; Salibe 1977; Wallace 1978; Garnsey et al. 1987a; Bar-Joseph 1989; Garnsey et al. 1996; Gottwald et al. 2002; Garnsey et al. 2005; Bar-Joseph et al. 2010; Yokomi et al. 2017). When infected with certain CTV isolates, some citrus trees develop yellowing, loss of leaves and/or wilting (referred to as decline), tree stunting or stem-pitting. Under greenhouse testing for cultivar specificity (which citrus cultivars display disease symptoms when exposed to a particular CTV isolate) and for isolate virulence (the amount and type of disease produced by the CTV isolate), seedling yellows, leaf curl symptoms (Roy and Brlansky 2009) and vein clearing symptoms are evaluated. Importantly, none of these symptoms are generally seen in field environmental settings (Bar-Joseph et al. 1979; Rocha-Peña et al. 1995; Dawson et al. 2015a), while stem pitting is both a greenhouse and a field symptom.

The ability for the cloned CTVs to cause disease was evaluated. In greenhouse tests CTV9R (a derivative of strain T36) and an isolate of strain T36 similarly displayed strong vein clearing and leaf cupping in Mexican lime (*Citrus aurantifolia* (Christm.) Swing.) and Alemow plants (*C. macrophylla* Wester), stem pitting on Mexican lime, seedling yellows on sour orange (*C. aurantium* L.) and Duncan grapefruit (*C. paradisi* Macf.), and stunting and chlorosis on Valencia orange (C. sinensis (L.) Osbeck.) grafted onto sour orange rootstock; no symptoms were observed on sweet orange and no stem pitting was observed on Duncan grapefruit (Satyanarayana et al. 2001). These greenhouse tests indicate the virulence of the CTV9R is similar to its parental T36 strain. CTV9R, and in some cases CTV9R-SoD, was also field tested under APHIS permits 08-330-101r-a1, 09-121-103r, 11-357-101r, 12-109-102r, 13-039-101r-a2, 14-320-101r, 16-036-101r, 16-067-104r-a1 and 16-308-101r-a1. In all cases the disease observed in the field was that typical of strain T36 isolates (data provided in field test reports).

[10] Horticultural and cultivated forms of citrus are derived from various *Citrus* species and related genera through crossing and clonal propagation referred to as cultivars or variety clones of citrus.

Figure 6. Field planting of CTV inoculated citrus trees showing stunted citrus (from (Dawson et al. 2015a).

In one field trial CTV9R caused stunting (Fig. 6) in 66% of the Valencia orange on sour orange rootstock inoculated trees (in 10 of 15 trees) (Dawson et al. 2015a). Because the virulence of CTV9R and CTV9R is similar to that of strain T36 Florida growers are not expected to be impacted any greater by disease damage caused by CTV9R-SoD than currently encountered with strain T36 CTV.

In greenhouse tests CTVT30 (a derivative of strain T30) did not produce any disease symptoms on Duncan grapefruit, Sun Chu Sha Mandarin, Sour orange and *C. macrophylla* (submitted with 17-044-101r permit application). While T30 strains generally are not known to produce field disease symptoms or yield loss sufficient to warrant intervention, in greenhouse tests some isolates of T30 are reported to cause stem pitting and stunting of grapefruit (Boz and Parlevliet 1992; Hull 2014b; Harper and Cowell 2016). However, strain T30 is considered a mild strain, widely prevalent in Florida and not regulated under the Florida Administrative Code (Harper et al. 2015; Harper and Cowell 2016). Because the virulence of CTVT30 is similar to strain T30 found in Florida, and because adding SoD to CTVR9 did not alter virulence, there is no reason to believe SoD will alter virulence in CTVT30. Thus, growers are not expected to be impacted any greater by disease damage caused by CTVT30-SoD than currently encountered with strain T30 CTV.

In the CTV9R/T30 hybrids, the p23 and 3' UTR region of CTV9R is removed and replaced with the p23 and 3' UTR region from strain T30 (Albiach-Marti et al. 2010). The CTV9R/T30 hybrid, similar to strain T30, failed to induce seedling yellows in sour orange and Duncan grapefruit in greenhouse studies (Albiach-Marti et al. 2010). Field trials were carried for CTV9R/T30 hybrids under permits 09-121-103r and 12-121-103r. Field observations using Valencia orange on sour orange rootstocks demonstrated a reduction in stunting in the CTV9R/T30 (4 of 18 trees, 22%) compared to CTV9R (in 10 of 15 trees, 66%). This reduction may be conferred by the plant RNAi suppressive activity conferred by the T30 p23 gene ((Lu et al. 2004; Dawson et al. 2015a). Because the type of disease symptoms (stunting) of CTV9R/T30 is similar to the T36 strain and

occurs at a reduced level compared to T36, Florida growers are not expected to be impacted any greater by disease damage caused by CTV9R-SoD than currently encountered with CTV strain T36.

Plant viruses genetically engineered to contain an additional gene or partial regions of a gene are routinely used as a tool to understand both plant and viral biological functions or for production of large amounts of proteins for later extractions for various research and therapeutic purposes (Dawson 2011; Dawson and Folimonova 2013). Other than the additional phenotype conferred by the introduced material, there is no evidence that adding genetic material in itself alters the ability of the virus to become a worse plant pest than the parental virus (Roberston 2004; Lange et al. 2013). Plant defensins are ubiquitous in the plant kingdom as part of the plant innate immune system to resist bacterial and fungal pathogens and do not themselves cause plant disease (Carvalho and Gomes 2009). The three CTV clones used in the current permit application will be engineered in one of three different locations in the CTV genome (see Fig. 4, p. 10) to contain one of three different promoters (claimed as CBI), with and without signal peptides and one or more of the seven spinach defensin genes (SoD2, SoD7, SoD8, SoD9, SoD11, SoD12 and/or SoD13), for a total of at least 378 possible permutations. The various combinations are expected impart changes to the expression of the different spinach defensin genes, including their stability within the cloned CTV (Folimonov et al. 2007). However, as provided in the field test reports, field trials with CTV9R-SoD inserted in various locations in the CTV genome did not show any greater symptoms than those already associated with strain T36. Therefore, CTV9R-SoD is not expected to cause any more disease than that of the strain T36 already widely present in Florida. Although not all of the 378 permutations have been field tested, there is no reason to believe that the use of other CTV clones or defensin genes or variations in promoter, signal peptide, or insertion site will alter the disease symptom phenotype compared to the CTV-SoD clones already field tested. However, since removal of CTV genes can in some cases lead to increased virulence (Tatineni and Dawson 2012), the permit will require that each CTV clone and insert combination be tested to verify intactness of viral genes before field trial .

Of the several factors taken into account including the historic and current status of CTV strains in Florida including T36, T30, greenhouse virulence tests, past field trials and that spinach defensins are not known to cause plant disease, introducing CTV9R-SoD, CTV9R/T30-SoD, and CTVT30-SoD is not expected to alter the ability of CTV to cause disease compared to non-engineered strains. Therefore the level of CTV disease due to the release of CTV-SoD is anticipated to be no different than what is already present in Florida.

Potential Changes to Host Range

One potential concern when CTV-SoD is applied by grafting onto citrus trees planted in the field, or graft-inoculated citrus trees are moved out into the environment, is whether the CTV-SoD could move to and infect plants beyond the virus's natural host range. Viruses that infect plants multiply within a restricted number of plant species, which establishes a virus's host range (Dawson and Hilf 1992; Hull 2014a). Natural plant hosts are those infected in either natural or agricultural settings (Dawson and Hilf 1992), while for research purposes plants can be experimentally inoculated with CTV through grafting, use of *Agrobacterium*, stem slash and bark flap inoculation

and using purified virus multiplied in plant protoplasts or tobacco plants (Gowda et al. 2005; Robertson et al. 2005; Ambros et al. 2011; Ambros et al. 2013). Both the natural and the experimental host ranges of CTV are well known (Knorr 1956; Muller and Garnsey 1984; Yoshida 1996; Lee and Bar-Joseph 2000; Moreno et al. 2008; Bar-Joseph et al. 2010; Dawson et al. 2013). The *Citrus* spp. and species in the closely related genera *Fortunella* are natural hosts (Yoshida 1996; Lee and Bar-Joseph 2000). Experimentally, other members of the Rutaceae family can also serve as host plants, including *Aegle marmelos* (L.) Corr. Serr., *Aeglopsis chevalieri* Swingle, *Afraegle paniculata* (Schumach.) Engl., *Citropsis gilletiana Swing. & M. Kell., Microcitrus australis* (Planch.) Swing. and *Pamburus missionis* (Wight) Swingle. In addition, plants in the Passifloraceae family can serve as experimental hosts (Knorr 1956; Rocha-Peña et al. 1995; Lee and Bar-Joseph 2000; Moreno et al. 2008) and *Nicotiana benthamiana* protoplasts have been infected experimentally by CTV (Ambros et al. 2011). Attempts to transmit CTV via aphids to over 200 other species outside the Rutaceae were not successful (Muller et al. 1974).

The genes in CTV directing host range are located in the 3' end of the CTV genome from p33 through p23 (Fig. 4) (Albiach-Marti 2013; Dawson et al. 2013; Dawson et al. 2015b). The cloned CTVs contain the entire CTV sequence including the genes for movement and infection (p33, p18 and p13) and silencing suppressors (p23, p25, p20). Therefore, the cloned CTVs (CTV9R, CTVT30, and CTV9R/T30) are expected to retain the current host range of strains T30 and T36 from which they were derived. In addition none of these virulence factors are altered in any CTV9R-SoD, CTVT9R/T30-SoD, or CTVT30-SoD clone. A permit condition will require Southern Gardens to confirm that the genes of CTV are not altered or disrupted in each CTV-SoD clone prior to its release. Therefore, there is no reason to believe the introduction of the spinach defensin genes along with non-translated regulatory elements will cause any plant pest harms by causing a change in host range.

Potential Changes to Distribution, Dispersal and Persistence

An overarching consideration is whether the genetically engineered changes to CTV alter the ability of the virus to move from citrus tree to citrus tree beyond the inoculated trees. As an obligate parasite CTV depends on citrus host plants for survival. It can be dispersed by moving CTV infected citrus plant material and by aphids. CTV was introduced and distributed in Florida during the development of the citrus industry, and it remains prevalent throughout Florida wherever citrus is grown (Grant 1952; Camp et al. 1953; Grant and Schneider 1953; Cohen 1956; University of Florida 1956; Cohen and Burnett 1961; Norman et al. 1961; Webber et al. 1967; Garnsey and Jackson 1975; Garnsey et al. 1980; Brlansky et al. 1986; Yokomi et al. 1992; Garnsey et al. 1993; Powell and Pelosi 1993; Lee et al. 1997; Stansly et al. 1999; Hilf and Garnsey 2002; Halbert et al. 2004; Sieburth and Nolan 2005; Harper et al. 2015; Harper and Cowell 2016). In the proposed APHIS permit application, CTV-SoD will be introduced into citrus trees by grafting bark, a leaf piece or stem bud according to SOPs provided in the permit application and in accordance with EPA label restrictions that will prohibit any further grafting to prevent unintended introduction. There are no reports of movement by field equipment, including pruning tools and through soil (Camp et al. 1953; CABI 2015) and the introductions into the environment will be carried out following USDA 7 CFR 301.76 and State of Florida Rule Chapter 5B-62 Florida Administrative Code. Therefore, there is no known means by which introducing a spinach defensin

would affect or require alteration of current measures that limit the spread of CTV by cultural propagation.

Although CTV can be found in citrus fruit the potential for the movement of CTV-infected fruit to provide an opportunity for dispersal to other citrus plants is negligible, because CTV is not found in the citrus seeds and the aphids that transport the virus feed on young growing tissue (Tsuchizaki et al. 1978; Broadbent 1995; CABI 2015). Therefore, fruit and seed are not considered a pathway for the dissemination of the virus (McClean 1957; Bar-Joseph 1989; European Food Safety Authority 2017; USDA APHIS PPQ 2017). The CTV-SoD genetic material is not expected to be inserted into the plant DNA because there is no plausible means by which the plant would become genetically engineered merely by containing a virus that is contained in the phloem. Thus, CTV-SoD genetic material would not be transferred to subsequent generations in seed or pollen (McClean 1957; Bar-Joseph 1989; Mink 1993; Nelson et al. 2011; USDA APHIS PPQ 2015). Therefore, the cloned CTV-SoD is expected to remain within the citrus trees and not move by seed, pollen and/or fruit.

A consideration is whether the introduced genetic material will enhance aphid-mediated virus movement between trees. After CTV is acquired by aphids during feeding on phloem tissue in young stems and leaves, winged forms of aphids can move the virus to neighboring trees (Costa and Grant 1951; McClean 1975; Raccah et al. 1989). Certain species of aphids but no other types of insects have the ability to move CTV from one citrus tree to another (Norman and Grant 1956; Roistacher and Bar-Joseph 1987; Yokomi and Garnsey 1987; Bar-Joseph 1989; Roistacher and Moreno 1991). The aphid species that can transmit the Florida CTV strains are present in Florida and have been so for decades (Costa and Grant 1951; Norman and Grant 1956; Bar-Joseph et al. 1979; Yokomi and Garnsey 1987; Yokomi et al. 1989; Yokomi et al. 1994; Halbert 1995; Gottwald et al. 1996; Halbert 1996; Pelosi et al. 1996; Gottwald and Garnsey 1997; Lee et al. 1997; Powell et al. 1997; Halbert and Brown 1998; Halbert et al. 2004; Powell et al. 2006; Fasulo and Halbert 2015). The most important aphid vector of CTV common in Florida citrus groves is the brown citrus aphid (*T. citricida* Kirkaldy), first introduced to Florida in 1995. The less efficient cotton or melon aphid (*Aphis gossypii* Glover) can also transmit CTV (Stansly and Rogers 2017).

Aphids can transmit CTV for only 24 hours after feeding and the brown citrus aphid that typically spreads the virus in Florida usually only transmits CTV to the neighboring 2-8 rows of citrus (Camp et al. 1953; Gottwald et al. 1993b; Gottwald et al. 1993a; Gottwald and Garnsey 1997; Gottwald et al. 1999). Thus, even though aphids can be carried long distances by winds, CTV spread is typically limited to within the grove or to neighboring trees. Environmental conditions and cultivation practices influence the spread of CTV by aphids. In Florida the aphid populations generally increase to their highest levels in spring (March-May) when new succulent leaves support aphid growth, with a second lower level of proliferation September through December (Pelosi et al. 1996; Powell et al. 2006). Citrus grove management practices such as hedge trimming, irrigation and fertilization may favor aphid population proliferation resulting in large colonies developing winged forms that may spread CTV to nearby trees (Lee and Bar-Joseph 2000).

Not all CTV isolates have the ability to be spread efficiently by aphids (data submitted with 17-044-101r) (Bar-Joseph and Loebenstein 1973). The parental T36 strain can be transmitted but with low efficiency (Powell et al. 1999; Harper et al. 2016). Prior to cloning, the strain T36 isolate used to generate CTV9R was propagated for 7 years in the greenhouse by graft transmission (Satyanarayana et al. 1999; Satyanarayana et al. 2003; Folimonov et al. 2007; Harper 2013). Aphid transmissibility tests with the brown citrus aphid (the most efficient aphid vector of CTV) were carried out in the greenhouse with CTV9R and CTVT30 on 172 recipient and 210 trees, respectively (data submitted with 17-044-101r). The aphid transmission rate with the cloned CTV9R was 0.6% compared with 24.1% for a wild-type T36 strain (FS577-1-8) typically found in the field (data submitted with 17-044-101r). No aphid movement was detected with CTVT30. These greenhouse tests indicate the CTV9R and CTVT30 are minimally transmitted by aphids.

The ability of CTV9R to be moved by aphids was further tested in the field environment. CTV9R and CTV9R-SoD, were field tested in citrus under APHIS permits since 2010 with requirements to test for movement under field conditions. During these trials the CTV9R was inoculated into field grown Valencia or Hamlin orange trees grafted onto either Carrizo citrange or Swingle citrumelo. Completely surrounding the CTV9R and CTV9R-SoD inoculated citrus trees were at least one row of non-inoculated sentinel citrus trees. The sentinel trees served as potential trap trees to detect movement of the CTV9R and CTV9R-SoD to adjacent citrus trees in a natural field setting. Every sentinel tree was tested for the presence of the CTV9R and CTV9R-SoD, thereby monitoring for movement of the virus by aphids beyond the inoculated trees. Testing was by using an antibody and/or by polymerase chain reaction (PCR). Of the over 6,800 trees tested, no movement of CTV9R or CTV9R-SoD was detected. Insecticides were applied during the field trial, and these are similar to what is currently typically used to control insects in Florida citrus groves. However, no movement was detected even when aphids were detected in the field. The greenhouse and field data indicate the CTV9R and the CTV9R-SoD are poorly transmitted by aphids. It is therefore expected that CTV9R and the CTV9R-SoD will likely be restricted to the inoculated tree and are unlikely to move to neighboring citrus trees.

The CTV9R/T30 contains the CTV9R p27, p65 and p61 genes associated with aphid vectoring (Harper et al. 2016), while the CTVT30 clone that provided the p23 and 3’UTR also does not allow for aphid transmission, thus the CTV9R/T30 is expected to be no more transmissible than CTV9R.

Another issue that could affect transmissibility is alteration of one or more genes known to be involved in aphid transmission. The genetic regions involved in aphid transmission are associated with the p27, p61 and p65 sequences (Harper et al. 2016; Killiny et al. 2016). Although one of locations for insertion of the spinach defensin is between the p27 and p25 genes, these genes are not expected to be altered. A permit condition will require Southern Gardens to confirm that the genes of CTV are not altered or disrupted in each CTV-SoD clone prior to its release.

A factor that can affect the movement and survival of CTV in other citrus is that infection of citrus trees with one CTV strain can reduce or prevent infection by a genetically similar strain of CTV, a

phenomena known as cross protection (or superinfection exclusion). Because of the current prevalence of CTV strains T36 and T30 in Florida citrus trees, any trees already infected with T36 and T30 will not be able to be reinfected by CTV-SoD clones based on the T36 and T30 strains (Cohen and Burnett 1961; Garnsey et al. 1980; Powell and Pelosi 1993; Hilf and Garnsey 2002; Halbert et al. 2004; Sieburth and Nolan 2005; Harper et al. 2015; Harper and Cowell 2016; Harper et al. 2017). This further limits the risk of spread of the CTV-SoD into surrounding citrus trees.

The CTV-SoD clones are expected to be relatively stable and able to compete effectively with the wildtype virus (Folimonov et al. 2007). This long-term stability of cloned CTV contrasts with the relatively low stability of most other recombinant plant virus based vectors, such as tobacco mosaic virus and potato virus X that lose their inserted sequences during systemic infection within a couple of weeks (Dawson 2011; Dawson and Folimonova 2013). Greenhouse experiments have shown CTV9R derived vectors can remain stable after more than 9 years (Folimonov et al. 2007; Dawson and Folimonova 2013). Greenhouse experiments using a mixed population of CTV9R and CTV9R expressing green fluorescent protein (GFP) inoculated into *C. macrophylla* trees found that after 2 and 4 months the ratio of the CTV RNAs from CTV9R and CTV9R containing green fluorescent protein (GFP) did not significantly change (Folimonov et al. 2007). Because the inserted material such as spinach defensin is not expected give a competitive advantage or disadvantage to CTV-SoD survival, the CTV-SoDs are expected to remain in the citrus trees for an extended period of time coexisting with the CTV strains currently present in Florida. Once in the field the CTV-SoD is expected to reach an equilibrium with the mixed populations of CTV already infecting the trees in the field.

Taking into account the above information, it is not likely that CTV-SoD will move beyond the inoculated trees. Furthermore there is no scientifically plausible hypothesis to suggest a means by which the spinach defensin gene might confer a greater advantage to the dispersal or persistence of CTV. Therefore, the CTV-SoDs assessed in this PRA are expected to have a reduced ability to be moved by aphids compared to CTV currently present in Florida, and there is not expected to be any distribution and dispersal of CTV-SoD beyond the inoculated trees.

Potential to Create a New Problematic Virus

A consideration is whether use of CTV-SoD for biological control of HLB in citrus could generate a new virus with altered virulence, transmissibility or persistence (Falk and Bruening 1994; AIBS 1995; Tepfer 2002; Fuchs and Gonsalves 2007; Thompson and Tepfer 2010; Tepfer et al. 2015). CTV has been widely present in Florida citrus since at least the 1950s (Grant 1952; Camp et al. 1953; Cohen 1956; University of Florida 1956; Brlansky et al. 1986; Powell and Pelosi 1993; Brown and Davison 1997; Halbert et al. 2004; Harper et al. 2015; Harper and Cowell 2016). Field grown citrus trees when infected with CTV typically contain mixtures of different strains and different individuals within a strain of CTV (Kong et al. 2000; Rubio et al. 2001; Weng et al. 2007; Martin et al. 2009; Roy and Brlansky 2009; Scott et al. 2013). This coexistence of genetically diverse populations of viruses in the same plant is the normal phenomena for long lived plants such as trees that are likely to have been repeatedly infected with different virus variants (Stubbs 1964; Harper et al. 2015; Harper and Cowell 2016; Mascia and Gallitelli 2016). In addition RNA viruses such as CTV often have a very high mutation frequency due to a high error rate during RNA synthesis (because viral RNA polymerases have poor proof-reading functions) (Lai 1992).

Thus both the presence of different viruses and the error-prone recombination leads to a naturally genetically diverse populations of CTV within an individual tree. It is possible these genetically variable viruses may interact synergistically or by recombination to form a new strain that exacerbates or ameliorates disease symptoms (Lai 1992; Weng et al. 2007; Bujarski 2013; Tepfer et al. 2015).

As previously discussed, CTV has been present in Florida at least since the 1950s. The non-engineered CTV from which the cloned CTV were derived have been widely present in Florida as mixtures of the different strains and populations of individuals typically coexisting within a single citrus tree (Garnsey et al. 1980; Powell and Pelosi 1993; Gottwald et al. 1996; Brown and Davison 1997; Hilf and Garnsey 2002; Sieburth and Nolan 2005; Harper et al. 2015; Harper and Cowell 2016). In one study 78 percent of trees were coinfected with both T30 and T36 (Harper and Cowell 2016).

A principle issue considered was whether genetic recombination between the CTV-SoD and CTV already present in Florida citrus will result in a novel problematic virus. Experimental evidence comparing genome sequences from different CTV isolates has demonstrated a diverse array of naturally occurring recombinants; however these recombinants rarely have a competitive advantage over non-recombinant viruses (AIBS 1995; Mawassi et al. 1996; Ayllón et al. 1999; Vives et al. 1999; Vives et al. 2005; Weng et al. 2007; Martin et al. 2009; Hilf 2010; Rubio et al. 2013). During the course of time where citrus in Florida has contained strains T30 and T36, it's highly likely that coinfection has provided the opportunity for genetic exchange between strain T30 and T36. Thus, T36/T30 recombinants may already be present in Florida.

Therefore, any CTVs arising from recombination of the CTV-SoD with the CTV already present in Florida are likely to have similar properties to those CTV already present in Florida, except for the presence of the defensin gene. Based on the analyses in the previous sections, the CTV-SoDs are unlikely to be any more virulent, stable, or transmissible than the T36 and T30 strains currently prevalent. Thus, there is no reason to believe that any novel recombinant of CTV-SoD with non-engineered CTV will confer any greater plant pest risk than that already present in the State of Florida.

Viral vectors are known to lose inserted genetic material over time (Dawson 2011). Thus, another consideration is whether loss of inserted genetic material could alter the ability of CTV to cause disease. Greenhouse tests with citrus plants (*C. macrophylla*) inoculated with CTV engineered to produce green fluorescent protein (GFP) demonstrated the inserted genetic material may remain stable within the CTV vector and produce GFP for up to 4 years (Folimonov et al. 2007). However, in some cases all or part of the added genetic material may be lost (Folimonov et al. 2007). A complete loss of the inserted genetic material would generate a virus that is no different than those already present in Florida. Since a partial gene would not confer function, there is no reason to believe that partial deletion of the spinach defensin gene will affect the virulence, transmissibility, persistence, or host range of the CTV. In addition since adding a promoter is not likely to change the competitiveness of CTV in plants (Folimonov et al. 2007), even if an intact promoter is retained it is not likely to alter virulence. This because changing the levels of expression of a gene is not likely to have effects on the expression of other genes (Dawson 2011).

Therefore, it is not likely that loss of all or part of the inserted genetic material will alter the ability of the resulting CTV to cause disease.

For consideration is the chance CTV-SoD would be altered genetically during movement by aphids (Garnsey et al. 2005; Roy and Brlansky 2009). CTV is transferred by its aphid vector without the virus actually replicating inside the aphid, so there would be no opportunity for genetic change to occur within the aphid (Hull 2014b). Even if CTV did become altered over time by aphid transmission the resulting virus is expected to be similar to the strains currently in Florida, because the CTVSoD were created using CTV viruses widely prevalent in Florida.

Another consideration is the possibility of virus spread through interaction between CTV-SoD and other closely related or distantly related viruses present in a citrus plant. One such interaction, in addition to the potential genetic recombinants discussed in the section Potential to Create a New Problematic Virus, is the encapsidation of CTV-SoD generated RNA by the coat protein of another virus (heteroencapsidation) (de Zoeten 1991; Fuchs and Gonsalves 2007). Such an interaction might then result in a virus that is aphid transmissible. Field trial data from previously released transgenic plants containing coat proteins demonstrate that heteroencapsidation may occur at low rates (Fuchs and Gonsalves 2007). However, even if heteroencapsidation and transmission did occur, subsequent virus replication would only produce viruses of the original parental isolate. Since the genome of the new virus is not changed this would result in a genetic dead end for the heteroencapsidated product and the resulting virus would be CTV-SoD. Therefore, the introduced CTV-SoD would not be substantively different than the CTV currently present in Florida.

Taking into account the above information, because the CTV-SoD are derived from stains already widely present, and the introduced CTV-SoD is not expected to be substantively different than the CTV currently present in Florida, release of the CTV-SoD is unlikely to result in a new problematic virus in Florida.

Change to the Ability to Control CTV

Since CTV is widely prevalent in Florida and has been so for a long time, researchers and growers have developed control measures that have allowed successful cultivation of citrus despite the presence of CTV (Roberts et al. 2016; Singerman and Burani-Arouca 2017). Genetic resistance and/or tolerance (where the virus multiplies with no noted symptom development or yield reduction) derived through traditional breeding techniques will continue to remain available to citrus growers (Hutchinson 1985; Garnsey et al. 1987b; Fang et al. 1998; Stover and Castle 2002; Febres et al. 2008; Moreno et al. 2008; Bar-Joseph et al. 2010; Soler et al. 2012; Roberts et al. 2016). There is no reason to believe the spinach defensin produced will have impact on the ability to control CTV.

C. Conclusion

APHIS has reviewed the information submitted in the permit application, supporting documents, and other relevant information to assess the plant pant pest risk of the genetically engineered CTV9R-SoD, CTVT30-SoD and CTV9R/T30-SoD (CTV-SoD) expressing spinach defensins for use in controlling HLB. APHIS concludes that the CTV-SoD is unlikely to pose a greater plant pest risk than CTV current present in Florida based on the following findings:

- CTV epidemiology and its mechanism of spread are well understood.
- CTV is widespread in the state of Florida, so the use of CTV-SoD in citrus groves in the state of Florida and would not expose Florida citrus to a new plant pest.
- The genetic material for the spinach defensin not expected be permanently retained so that the introduced virus will revert back to CTV strains already widely prevalent in Florida.
- No changes are expected in plant disease caused by CTV due to the cloning or presence of the spinach defensins expressed in CTV-SoD.
- The host range of the CTV is not expected to be impacted by the presence of the genetic material coding for spinach defensins.
- The potential for spread from the inoculated trees is low because CTV-SoD will be graft inoculated, not transmitted by pollen or seed, and lacks the ability to be vectored by aphids.
- Additionally, CTV-SoD is likely to be limited to the inoculated tree due to cross protection, which limits the ability of a strain to infect a plant already infected with a similar strain of the virus.
- The inserted genetic material is not expected to give CTV-SoD a selective advantage to increase survival and/or fitness. The CTV-SoD are expected to persist at similar levels as CTV currently present in Florida.
- Any recombination between CTV-SoD and viruses naturally present in the inoculated trees are likely to result in viruses which are similar to others already present in Florida which have resulted from natural recombination. The only difference would be the presence of the spinach defensin conferring resistance to plant pests.
- The CTV-SoD are not expected become aphid vectored by interaction with closely or distantly related viruses.

D. Draft Permit Conditions

DRAFT Supplemental Permit Conditions

1. This permit authorizes use of the regulated article only as described in the current permit, permit conditions, and associated design protocols/standard operating procedures (SOPs), and only at locations described in the current permit. If design protocols/SOPs are conflicting or conflict with the permit or permit conditions, the permit and permit conditions supersede the conflicting design protocols/SOPs and must be followed. This authorization for release under permit is valid for a period of three years from issuance. Plants inoculated with the regulated article must be treated as a regulated article. Until such time as APHIS determines otherwise, inoculated plants remain a regulated article even upon expiration of the permit and must continue to be treated as such.

2. Permittee must confirm and keep records that the genes of CTV are not altered or disrupted for each CTV cloned vector after its manufacture and prior to its first release.

3. Permittee must have a diagnostic probe and/or method to identify each CTV cloned vector after its manufacture and prior to its first release.

4. Permittee or designated Authorized Representatives (as defined in the SOPs) of the Permittee must keep records of where the regulated material is moved to and released into the environment. Permittee's records must be updated on a quarterly basis.

5. Standard permit condition #6 can be met by identifying regulated articles using field maps or planting records in lieu of individual labels on trees.

6. Permittee must provide BRS with the location of the Authorized Representatives (as defined in the SOPs) and Licensed Facilities (as defined in the SOPs) that are authorized to propagate plants containing the CTV cloned vectors. Authorized representatives must follow the permit conditions and the SOPs. BRS must be notified in writing of any proposed changes to the permit application or Design Protocols/Standard Operating Procedures (SOPs) submitted with the permit application; additional constructs (including any other identifying information, e.g., lines/events); and new authorized representatives/facilities. Changes to the issued permit, including Design Protocols/SOPs must be approved by BRS and usually require amendments. Questions should be directed to the USDA APHIS BRS Biotechnology Risk Analysis Programs (BRAP), Biotechnology Permit Services via phone at 301-851-3935 or electronically at BRSPermits@aphis.usda.gov.

7. Permittee must maintain records sufficient for APHIS to verify compliance with the procedures, processes, and safeguards used to prevent escape and dissemination of the regulated article, as specified in the current permit, permit conditions and associated design protocols/SOPs.

8. Reporting Unintended Effects under Standard Permit Condition 10(ii) :

   For purposes of this permit, the permittee will be deemed to have "found" any occurrence enumerated in standard permit condition 10(ii) upon notification (as defined by the SOPs) by the authorized representative (as defined by the SOPs), end user/grower (as defined by the SOPs) or another party of that occurrence.

   For purposes of standard permit condition 10(ii), written notification should be sent by the permittee by email to:
   By e-mail:
   BRSCompliance@aphis.usda.gov, or by mail to:
   Biotechnology Regulatory Services (BRS)
   Regulatory Operations Program
   USDA/APHIS
   4700 River Rd. Unit 91
   Riverdale, MD 20737

9. Reporting an Unauthorized or Accidental Release Under Standard Permit Condition 10(i):
REQUIRED:
APHIS must be notified verbally by the permittee immediately upon discovery and notified in writing within 24 hours of discovery in the event of any accidental or unauthorized release of the regulated article. Examples include, but are not limited to: loss during movement of the regulated article, release of an article with an unauthorized construct, and any other release or suspected release of a regulated article in an unauthorized location. For purposes of this permit, the permittee will be deemed to have "discovered" the event upon notification (as defined by the SOP) by the authorized representative or another party of that occurrence.

Call APHIS/BRS Compliance Staff at (301) 851-3935. Leave a verbal report on voicemail if the phone is not answered. Written notification must be sent to BRSCompliance@aphis.usda.gov. Additional instructions may be found at: https://www.aphis.usda.gov/aphis/ourfocus/biotechnology/sa_compliance_and_inspections/ct_compliance_incident

OPTIONAL:
In addition, as a resource to find out information and if you would like to speak immediately to APHIS personnel regarding the incident, call:

A) APHIS/BRS Regional Biotechnologist assigned in the region where the incident occurred: For Western Region, contact the Western Region Biotechnologist at (970) 494-7513; for Eastern Region, contact the Eastern Region Biotechnologist at (919) 855-7622

Or

B) APHIS/PPQ State Plant Health Director for the state where the incident occurred: The list of APHIS State Plant Health Directors is available at: http://www.aphis.usda.gov/wps/portal/aphis/home/?urile=wcm%3apath%3a%2Faphis_content_library%2Fsa_our_focus%2Fsa_plant_health%2Fsa_program_overview%2Fct_sphd

10. Reports, Notices, and Other Requirements

A) Environmental Release Report

Release Reports are required for all authorized field trial (as defined in the SOPs) sites. Release Reports for Field Trials must be submitted to BRS by the 15th of the month following the month in which the release occurred. Release reports for commercial tree plantings (as defined in the SOPs) must be submitted for the initial commercial release by the 15th of the month in which the first release occurred and then every 6 months for any future releases, and must include the following

i. Permit number
ii. Regulated article and list of all constructs released
iii. State
iv. County

v. Location Name (Unique Grove/Block identifier)
vi. GPS coordinates, in decimal degrees for the northwest corner of the release
vii. Planting date
viii. Number of trees planted
ix. Type of planting (solid set, resets, etc.)

1. Planting date and location information will suffice as a Planting Unique ID for a given location. For contiguous days of planting, the starting date will be used as the planting date for the Unique ID

B. Termination Notice

If a planting is a field test a termination notice must be submitted as follows:

At least ten (10) calendar days prior to anticipated termination, a notice must be emailed to brscompliance@aphis.usda.gov indication the planned date of the termination and the contact information for each site.

C) Field Test Report under Standard Permit Condition 9

If planting is a commercial planting (as defined by the SOPs) (i.e., a planting primarily intended for production of fruit for commerce) no field test report is required.

If planting is a field test (i.e., field trial), a field test report must be submitted for any site where a field test has been terminated. The report must be submitted within six months after the termination of the field test.

Field Test Reports provide the final status and observations at each location and must include:
- Permit number
- State
- County
- Location Name (Unique Grove/Block identifier)
- Regulated article and a list of all constructs releases at the location
- GPS coordinates for the release
- Size of the release (in acres) at each location
- Provide the termination date and describe how the regulated material was terminated
- If material was removed from the field and terminated off site describe how it was disposed and provide the date of off-site destruction.
- If material was removed from the field and placed in storage, provide the amount of material that was stored and provide a description of the storage location
- Describe any other disposition methods that may be applicable
- Describe any deleterious effects on plants, non-target organisms, or the environment
- Describe methods of observations and resulting data and analyses
- Indicate if you have submitted any of the following:

1. A report on the accidental or unauthorized release of the regulated article;
2. A report that characteristics of the permitted species are substantially different from those listed in the application; or
3. A report of any unusual occurrence

We encourage the inclusion of other types of data if the applicant anticipates submission of a petition for determination of non-regulated status for their regulated article.
APHIS considers these data reports as critical to our assessment of plant pest risk and development of regulatory policies based on the best scientific evidence. Failure by an applicant to provide data reports in a timely manner for a field trial may result in the withholding of permission by APHIS for future field trials.

Reports and notices can be submitted via email or mail to:

Email: BRSCompliance@aphis.usda.gov
Mail:
Animal and Plant Health Inspection Service (APHIS)
Biotechnology Regulatory Services (BRS)
Document Control Officer
4700 River Rd. Unit 146
Riverdale, MD 20737

Standard Permit Conditions for the Introduction of a Regulated Article
(7 CFR 340.4 (f))
Permit Conditions: A person who is issued a permit and his/her employees or agents shall comply with the following conditions, and any supplemental conditions which shall be listed on the permit, as deemed by the Deputy Administrator to be necessary to prevent the dissemination and establishment of plant pests:

(1) The regulated article shall be maintained and disposed of (when necessary) in a manner so as to prevent the dissemination and establishment of plant pests.

(2) All packaging material, shipping containers, and any other material accompanying the regulated article shall be treated or disposed of in such a manner as to prevent the dissemination and establishment of plant pests.

(3) The regulated article shall be kept separate from other organisms, except as specifically allowed in the permit.

(4) The regulated article shall be maintained only in areas and premises specified in the permit.

(5) An inspector shall be allowed access, during regular business hours, to the place where the regulated article is located and to any records relating to the introduction of a regulated article.

(6) The regulated article shall, when possible, be kept identified with a label showing the name of the regulated article, and the date of importation.

(7) The regulated article shall be subject to the application of measures determined by the Administrator to be necessary to prevent the accidental or unauthorized release of the regulated article.

(8) The regulated article shall be subject to the application of remedial measures (including disposal) determined by the administrator to be necessary to prevent the spread of plant pests.

(9) A person who has been issued a permit shall submit to APHIS a field test report within 6 months after the termination of the field test. A field test report shall include the APHIS reference number, methods of observation, resulting data, and analysis regarding all deleterious effects on plants, nontarget organisms, or the environment.

(10) APHIS shall be notified within the time periods and manner specified below, in the event of the following occurrences:

(i) Orally notified immediately upon discovery and notify in writing within 24 hours in the event of any accidental or unauthorized release of the regulated article;

(ii) In writing as soon as possible but not later than within 5 working days if the regulated article or associated host organism is found to have characteristics substantially different from those listed in the application for a permit or suffers any unusual occurrence (excessive mortality or morbidity, or unanticipated effect on non-target organisms).

(11) A permittee or his/her agent and any person who seeks to import a regulated article into the United States shall:

(i) Import or offer the regulated article for entry only through any USDA plant inspection station listed in 7 CFR 319.37-14;

(ii)Notify APHIS promptly upon arrival of any regulated article at a port of entry, of its arrival by such means as a manifest, customs entry document, commercial invoice, waybill, a broker's document, or a notice form provided for such purpose; and

(iii) Mark and identify the regulated article in accordance with 7 CFR 340.7.

E. References

51 FR 23302. 1986. *Coordinated Framework for Regulation of Biotechnology*.

57 FR 22984. 1992. *Statement of Policy: Foods Derived from New Plant Varieties*.

82 FR 17179. 2017. *Southern Gardens Citrus Nursery, LLC; Notice of Intent To Prepare an Environmental Impact Statement for Permit for Release of Genetically Engineered Citrus tristeza virus*.

AIBS. 1995. *Transgenic Virus-Resistant Plants and New Plant Viruses*. American Institute of Biological Sciences Workshop.

Albiach-Marti MR. 2013. The Complex Genetics of Citrus tristeza virus. In: *Current Issues in Molecular Virology - Viral Genetics and Biotechnological Applications, V. Romanowski (ed.)* (InTech), pp. 1-25.

Albiach-Marti MR, Robertson C, Gowda S, and et al. 2010. *The pathogenicity determinant of Citrus tristeza virus causing the seedling yellows syndrome maps at the 3'-terminal region of the viral genome*. Molecular Plant Pathology 11, pp. 55-67.

Ambros S, El-Mohtar C, Ruiz-Ruiz S, and et al. 2011. *Agroinoculation of Citrus tristeza virus causes systemic infection and symptoms in the presumed nonhost Nicotiana benthamiana*. Molecular Plant-Microbe Interactions 24, pp. 1119-1131.

Ambros S, Ruiz-Ruiz S, Pena L, and et al. 2013. *A genetic system for Citrus tristeza virus using the non-natural host Nicotiana benthamiana: an update*. Frontiers in Microbiology 4 Article 165, pp. 1-14.

Atta S, Zhou C, Zhou Y, and et al. 2012. *Distribution and research advances of Citrus tristeza virus*. Journal of Integrative Agriculture 11, pp. 346-358.

Ayllón MA, Rubio L, Moya A, and et al. 1999. *The haplotype distribution of two genes of Citrus tristeza virus is altered after host change or aphid transmission*. Virology 255, pp. 32-39.

Baldwin E, Bai J, Plotto A, and et al. 2014. *Citrus fruit quality assessment: producer and consumer perspectives*. Stewart Postharvest Review 2:1, pp. 1-7.

Bar-Joseph M. 1989. *The continuous challenge of Citrus tristeza virus control*. Annual Review of Phytopathology 27, pp. 291-316.

Bar-Joseph M and Loebenstein G. 1973. *Effect of strain, source plant, and temperature on the transmissibility of Citrus tristeza virus by the melon aphid*. Phytopathology 63, pp. 716-720.

Bar-Joseph M, Garnsey SM, and Gonsalves D. 1979. *The Closteroviruses: A distinct group of elongated plant viruses*. Advances in Virus Research 25, pp. 93-168.

Bar-Joseph M, Batuman O, and Roistacher CN. 2010. The History of Citrus triseza virus - Revisited. In: *Citrus tristeza virus Complex and Tristeza Diseases, A.V. Karasey & M.E. Hilf (eds.), American Phytopathological Society, St. Paul, MN*, pp. 3-26.

Bove J. 2006. *Huanglongbing: A destructive, newly-emerging, century-old disease of citrus*. Journal of Plant Pathology 88, pp. 7-37.

Brlansky RH, Pelosi RR, Garnsey SM, and et al. 1986. *Tristeza quick decline epidemic in South Florida*. Florida State Horticultural Society Proceedings 99, pp. 66-69.

Broadbent P. 1995. *Quarantine in relation to Australian citrus imports and exports*. Australasian Plant Pathology 24, pp. 145-156.

Broekaert WF, Terras FRG, Cammue BPA, and et al. 1995. *Plant defensins: novel antimicrobial peptides as componenets of the host defense system*. Plant Physiology 108, pp. 1353-1358.

Brown L and Davison D. 1997. *Citrus tristeza virus survey in Florida: phase one results*. International Organization of Citrus Virologists Newsletter 14, pp. 17-19.

Bujarski JJ. 2013. *Genetic recombination in plant-infecting messenger-sense RNA viruses: overview and research perspectives*. Frontiers in Plant Science 4 Article 68, pp. 1-9.

CABI. 2015. *Citrus tristeza virus (grapefruit stem pitting)*. Wallingford, UK. Crop Protection Compendium, Centre for Agriculture and Biosciences International.

Camp AF, DuCharme EP, and Knorr LC. 1953. *Tristeza Information for Florida Citrus Growers*. State Plant Board of Florida, Bulletin 2.

Carvalho AO and Gomes VM. 2009. *Plant defensins-Prospects for the biological functions and biotechnological properties*. Peptides 30, pp. 1007-1020.

Castle WS, Bowman KD, Grosser JW, and et al. 2016. *Florida Citrus Rootstock Selection Guide, 3rd Edition*. University of Florida, Institute of Food and Agricultural Sciences, HS1260.

Cohen M. 1956. *Injury and loss of citrus trees due to tristeza disease in an Orange County grove*. Florida State Horticultural Society Proceedings 69, pp. 19-24.
Cohen M and Burnett HC. 1961. *Tristeza in Florida*. International Organization of Citrus Virologists Conference Proceedings, pp. 107-112.
Cools T, Struyfs C, Cammue BPA, and et al. 2017. *Antifungal plant defensin: increased insight in their mode of action as a basis for theri use to combat fungal infections*. Future Microbiology 12, pp. 441-453.
Costa AS and Grant TT. 1951. *Studies on transmission of the Tristeza virus by the vector, Aphis citricidus*. Phytopathology 41, pp. 105-113.
Davies FS and Jackson LK. 2009. *Citrus Growing in Florida, pp 310*. Gainsville, FL: University Press of Florida.
Dawson WO. 2011. *A personal history of virus-based vector construction*. Current Topics in Microbiology and Immunology 375, pp. 1-18.
Dawson WO and Hilf ME. 1992. *Host-range determinants of plant viruses*. Annual Review of Phytopathology 43, pp. 527-555.
Dawson WO and Folimonova SY. 2013. *Virus-based transient expression vectors for woody crops: a new frontier for vector design and use*. Annual Review of Phytopathology 51, pp. 321-337.
Dawson WO, Garnsey SM, Tatineni S, and et al. 2013. *Citrus tristeza virus-host interactions*. Frontiers in Microbiology 4 (88), pp. 1-10.
Dawson WO, Robertson CJ, Albiach-Marti MR, and et al. 2015a. *Mapping sequences involved in induction of decline by Citrus tristeza virus T36 on the sour orange rootstock.* Journal of Citrus Pathology doi: 10.3389/fmicb.2013.00088.
Dawson WO, Bar-Joseph M, Garnsey SM, and et al. 2015b. *Citrus tristeza virus: making an ally from an enemy*. Annual Review of Phytopathology 53, pp. 137-155.
De Coninck B, Cammue B, and K T. 2013. *Modes of antifungal action and in planta functions of plant defensins and defensin-like peptides*. Fungal Biology Reviews 26, pp. 109-120.
de Zoeten G. 1991. *Risk assessment: Do we let history repeat itself*. Phytopathology 81, pp. 585-586.
Dias R and Franco O. 2015. *Cysteine-stabilized αß defensins: From a common fold to antibacterial activity*. Peptides 72, pp. 64-72.
Dohm JC, Minoche AE, Hotgrawe D, and et al. 2014. *The genome of the recently domesticated crop plant sugar beet (Beta vulgaris)*. Nature 505, pp. 546-549.
EPA. 2011. *Cydia pomonella granulovirus EPA Pesticide Registration*
European Food Safety Authority. 2017. *Pest categorisation of Citrus tristeza virus (non-Euopean isolates)*.
Fagen JR, Leonard MT, Coyle JF, and et al. 2014. *Liberibacter crescens gen. nov., sp. nov., the first cultured member of the genus Liberibacter*. International Journal of Systematic and Evolutionary Microbiology 64, pp. 2461-2466.
Falk B and Bruening G. 1994. *Will transgenic crops generate new viruses and new diseases*. Science 263, pp. 1395-1396.
Fang DQ, Federici CT, and Roose ML. 1998. *A high-resolution linkage map of the Citrus triseza virus resistance gene region in Poncirus trifoliata (L.) Raf.* Genetics 150, pp. 883-890.
Fasulo TR and Halbert SE. 2015. *Aphid Pests of Florida Citrus, University of Florida, Institute of Food and Agricultural Sciences Extension, ENY811*.

Febres VJ, Lee RF, and Moore GA. 2008. *Transgenic resistance to Citrus tristeza virus in grapefruit*. Plant Cell Rep. 27, pp. 93-104.
Fishman S, Marcus R, Talpaz H, and et al. 1983. *Epidemiological and economic models for spread and control of citrus tristeza virus disease*. Phytoparasitica 11, pp. 39-49.
Florida Administrative Code Rule Chapter 5B-62. 2015. *Citrus Nursery Stock Certification Program*.
Florida Department of Agriculture and Consumer Services. 2016. *Citrus Budwood Annual Report 2015-2016*.
Folimonov AS, Folimonova SY, Bar-Joseph M, and et al. 2007. *A stable RNA virus-based vector for citrus trees*. Virology 368, pp. 205-216.
Folimonova SY and Achor DS. 2010. *Early event of citrus greening (Haunglongbing) disease development at the ultrastructural level*. Phytopathology 100, pp. 949-958.
Franco O. 2011. *Peptide promiscuity: An evolutionary concept for plant defense*. FEBS Letters 585, pp. 995-1000.
Frost HB and Soost RK. 1968. Seed Reproduction: Development of Gametes and Embryos. In: *The Citrus Industry Vol. II, W. Reuther, L.D. Batchelor, H.J. Webber (eds.)* (University of California), pp. 290-324.
Fuchs M and Gonsalves D. 2007. *Safety of virus-resistant transgenic plant two decases after their introduction: lessons from realistic field risk assessment studies*. Annual Review of Phytopathology 45, pp. 173-202.
Garnsey S, Lee RF, Youtsey CO, and et al. 1980. *A survey for citrus tristeza virus in registered budwood sources commercially propagated on sour orange rootstocks in Florida*. Proceedings of the Florida State Horticultural Society 93, pp. 7-9.
Garnsey S, Gumpf DJ, Roistacher CN, and et al. 1987a. *Toward a standardization evaluation of the biological properties of Citrus tristeza virus*. Phytophylactica 19, pp. 151-157.
Garnsey S, Civerolo EL, Gumpf DJ, and et al. 1999. *Development of a worldwide collection of citrus tristeza virus isolates*. International Organization of Citrus Virologists Conference Proceedings, pp. 113-120.
Garnsey SM and Jackson JL. 1975. *A destructive outbreak of tristeza in Central Florida*. Florida State Horicultural Society 88, pp. 65-69.
Garnsey SM, Barrett HC, and Hutchinson DJ. 1987b. *Identification of Citrus tristeza virus resistance in relatives and its potential applications*. Phytophylactica 19, pp. 187-191.
Garnsey SM, Su HF, and Tsai MC. 1996. *Differential susceptibility of pummelo and swingle citrumelo to isolates of Citrus tristeza virus*. International Organization of Citrus Virologists Conference Proceedings, pp. 138-146.
Garnsey SM, Permar TA, Cambra M, and et al. 1993. *Direct tissue blot immunoassay (DTBIA) for detection of Citrus Tristeza Virus (CTV)*. International Organization of Citrus Virologists Conference Proceedings, pp. 39-50.
Garnsey SM, Civerolo EL, Gumpf DJ, and et al. 2005. *Biological characterization of an international collection of citrus tristeza virus (CTV) isolates*. International Organization of Citrus Virologists Conference Proceedings, pp. 75-93.
GenBank. 2017. *BLAST*.
Gottwald TR and Garnsey SM. 1997. *The influence of aphid species and biology on the spread of CTV*. Annual Meeting APS-Caribbean Division San Jose, Nov. 10-12.

Gottwald TR, Garnsey SM, and Yokomi RK. 1993a. *Potential for spread of citrus tristeza virus and its vector the brown citrus aphid*. Proceedings of the Florida State Horticultural Society 106, pp. 85-94.
Gottwald TR, Cambra M, and Moreno P. 1993b. *The use of serological assays to monitor spatial and temporal spread of Citrus tristeza virus in symptomless trees in Eastern Spain*. International Organization of Citrus Virologists Conference Proceedings, pp. 51-61.
Gottwald TR, Garnsey SM, and Yokomi RK. 1996. *Studies on the increase and spread of Citrus tristeza virus in the presence of the brown citrus aphid and the implications for the U.S. citrus industry*. Citrus Industry 77, pp. 41-58.
Gottwald TR, Garnsey SM, and Riley TK. 2002. *Latency of systemic infection in young field-grown sweet orange trees following graft-inoculation with citrus tristeza virus*. International Organization of Citrus Virologists Conference Proceedings, pp. 48-53.
Gottwald TR, Gibson GJ, Garnsey SM, and et al. 1999. *Examination of the effect of aphid vector population composition on the spatial dynamics of citrus tristeza virus spread by stochastic modeling*. Phytopathology 89, pp. 603-608.
Gottwald TR, Garnsey S, Irey M, and et al. 2013. *An Overview of the Epidemiology of Citrus Tristeza Virus*. Fort Pierce, FL.
Gowda S, Satyanarayana T, Robertson CJ, and et al. 2005. *Infection of citrus plants with virions generated in Nicotiana benthamiana plants Agroinfiltrated with a binary vector based citrus tristeza virus*. International Organization of Citrus Virologists Conference Proceedings, pp. 23-33.
Grant TJ. 1949. *Studies in tristeza disease of citrus in Brazil*. Proceedings of the Florida State Horticultural Society 62, pp. 72-79.
Grant TJ. 1952. *Evidence of tristeza, or quick decline, virus in Florida*. Florida State Horicultural Society 65, pp. 28-31.
Grant TJ and Costa AS. 1948. *A progress report on studies of tristeza disease of citrus in Brazil*. Proceedings of the Florida State Horticultural Society 61, pp. 20-33.
Grant TJ and Schneider H. 1953. *Initial evidence of the presence of tristeza or quick decline of citrus in Florida*. Phytopathology 43, pp. 51-52.
Guerrieri E and Digilio MC. 2008. *Aphid-plant interactions: a review*. Journal of Plant Interactions 3, pp. 223-232.
Halbert SE. 1995. Trilogy Vol. 34, Entomology Section. Florida Department of Agriculture and Consumer Services, Division of Plant Industry.
Halbert SE. 1996. Trilogy Vol. 35, Entomology Section. Florida Department of Agriculture and Consumer Services, Division of Plant Industry.
Halbert SE and Brown LG. 1998. *Toxoptera citricida (Kirkaldy), Brown Citrus Aphid-Identificatiton, Biology, and Managment Strategies*. Florida Department of Agriculture & Consumer Services, Division of Plant Industry, Entomology Circular No. 374.
Halbert SE and Manjunath KL. 2004. *Asian citrus psyllids (Sternorrhyncha: Psyllidae) and greening disease of citrus: a literature review and assessment of risk in Florida*. Florida Entomologist 87, pp. 330-353.
Halbert SE, Genc H, Cevik B, and et al. 2004. *Distribution and Characterization of Citrus tristeza virus in South Florida Following Establishment of Toxoptera citricida*. Plant Disease 88, pp. 935-941.

Harper SJ. 2013. *Citrus tristeza virus: evolution of complex and varied genotypic groups*. Frontiers in Microbiology 4 (93), pp. 1-18.
Harper SJ and Cowell SJ. 2016. *The past and present status of Citrus tristeza virus in Florida*. Journal of Citrus Pathology 3, pp. 1-6.
Harper SJ, Cowell SJ, and Dawson WO. 2017. *Isolate fitness and tissue tropism determine superinfection success*. Virology 511, pp. 222-228.
Harper SJ, Cowell SJ, Halbert SE, and et al. 2015. *CTV status in Florida*. Citrus Industry Magazine April, pp. 8-12.
Harper SJ, Killiny N, Tatineni S, and et al. 2016. *Sequence variation in two genes determines the efficacy of transmission of citrus tristeza virus by the brown citrus aphid*. Achives of Virology, pp. 3555-3559.
Hilf ME. 2010. *Two distinct evolutionary pathways for Citrus tristeza virus: recombination defines two gene modules and provides for increased genetic diversity in a narrow host range plant virus. (Abstract).* International Organization of Citrus Virologists Conference Proceedings, p. 248.
Hilf ME and Garnsey SM. 2002. *Citrus tristeza virus in Florida: a synthesis of historical and contemporary biological, serological, and genetic data*. International Organization of Citrus Virologists Conference Proceedings, pp. 13-20.
Hodges AW and Spreen TH. 2012. *Economic Impacts of Citrus Greening (HLB) in Florida*. University of Florida, Institute of Food and Agriculture.
Hughes WA and Lister CA. 1949. *Lime disease in the Gold Coast*. Nature 154, pp. 880.
Hull R. 2014a. Symptoms and Host Range, in Plant Virology, Fifth edition, pp. 145-197.
Hull R. 2014b. *Plant Virology, Fifth Edition, 687 pp.* New York, NY: Elsevier.
Hutchinson DJ. 1985. *Rootstock development screening and selection for disease tolerance and horticultural characteristics*. Fruit Varieties Journal 39, pp. 21-25.
Karasev AV, Nikolaeva OV, Koonin EV, and et al. 1994. *Screening of the closteroviurs genome by degenerative primer-mediated polymerase chain reaction*. Journal of General Virology 75, pp. 1415-1422.
Karasev AV, Boyko VP, Gowda S, and et al. 1995. *Complete sequence of the Citrus tristeza virus RNA genome*. Virology 208, pp. 511-520.
Kaur J, Sagaram U, and Shah D. 2011. *Can plant defensins be used to engineer durable commercially useful fungal resistance in crop plants?* Fungal Biology Reviews 25, pp. 128-135.
Killiny N, Harper SJ, Alfaress S, and et al. 2016. *Minor coat and heat-shock proteins are involved in binding of citrus tristeza virus to the foregut of its aphid vector, Toxoptera citricida*. Applied and Environmental Microbiology AEM.01914-16.
King AMQ, Lefkowitz E, Adams MJ, and et al. 2012. *Virus taxonomy : Classification and Nomenclature of Viruses : Ninth Report of the International Committee on Taxonomy of Viruses*. London, Academic Press. International Union of Microbiological Societies, Virology Division.
Knorr LC. 1956. *Suscepts, indicators, and filters of tristeza virus, and some differences beteween tristeza in Argentina and in Florida*. Phytopathology 46, pp. 557-560.
Kong P, Rubio L, Polek M, and et al. 2000. *Population structure and genetic diversity within California Citrus tristeza virus (CTV) isolates*. Virus Genes 21, pp. 139-145.

Lai MM. 1992. *RNA recombination in animal and plant viruses*. Microbiological Reviews 56, pp. 61-79.
Lange M, Yellina AL, Orashakova S, and et. al. 2013. *Virus-Induced Gene Silencing (VIGS) in Plants: An Overview of Target Species and the Virus-Derived Vector Systems*. Methods in Molecular Biology 975, pp. 1-12.
Lee JG and Rocha-Pena MA. 1992. Citrus tisteza virus. In: *Plant Disease of International Importance Vol. 3, A. Mukhopadhyay (eds.)* (Englewood Cliffs, NJ: Prentice Hall), pp. 226-249.
Lee R, Barber B, Brown L, and et al. 1997. *Is Meyer lemon a threat to citrus?* Citrus Industry Magazine 78, pp. 46-50.
Lee RF and Bar-Joseph M. 2000. Tristeza. In: *Compendium of Citrus Diseases, L.W. Timmer, S.M. Garnsey & J.G. Graham (eds.)* (St. Paul, MN: American Phytopathological Society), pp. 61-63.
Lu R, Folimonov A, Shintaku M, and et al. 2004. *Three distinct suppressors of RNA silencing encoded by a 20-kb viral RNA genome*. Proceeding of the National Academy of Sciences 101, pp. 15742-15747.
Martin S, Sambade A, Rubio L, and et al. 2009. *Contribution of recombination and selection to molecular evolution of Citrus tristeza virus*. Journal of General Virology 90, pp. 1527-1538.
Mascia T and Gallitelli D. 2016. *Synergies and antagonisms in virus interactions*. Plant Science 252, pp. 176-192.
Mawassi M, Mietkiewska E, Gofman R, and et al. 1996. *Unusual sequence relationships between two isolates of citrus tristeza virus*. Journal of General Virology 77, pp. 2359-2364.
McClean APD. 1957. *Tristeza virus of citrus: evidence for absence of seed transmission*. Plant Disease Reporter 41, pp. 821.
McClean APD. 1974. *The tristeza virus complex*. International Organization of Citrus Virologists Conference Proceedings, pp. 59-66.
McClean APD. 1975. *Tristeza virus complex: its transmission by the aphid Toxoptera citricidus*. Phytophylactica 7, pp. 109-114.
Mink GI. 1993. *Pollen and seed transmitted viruses and viroids*. Annual Review of Phytopathology 31, pp. 375-402.
Moreno P and Garnsey SM. 2010. Citrus Tristeza Diseases- A Worldwide Perspective. In: *Citrus tristeza virus Complex And Tristeza Diseases, A.V. Karasev, M.E. Hilf (eds.)* (St. Paul, MN: American Phytopathological Society), pp. 27-49.
Moreno P, Ambros S, Albiach-Marti MR, and et al. 2008. *Citrus tristeza virus: a pathogen that changed the course of the citrus industry*. Molecular Plant Pathology 9, pp. 251-268.
Muller GW and Garnsey SM. 1984. *Susceptibility of citrus varieties, species, citrus relatives, and non-rutaceous plants to slash-cut mechanical inoculation with citrus tristeza virus (CTV)*. International Organization of Citrus Virologists Conference Proceedings, pp. 33-40.
Muller GW, Costa AS, Kitajima EW, and et al. 1974. *Additional evidence that tristeza virus multiplies in Passiflora spp.* International Organization of Citrus Virologists Conference Proceedings, pp. 75-78.
National Academy of Sciences. 2010. *Strategic Planning for the Florida Citrus Industry: Addressing Citrus Greening*. Washington, DC: National Academy of Sciences.

Nelson S, Melzer M, and Hu J. 2011. *Citrus Tristeza Virus in Hawai'i*. University of Hawai'i at Manoa. College of Tropical Agriculture and Human Resources, PD-77.
Norman G, Price WC, and Grant TJ. 1961. *Ten years of tristeza in Florida*. Florida State Horicultural Society 74, pp. 107-111.
Norman PA and Grant TJ. 1956. *Transmission of tristeza virus by aphids in Florida*. Florida State Horicultural Society 69, pp. 38-42.
Organic Materials Review Institute. 2017. *OMRI Products List*. Retrieved from https://www.omri.org/sites/default/files/opl_pdf/CompleteCompany-NOP.pdf, accessed 6/29/17
Pappu HR, Karasev AV, Anderson EJ, and et al. 1994. *Nucleotide sequence and organization of eight 3' open reading frames of the citrus tristeza closterovirus genome*. Virology 199, pp. 35-46.
Pelegrini P, del Sarto R, Silva O, and et al. 2011. *Antibiacterial peptides from plants: what they are and how they probably work*. Biochemistry Research International 2011:250349, pp. 1-9.
Pelosi RR, Killer EE, and Bullock RC. 1996. *Aphid populations in a Florida citrus tristeza virus supression trial*. Proceedings of the Florida State Horticultural Society 109, pp. 69-72.
Permar TA, Garnsey SM, Gumpf DJ, and et al. 1990. *A monolonal antibody that discriminates strains of citrus tristeza virus*. Phytopathology 80, pp. 224-228.
Pogue GP, Lindbo JA, Garger SJ, and et al. 2002. *Making an ally from an enemy: Plant virology and the new agriculture*. Annual Review of Phytopathology 40, pp. 45-74.
Powell CA and Pelosi RR. 1993. *Prevalence of severe strains of citrus tristeza virus in Florida citrus nurseries*. HortScience 28, pp. 699-700.
Powell CA, Pelosi RR, and Bullock RC. 1997. *Natural field spread of mild and severe isolates of Citrus tristeza virus in Florida*. Plant Disease 81, pp. 18-20.
Powell CA, Lin Y, and Brlansky RH. 1999. *Transmission of mild and decling-inducing isolates of citrus tristeza virus by the brown citrus aphid*. Phytopathology 89 No. 6 Supplement, pp. S61.
Powell CA, Burton MS, Pelosi RR, and et al. 2006. *Six-year evaluation of brown citrus and spirea aphid populations in a citrus grove and the effects of insecticides on these populations.* HortScience 41, pp. 688-690.
Pringle CR. 1996. *Virus Taxonomy 1996 - A bulletin from the Xth International Congress of Virology in Jerusalem*. Archives of Virology 141, pp. 2251-2256.
Raccah B, Roistacher CN, and Barbagallo S. 1989. Semiperistant transmission of viruses by vectors with special emphasis on Citrus tristeza virus. In: *Advances in Disease Vector research, Vol. 6* (New York, NY: Springer-Verlag), pp. 301-340.
Roberston D. 2004. *VIGS Vectors for gene silencing: many targets, many tools*. Annual Review of Plant Biology 55, pp. 495-519.
Roberts PD and et al. 2017. *2017-2018 Florida Citrus Production Guide, pp. 137-138, Tristeza*. University of Florida, Institute of Food and Agricultural Sciences.
Roberts PD, Hilf ME, Sieburth PJ, and et al. 2016. *2016 Florida Citrus Pest Managment Guide: Ch.25 Tristeza*. University of Florida IFAS Extension.
Robertson CJ, Garnsey SM, Satyanarayana T, and et al. 2005. *Efficient infection of citrus plants with different cloned constructs of citrus tristeza virus amplified in Nicotiana benthamiana*

protoplasts. International Organization of Citrus Virologists Conference Proceedings, pp. 187-195.
Rocha-Peña MA, Lee RF, Lastra R, and et al. 1995. *Citrus tristeza virus and its aphid vector Toxoptera citricida: Threats to citrus production in the Caribbean and Central and North America*. Plant Disease 79, pp. 437-445.
Roistacher CN and Bar-Joseph M. 1987. *Aphid transmission of citrus tristeza virus: a review*. Phytophylactica 19, pp. 163-167.
Roistacher CN and Moreno P. 1991. *The worldwide threat from destructive isolates of citrus tristeza virus-a review*. International Organization of Citrus Virologists, Proceedings pp. 7-19.
Roy A and Brlansky RH. 2009. *Population dynamics of a Florida Citrus tristeza virus isolate and aphid-transmitted subisolates: identification of three genotypic groups and recombinants after aphid transmission*. Phytopathology 99, pp. 1297-1306.
Rubio L, Guerri J, and Moreno A. 2013. *Genetic variability and evolutionary dynamics of viruses of the family Closeroviridae*. Frontiers in Microbiology 4 Article 151, pp. 1-15.
Rubio L, Ayllon MA, Kong P, and et al. 2001. *Genetic variation of Citrus tristeza virus isolated from California and Spain: evidence for mixed infections and recombination*. Journal of Virology 75, pp. 8054-8062.
Salibe AA. 1977. *The stem-pitting effects of tristeza on different citrus hosts and their economic significance*. Proceedings of the International Society of Citriculture 3, pp. 953-955.
Satyanarayana T, Gowda S, Boyko VP, and et al. 1999. *An engineered closterovirus RNA replicon and analysis of heterologous terminal sequences for replication*. Proceeding of the National Academy of Sciences 96, pp. 7433-7438.
Satyanarayana T, Bar-Joseph M, Mawassi M, and et al. 2001. *Amplification of Citrus tristeza virus from a cDNA clone and infection of citrus trees*. Virology 280, pp. 87-96.
Satyanarayana T, Gowda S, Ayllón MA, and et al. 2003. *Frameshift mutations in infectious cDNA clones of Citrus tristeza virus: a strategy to minimize the toxicity of viral sequences to Escherichia coli*. Virology 313, pp. 481-491.
Schneider H. 1968. The Anatomy of Citrus. In: *The Citrus Industry Vol. II* (University of California), pp. 1-85.
Scott KA, Hlela Q, AZablocki O, and et al. 2013. *Genotype composition of populations of grapefruit-cross-protecting plant and aphid-transmitted sub-isolates*. Achives of Virology 158, pp. 27-37.
Segura A, Moreno M, Molina A, and et al. 1998. *Novel defensin subfamily from spinach (Spinacia oleracea)*. FEBS Letters 435, pp. 159-162.
Shafee T, Lay F, Phan T, and et al. 2017. *Convergent evolution of defensin sequence, structure and funtion*. Cellular and Molecular Life Sciences 74, pp. 663-682.
Sieburth PJ. 2000. *Pathogen testing in the Florida mandatory citrus budwood protection program*. Conference International Organization of Citrus Virologists Conference Proceedings, pp. 408-410.
Sieburth PJ and Nolan KG. 2005. *Survey of stem petting citrus tristeza virus in commercial citrus groves in Florida*. Proceedings of the Florida State Horticultural Society 118, pp. 40-42.
Singerman A and Burani-Arouca M. 2017. *Evolution of Citrus Disease Management Programs and Their Economic Implications: The Case of Florida's Citrus Industry*. University of Florida, Institute of Food and Agriculture Sciences, FE915

Soler N, Plomer M, Fagoaga C, and et al. 2012. *Transformation of Mexian lime with an intron-hairpin construct expressing untranslatable versions of the genes coding for the three silencing suppressors of Citrus tristeza virus confers complete resistance to the virus*. Plant Biotechnology Journal 10, pp. 597-608.

Stansly P, Rouse B, and Roberts P. 1999. *Citrus tristeza heats up in Southwest Florida*. Citrus & Vegetable Magazine February, pp. 8-10.

Stansly PA and Rogers ME. 2017. *2017-2018 Florida Citrus Production Guide: Soft-Bodied Insects Attacking Foliage and Fruit, pp. 87-89*. University of Florida, Institute of Food and Agricultural Sciences.

Stover E and Castle W. 2002. *Citrus Rootstock Usage, Characteristics, and Selection in the Florida Indian River Region*. HortTechnology 12, pp. 143-147.

Stover E, Stange RR, McCollum TG, and et al. 2013. *Screening antimicrobial peptides In Vitro for use in developing transgenic citrus resistant to Huanglongbing and citrus canker*. Journal of the Americal Society of Horticultural Science 138, pp. 142-148.

Stubbs LL. 1964. *Tansmission and protective inoculation studies with viruses of the citrus tristeza complex*. Australian Journal of Agricultural Research 15, pp. 752-770.

Tatineni S and Dawson WO. 2012. *Enhancement or attenuation of disease by deletion of genes from Citrus tristeza virus*. Journal of Virology 86, pp. 7850-5857.

Tepfer M. 2002. *Risk assessment of virus-resistant transgenic plants*. Annual Review of Phytopathology 30, pp. 467-491.

Tepfer M, Jacquemond M, and Garcia-Arenal F. 2015. *A critical evaluation of whether recombination in virus-resistant transgenic plants will lead to the emergence of novel viral diseases*. New Phytologist 207, pp. 537-541.

Thomma B, Cammue B, and Thevissen K. 2002. *Plant defensins*. Planta 216, pp. 193-202.

Thompson JR and Tepfer M. 2010. Assessment of the Benefits and Risk for Engineered Virus Resistance. In: *pp. 33-56, Advances in Virus Research, J.P. Carr, G. Loebenstein (eds.)* (Academic Press).

Title 3 California Code of Regulations § 3407. *Citrus Tristeza Virus Interior Quarantine*.

Tsuchizaki T, Sasaki A, and Saito Y. 1978. *Purification of citrus tristeza virus from diseased citrus fruits and the detection of the virus in citrus tissues by fluorescent antibody techniques*. Phytopathology 68, pp. 139-142.

University of Florida AES. 1956. *Distribution and rate of spread of tristeza*.

USDA APHIS PPQ. 2015. *Global pest list of Citrus spp. pathogens and an examination of evidence for seed transmission*. Raleigh, NC. United States Department of Agriculture, Animal Plant Health Inspection Service, Plant Protection and Quarantine.

USDA APHIS PPQ. 2017. *Importation of Fresh Citrus Fruit, Citrus spp., from Brazil into the Continental United States, A Qualitative, Pathway-Initiated Pest Risk Assessment, March 29, 2017*.

Vives MC, Rubio L, Lopez C, and et al. 1999. *The complete genome sequence of the major component of a mild citrus tristeza virus isolate*. Journal of General Virology 80, pp. 811-816.

Vives MC, Rubio L, Sambade A, and et al. 2005. *Evidence of multiple recombination events between two RNA sequence variants with a Citrus tristeza virus isolate*. Virology 331, pp. 232-237.

Wallace JM. 1956. *Tristeza disease of citrus, with special reference to its situation in the United States* (FAO Plant Protection Bulletin, 6:77-87).
Wallace JM. 1978. Virus and Viruslike Diseases- The Tristeza-Disease Complex. In: *The Citrus Industy Vol. IV, W. Reuther, E.C. Calavan, G.E. Carman (eds.)* (University of California), pp. 87-109.
Webber HJ. 1943. *The "Tristeza" disease of sour-orange rootstock.* American Society for Horticultural Science 43, pp. 160-168.
Webber HJ, Reuther W, and Lawton HW. 1967. History and Development of the Citrus Industry, Florida. In: *The Citrus Industry Vol. I, W. Reuther, H.J. Webber, L.D. Batchelor (eds.)* (University of California), pp. 83-87.
Weng Z, Barthelson R, Gowda S, and et al. 2007. *Persistent infection and promiscuous recombination of multiple genotypes of an RNA virus within a single host generate extensive diversity*. PLoS ONE September Issue 9, pp. 1-9.
Wozniak CA, McClung G, Gagliardi J, and et al. 2013. Regulation of Genetically Engineered Microorganisms Under FIFRA, FFDCA and TSCA. In: *Regulation of Agricultural Biotechnology: The United States and Canada, C.A. Wozniak & A. McHugen (eds.)* (Springer), pp. 57-93.
Yokomi R and Garnsey S. 1987. *Transmission of citrus tristeza virus by Aphis gossypii and Aphis citricola in Florida*. Phytophylactica 19, pp. 169-172.
Yokomi RK, Garnsey SM, and Lee RF, et al.,. 1992. *Spread of decline-inducing isolates of citrus tristeza virus in Florida*. Proceedings of the International Society of Citriculture 2, pp. 778-780.
Yokomi RK, Garnsey SM, Civerolo EL, and et al. 1989. *Transmission of exotic Citrus tristeza virus isolates by a Florida colony of Aphis gossypii*. Plant Disease 73, pp. 552-556.
Yokomi RK, Lastra R, Stoetzel MB, and et al. 1994. *Establishment of the Brown citrus aphid (Homoptera: Aphididae) in Central America and the Caribbean Basin and transmission of Citrus tristeza virus*. Journal of Economic Entomology 87, pp. 1078-1085.
Yokomi RK, Selvaraj V, Maheshwari Y, and et al. 2017. *Identification and characterization of Citrus tristeza virus isolates breaking resistance in trifoliate orange in California*. Phytopathology 107, pp. 901-908.
Yoshida T. 1996. *Graft compatibility of Citrus with plants in the Aurantioideae and their susceptibility to Citrus tristeza Vvirus*. Plant Disease 80, pp. 414-417.