Fig1. Quick decline of sweet orange (Valencia) trees in the Central Valley during the summer of 2009.

Citrus quick decline: *a disease complex*

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In August 2009, the UCCE farm advisor for Tulare County reported citrus quick decline (QD) of sweet orange trees in old orchards (at least 50 years) that presumably were grafted on sour orange (SO) rootstock (Fig. 1). These orchards were primarily in the Lindsay and Exeter area within the San Joaquin Valley of California. It is estimated that 150-200 acres of orchards were afflicted with declining trees. After showing general decline symptoms, affected trees collapsed within a few weeks and died. Bud union symptoms included "honeycombing" or "inverse stem pitting," which appears as numerous tiny pegs on the side of the wood and corresponding pinholes on the side of the bark. Just below the bud-union a yellow to light brown discolored zone was also observed. This combination of symptoms is seen on sweet orange, grapefruit, and tangerines on SO and is typical of quick decline of citrus, caused by the *Citrus tristeza virus* (CTV) (Fig.2). Additionally, roots showed stained grayish brown to purple lesions in the bark of large scaffold roots, which is characteristic of dry root rot symptoms caused by *Fusarium solani* (Fig.3A).

Tristeza-CTV: During the nineteenth century, a root rot epidemic caused by *Phytophthora* spp. destroyed seedlings of sweet orange trees and forced the adaptation of *Phytophthora*-tolerant SO rootstock (6). *Phytophthora* species are Oomycetes, closely related to water molds that behave like fungi. In the beginning of the twentieth century, citriculture expanded worldwide and large quantities of citrus plants were shipped from areas where CTV originated to

Fig 2. Bud union symptoms of CTV infected sweet orange on sour orange rootstock. Bud union of declining tree with honeycombing on the wood and small pinholes on the bark (left). B) Bud union of healthy tree (right).

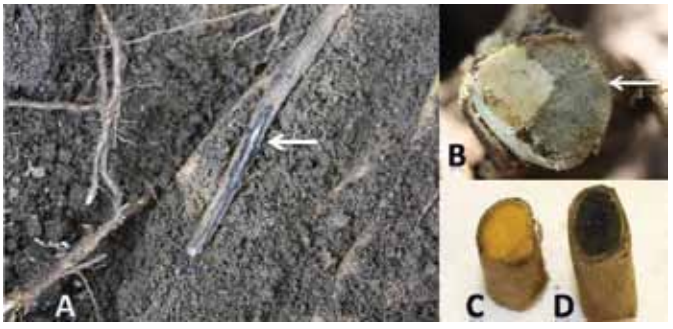

Fig 3. Root symptoms of quick declining trees on sour orange rootstock. A) Dark decay in the bark of large scaffold roots B) Cross section of dark decay in a root. C) Potassium iodide test of declining tree lacking starch (no color) D) and healthy tree with starch (dark blue color).

CTV-free areas, spreading the disease around the world (1,7). During this time, the first serious decline epidemic of sweet orange trees on SO rootstock was recorded in most citrus growing areas of the world. More than 60 million trees died worldwide, and the disease was named "tristeza" from the Spanish and Portuguese word for "sadness". California was hit by this tristeza epidemic in 1939 and since then CTV has become established throughout the state (1, 3).

CTV is a phloem-limited virus that is transmitted by aphids. It can also be spread through grafting infected plant material. In addition to the above symptoms, previous studies showed that CTV causes a girdling effect at the bud union of trees on SO, thereby depleting starch to the root system and debilitating feeder roots (4, 5).

Dry root rot- *Fusarium solani*: Dry root rot is a destructive disease of citrus, caused by the fungus *Fusarium solani*. The fungus begins as a weak pathogen, colonizing the outer portion of larger roots and spreads up into the crown (Fig. 3A). The wood below the dead bark is hard, dry, and stained grayish brown to purple and the lesion extends deep into the wood (Fig. 3B). When the plant is under stress, and/or root starch is depleted, the fungus colonizes rapidly and causes extensive root damage, with leaves suddenly wilting and dying on the tree. (2). Dry root rot has been reported from citrus growing areas worldwide including California. *F. solani* is also often involved in Phytophthora root rot caused by *Phytophthora nicotianae*.

Hypothesis and Study: Based on the observed symptoms and the biology of the fungus, we hypothesized that interactions between CTV, *F. solani*, and/or *Phytophthora nicotiana* potentially play a role in the QD problem observed in Tulare County.

Three orchards with quick declining citrus were studied in Tulare County in August of 2009. Leaf, shoot, bark, and root samples from advanced declining, declining, and healthy looking trees were collected. Leaf, shoot, and bark material were tested for CTV using ELISA (Enzyme-Linked Immunosorbent Assay), and RT-PCR (Reserve Transcription Polymerase Chain Reaction). Rootstock bark was genetically analyzed at Dr. M. Roose's UCR laboratory to verify that they were SO rootstocks. Roots were plated onto different culture media in order to isolate fungal and bacterial pathogens. Fungal and bacterial cultures were further processed for molecular identification. To assess tree girdling at the bud union by CTV, presence of starch in roots was determined by dipping roots in 2% potassium iodide and 0.2% elemental iodine solution (5). Starch absence (no color) indicates a tree with starch depleted roots caused by a girdling effect (Fig 3C). Roots with starch (dark blue color) are indicated as not stressed or girdled at the bud union (Fig. 3D).

Table 1. Pathogen detection, starch analysis, and rootstock genetic profile of sweet orange from Tulare County in California

	Tree Condition	Rootstock	CTV	Iodine Starch test	*F. solani*	*Phytophthora sp.*
Orchard 1	advanced decline	Sour Orange	+	-	+	+
	declining	Sour Orange	+	-	+	-
	healthy looking	Sour Orange	-	++*	-	-
Orchard 2	advanced decline	Sour Orange	+	-	+	-
	declining	Sour Orange	+	-	+	-
	healthy looking	Sour Orange	-	+	-	-
Orchard 3	advanced decline	Sour Orange	+	-	+	-
	declining	Sour Orange	+	-	+	-
	healthy looking	Cleopatra mandarin	-	++*	-	-

*++Indicates high levels of starch

Laboratory tests showed that the advanced decline and declining trees were consistently infected with CTV and *F. solani*. *F. solani* and CTV were never recovered from the healthy looking trees (Table 1). The roots of declining trees were also starch depleted (Table 1, Fig 3. C), which indicates that the plant were under stress because of the girdling at the bud union caused by infection of CTV.

Previous studies have shown that *F. solani* infections occur when trees are stressed by lack of water, poor nutrition, freeze damage and most often when trees are girdled (2). In this investigation, the presence of *F. solani* on starch depleted CTV girdled trees corroborates results from previous studies. Because *F. solani* was recovered only from trees on which CTV was present, it is likely that the QD observed in Tulare County is not the result of a single pathogen, but the result of a disease complex in which trees are predisposed to root stress by CTV and are rapidly dying by an additional infestation of *F. solani*. Control for this disease complex is currently unknown; however removal of trees infected with CTV to prevent QD spread to healthy trees in California should be considered. The Eskalen laboratory is currently investigating to determine control measures for dry root rot.

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