Presence of *Xylella fastidiosa* in Sweet Orange Fruit and Seeds and Its Transmission to Seedlings

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ABSTRACT

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Xylella fastidiosa, a xylem-limited bacterium, causes several economically important diseases in North, Central, and South America. These diseases are transmitted by sharpshooter insects, contaminated budwood, and natural root-grafts. *X. fastidiosa* extensively colonizes the xylem vessels of susceptible plants. Citrus fruit have a well-developed vascular system, which is continuous with the vascular system of the plant. Citrus seeds develop very prominent vascular bundles, which are attached through ovular and seed bundles to the xylem system of the fruit. Sweet orange (*Citrus sinensis*) fruit of cvs. Pera, Natal, and Valencia with characteristic symptoms of citrus variegated chlorosis disease were collected for analysis. *X. fastidiosa* was detected by polymerase chain reaction (PCR) in all main fruit vascular bundles, as well as in the seed and in dissected seed parts. No visual abnormalities were observed in seeds infected with the bacterium. However, the embryos of the infected seeds weighed 25% less than those of healthy seeds, and their germination rate was lower than uninfected seeds. There were about 2,500 cells of *X. fastidiosa* per infected seed of sweet orange, as quantified using real-time PCR techniques. The identification of *X. fastidiosa* in the infected seeds was confirmed by cloning and sequencing the specific amplification product, obtained by standard PCR with specific primers. *X. fastidiosa* was also detected in and recovered from seedlings by isolation in vitro. Our results show that *X. fastidiosa* can infect and colonize fruit tissues including the seed. We also have shown that *X. fastidiosa* can be transmitted from seeds to seedlings of sweet orange. To our knowledge, this is the first report of the presence of *X. fastidiosa* in seeds and its transmission to seedlings.

Xylella fastidiosa Wells et al., a gram-negative and xylem-limited bacterium (33), causes numerous diseases on many economically important fruit and urban shade trees as well as grapevine (22). Citrus variegated chlorosis (CVC), a destructive disease of sweet orange, *Citrus sinensis* (L.) Osbeck, was first discovered in São Paulo, Brazil, in 1987 and subsequently was shown to be caused by novel strains of *X. fastidiosa* (4,10,28). CVC disease can be transmitted by sharpshooter insects (family Cicadellidae) (26), contaminated plant budwood (13), and natural root grafts (11). CVC has rapidly spread to all citrus production areas in Brazil (2), reaching an average incidence of 35% in São Paulo State. A similar disease known as "pecosita" was found in some regions in Argentina (3). Although sweet orange trees affected by the disease do not die, fruit from the trees may be severely undersized, hard, and lacking juice. Such fruit is unacceptable for either the juice or fresh market.

The English language name for the citrus disease, CVC, comes from the striking chlorotic variegation induced on sweet orange leaves by the pathogen. However, the disease is also known as "*amarelinho de citros*" in Portuguese. This name roughly translates as "little yellow citrus" and emphasizes the importance of the fruit symptoms of the disease, which include premature coloration and failure to fill to normal size. Typical fruit symptoms of the disease can be easily recognized from a distance. Because these fruit symptoms are easy for producers to recognize and it is the smaller fruit size that causes economic loss, most Brazilian citrus producers prefer the name *amarelinho de citros* for the disease.

The severity of the CVC disease problem contributed to the selection of a citrus strain of *X. fastidiosa* as the first plant-pathogenic bacterium to have its entire genome sequenced (31). The strain selected was 9a5c, obtained from CVC-infected sweet orange plants in Brazil (4,15). Many important advances have been made in research using the citrus strains of *X. fastidiosa*, including improved pathogenicity tests, identification of alternate experimental hosts (17,18), genetic transformation (24), and molecular methods for strain differentiation (25). Similar attention has been paid to the Pierce's disease strain (9,12). At least some strains of *X. fastidiosa*, including the peach (1,8) and citrus strains (11), can colonize the entire vascular system of the plant, including the roots. However, the presence and distribution of the bacterium in fruit and seed has received little attention. In one report, sharpshooter insect vectors of *X. fastidiosa* were not able to transmit the pathogen after feeding on grape clusters harvested from vines with Pierce's disease. However, in this experiment, the bulk of the insect feeding occurred on the stems of the fruit cluster rather than on the fruit themselves. The possible presence of *X. fastidiosa* in the grape seed from such fruit was not addressed (23).

Although *X. fastidiosa* was found in peduncles of sweet oranges when the bacterium was first associated with the disease in 1990 (28), the colonization of the sweet orange fruit and seed by the pathogen has not received attention. The objectives of our research were to characterize the distribution of *X. fastidiosa* in various parts of sweet orange fruit and seeds and to determine whether the pathogen can be transmitted through seed to seedlings.

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MATERIALS AND METHODS

Grove history and collection of fruit and seed. The most economically important sweet orange cultivars in Brazil, 'Pera', 'Valencia', and 'Natal', were selected for study. All three cultivars were grafted on 'Rangpur' lime (*Citrus limonia* L.) rootstocks and had been planted in 1993 and 1994 in separate blocks of 2,700 or 2,800 trees in the region of Bebedouro, São Paulo, Brazil. More than 60% of the plants in each block had shown CVC leaf and fruit symptoms before these experiments were initiated in 1999. Three trees at CVC disease severity level 3, as well as three apparently healthy trees were selected from each cultivar. Disease severity level 3 means that all of the main branches show both leaf and fruit symptoms (2). Undersized fruit were harvested from the symptomatic trees. The apparently healthy trees did not have any CVC symptoms and were negative for *X. fastidiosa* in diagnostic tests of leaf petioles using polymerase chain reaction (PCR) (21). The fruit from the apparently healthy trees were normal in size.

More than 400 abnormally small fruits and another 400 apparently healthy fruit were harvested in August 1999 for extraction of seeds and detection of *X. fastidiosa* in fruits. The seeds, usually three to five per fruit, were removed from the fruit, washed three times in tap water, and dried for 24 h in shade at room temperature. Seeds were divided into lots and either sown in soil or set aside for dissection and assay for *X. fastidiosa* by PCR. Fruits not needed to provide seeds were stored at 4°C for 2 weeks until they were dissected and the internal tissues were assayed for *X. fastidiosa* by PCR (21).

Seed morphology, sowing, and germination. Three hundred seeds of each cultivar from CVC-affected fruits and another 300 seeds from apparently healthy fruits were examined visually for any abnormalities and then separated into lots of 100 seeds each (replicates), which were then weighed. The seeds were dissected into two parts, the embryos and the seed coats. Because it was difficult to completely separate the inner coat (tegma) from either the outer coat (testa) or embryo, portions of the inner coat (tegma) were included in both the outer coat (testa) and embryo samples. For seed germination tests and seedling analysis, four lots of 50 seeds each from CVC-affected fruit and another four lots of 50 seeds each from apparently healthy fruit of each cultivar were sown in 18-liter pots that contained commercial potting mix (Plantmax; Eucatex, São Paulo, Brazil). Pots were watered twice daily. The number of germinated seeds was recorded each day at 5:00 p.m. and tallied until 45 days, after which the remaining seeds did not germinate. Two months after sowing, 100 seedlings from seeds of CVC-affected fruits and another 100 seedlings from apparently healthy fruit were transplanted into 2-liter pots (15) for further growth and observation. Total DNA was extracted from the primary roots and aerial parts (leaves and stems) of the remaining seedlings and tested for the presence of *X. fastidiosa* by PCR (21). All plants were maintained in screen-protected greenhouses throughout the experiments to preclude unintended transmission of the pathogen by insect vectors. Analysis of variance was performed on the means and, when significant, was followed by a means comparison test (Tukey's test) using a confidence level of $P = 0.05$.

Extraction of genomic DNA from sweet orange fruit. Five CVC-affected fruits and another five apparently healthy fruits of each cultivar were sampled for detection of *X. fastidiosa*. The fruits were separated into six parts: peduncle, peel, or pericarp, (including the exocarp or flavedo), the mesocarp or albedo, endocarp, septum, locular membrane, and central axis. Three 0.2-g samples were prepared as replications from each anatomical part per fruit for DNA extraction, for a total of 180 samples of fruit parts. An additional 100 seeds from CVC-affected fruits, and another 100 seeds from apparently healthy fruit of each of the three cultivars were used individually for detection for *X. fastidiosa*. The seed coat (about 0.03 g) and embryos (about 0.10 g) from each seed were used separately for DNA extraction and PCR testing, for a total of 1,200 samples of seed parts.

Genomic DNA was prepared from fruit or seed with a modified CTAB (cetyltetramethyl ammonium bromide) procedure (29) as follows: 0.2 g of fruit tissue (for seeds, less than 0.2 g) was placed in tubes with 1 ml of phosphate buffered saline and homogenized with a Polytron blender (Pro Scientific, Monroe, CT). Extracts were filtered through four layers of cheesecloth and centrifuged for 5 min at 420 × *g*. The supernatant was transferred to a fresh tube and centrifuged for 20 min at 17,900 × *g*. The pellet was resuspended in 250 µl of TE buffer (0.5 M Tris-HCl, 0.5 M EDTA, pH 8.0) containing 10% *N*-lauryl sarcosine, proteinase K at 10 mg/ml, and ribonuclease at 10 mg/ml, and incubated for 1 h at 37°C. NaCl (5 M, 100 µl) was added to the solution, which was incubated for 10 min at 65°C. A CTAB/NaCl (50 µl) solution was added to the mixture and incubated for 20 min at 65°C. Chloroform/isoamyl alcohol (24:1, vol/vol; 400 µl), was added and mixed thoroughly. The mixture was centrifuged for 5 min at 17,900 × *g*. The aqueous phase was transferred to a fresh tube, 0.6 volumes of isopropanol were added, and the mixture was incubated for 1 h at –20°C and centrifuged for 15 min at 17,900 × *g*. The supernatant was removed and the pellet was washed with 500 µl of 70% ethanol and centrifuged for 5 min at 10,600 × *g*. The supernatant was removed and the pellet was dried for 5 min in a vacuum. The pellet was dissolved in 20 µl of TE buffer (10 mM Tris and 1 mM EDTA) and stored at –20°C.

PCR. Standard PCR assays were performed with primers specific for *X. fastidiosa*, 272-1-int and 272-2-int (21), in a final reaction volume of 40 µl. The amplification program began with incubation at 94°C for 4 min followed by 35 cycles of 94°C for 1 min, 64°C for 1 min, and 72°C for 1.5 min followed by a final extension cycle of 10 min at 72°C. PCR products were visualized by staining with ethidium bromide following electrophoresis through agarose gels. The expected amplification product was 472 bp (21).

Quantitative real-time PCR detection. The LightCycler instrument (Roche Diagnostics, Mannheim, Germany) was used to quantify *X. fastidiosa* cells in seeds. Hybridization probes (CVC1: 5′-GCC GCA AGA TGT TTC ATA G [FL-Q]-3′; and CVC2: 5′-[AmC6+LCRed640] GAC ACC GTT TCA TGG ATG-[Phop-Q]-3′) were designed based on the sequence of the 472-bp PCR product of primers 272-1-int and 272-2-int (21). Each reaction was carried out in 18 µl of PCR mixture containing LightCycler DNA master mix (consisting of *Taq* polymerase, PCR buffer, and dNTPs), 200 nM primer 272-1-int, 200 nM primer 272-2-int, 200 nM probe CVC1, 400 nM probe CVC2, and 2.5 mM $MgCl_2$. The amplification program began with 1 cycle of primary denaturation at 95°C for 30 s followed by 45 cycles of 95°C for 0 s with a ramp rate of 20°C/s, 58°C for 10 s with a ramp rate of 20°C/s, and 72°C for 20 s with a ramp rate of 20°C/s. The signal detection setting was F2/F1, and the fluorescence gains were F1 = 1, F2 = 15, and F3 = 30. A standard curve was established using 10-fold serial dilutions ($10^0 - 10^{-6}$) of a colony of *X. fastidiosa* strain 9a5c suspended in 100 µl of water.

PCR product cloning and sequencing. The PCR product amplified using primer pair 272-1-int and 272-2-int was isolated and purified using the Geneclean Spin Kit (Qbiogene, Carlsbad, CA), cloned into the TOPO TA cloning vector pCR2.1, and the resulting plasmids were introduced into One Shot TOP10 chemically competent *Escherichia coli* (Invitrogen, Carlsbad, CA) according to the manufacturer's instructions. Plasmid DNA was purified using the RPM kit (Qbiogene). DNA sequencing was done at the Genomics and Sequencing Laboratory, Auburn University, AL.

RESULTS

Presence of *X. fastidiosa* in fruit. The fresh weight of CVC-affected fruit of the three cultivars tested was about 25% less than

that of normal healthy fruit. *X. fastidiosa* was detected by PCR (Fig. 1) in samples of peduncle, peel, endocarp, septum, locular membrane, and central axis of CVC-affected fruits of the three sweet orange cvs. Pera, Natal, and Valencia. Of the 15 symptomatic fruits analyzed, all samples from the peduncles and central axis septa contained *X. fastidiosa* DNA, based on PCR tests. Of the samples of fruit peels and endocarp, 80% were positive for the presence of *X. fastidiosa* DNA, as well as 50% of the samples prepared from locular membranes. *X. fastidiosa* was not detected in any parts of the fruits collected from apparently healthy trees of the three cultivars.

Seed symptoms. No discoloration, lesions, or spots were observed on seed collected from these CVC-affected fruit. No significant differences were observed between CVC-affected and apparently healthy fruit in either number of seeds per fruit or the ratio of normal to aborted seed (W.-B. Li, W. D. Pria, Jr., and P. M. Lacava, *unpublished data*). There was also no obvious difference in the weight of the seeds from the healthy and CVC-affected fruits or in the weight of the seed coats. However, the weight of embryos of the seeds from CVC-affected fruits was 25% less than those from healthy fruits (Table 1). The reduction of the fresh weight of the embryos was the only symptom observed in the seeds from CVC-affected fruits of the three cultivars.

Seed germination. Seeds from CVC-affected and healthy fruits started to germinate at the same time, 12 days after sowing (Fig. 2). However, the cumulative total of seeds germinated from CVC-affected fruits consistently lagged behind that of the seeds from healthy fruits through the entire germination period of 44 days. The germination rate for seeds from healthy fruits 33 days after sowing reached 100%, but the final germination rate for seeds from CVC-affected fruits was only 91% 36 days after sowing. There were no apparent differences in seed germination among the three sweet orange cultivars.

Presence of *X. fastidiosa* in seeds. It was not possible to completely separate the inner coat (tegma) from either the outer coat (testa) or from cotyledons and embryos so portions of the inner seed coat (tegma) were present in both embryo and seed coat samples as summarized in Table 2. *X. fastidiosa* was detected by PCR (Fig. 3) in 20 to 22% of the seed coat samples and in 15 to 16% of the embryo samples from seeds of CVC-affected fruits of the three sweet orange cultivars (Table 2). This difference was statistically significant, but no significant differences were observed for the presence of *X. fastidiosa* in seed among the three cultivars. *X. fastidiosa* was not detected in any part of the seeds from healthy fruits of the three sweet orange cultivars.

Presence of *X. fastidiosa* in seedlings. One month after sowing, 250 seedlings were evaluated by PCR for *X. fastidiosa* (Table 3). The bacterium was detected in seedlings from 'Pera', 'Natal', and 'Valencia' sweet orange obtained from seeds collected from CVC-affected fruits. Among the 59 positive seedlings, *X. fastidiosa* was detected in aerial parts of 51 seedlings, in the primary roots of 14 seedlings, and in both aerial parts and primary roots of 6 seedlings. *X. fastidiosa* was not detected in any seedling obtained from the seeds of healthy fruits from the three sweet orange cultivars.

Isolation of *X. fastidiosa* from seedlings and observation of CVC symptoms. Fastidious bacteria were isolated on PW (5) medium from 3-month-old PCR-positive seedlings of 'Pera', 'Natal', and 'Valencia' (Table 3). The identity of isolates as *X. fastidiosa* was confirmed by PCR (Fig. 3). CVC-like foliar symptoms were observed in some of the 4-month-old PCR-positive seedlings (five

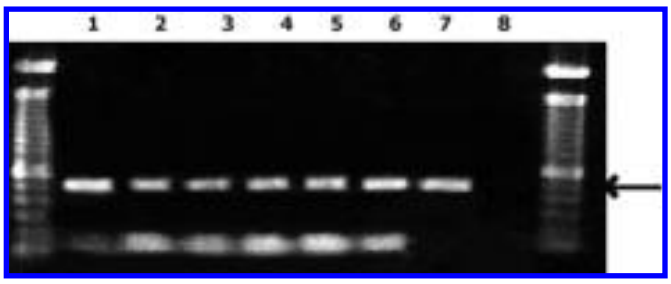

Fig. 1. Detection of *Xylella fastidiosa* in portions of sweet orange fruits assayed by polymerase chain reaction (PCR). Lane 1, peduncle; lane 2, exocarp; lane 3, mesocarp; lane 4, endocarp; lane 5, septa; lane 6, central axis; lane 7, *X. fastidiosa* strain 9a5c from culture in PW (5) medium; and lane 8, central axis from healthy fruit as negative control. The outside lanes contain a 100-bp ladder as a size marker. The PCR product, indicated by the arrow, is 472 bp.

TABLE 1. Fresh weight in grams of 100 seeds from citrus variegated chlorosis (CVC)-affected and healthy sweet orange fruit[x]

	Seed coat[y]		Embryo[z]	
Cultivar	Healthy	CVC-affected	Healthy	CVC-affected
Pera	3.45	3.40	11.01 a	8.84 b
Natal	3.62	3.60	11.35 a	9.12 b
Valencia	3.50	3.42	10.98 a	8.80 b

[x] Means followed by the same letter in each row are not different ($P < 0.05$) according to Tukey's test.
[y] Testa (outer coat), but includes some tegma (inner coat).
[z] Includes cotyledons and some tegma (inner coat).

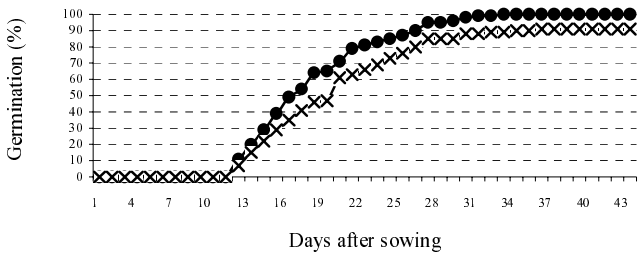

Fig. 2. Germination of seeds from apparently healthy and citrus variegated chlorosis (CVC)-affected 'Pera' sweet orange fruit: ●, apparently healthy fruit; and ×, CVC-affected fruit.

TABLE 2. Detection by polymerase chain reaction of *Xylella fastidiosa* in seeds from citrus variegated chlorosis (CVC)-affected sweet orange fruit[x]

Cultivar	Seed coat[y] (%)	Embryo[z] (%)
Pera	22.3	16
Natal	21.7	14.7
Valencia	20.3	15.3
Means	21.4	15.3

[x] One hundred seeds were dissected and tested per cultivar.
[y] Testa (outer coat), but includes some tegma (inner coat).
[z] Includes cotyledons and some tegma (inner coat).

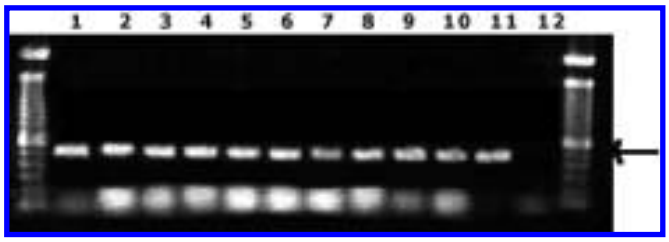

Fig. 3. Detection of *Xylella fastidiosa* in seed parts and seedlings and identification of isolates from seedlings as *X. fastidiosa*. Lanes 1 and 11, strain 9a5c of *X. fastidiosa* from culture in PW (5) medium; lane 2, seed coat; lanes 3 and 4, embryos; lanes 5 to 7, in vitro isolates from 'Valencia', 'Natal', and 'Pera' seedlings, respectively; lanes 8 to 10, seedlings of 'Pera', 'Natal', and 'Valencia'; and lane 12, water. The outside lanes contain a 100-bp DNA ladder as a size marker. The polymerase chain reaction product, indicated by the arrow, is 472 bp.

'Valencia', four 'Pera', and two 'Natal'). However, these symptoms disappeared 1 month later. Observation of further CVC symptom development was abruptly terminated when a tornado destroyed the greenhouse in September 2001.

DNA cloning and sequencing of *X. fastidiosa*. The products amplified by PCR from seeds of CVC-affected fruits of the three sweet orange cultivars (two per cultivar) were isolated from an agarose gel, purified, cloned, and sequenced. The sequences were compared with sequences in the NCBI GenBank database using BlastN. All of these sequences were identical and perfectly matched the corresponding sequence of strain 9a5c of *X. fastidiosa* isolated from sweet orange twigs (31).

Quantification of *X. fastidiosa* in seeds. Ten PCR-positive seed coats and embryos of each cultivar were subjected to quantitative PCR. A typical result is shown in Figure 4. The colony of *X. fastidiosa* used to establish the standard curve had about 200,000 live colony forming units, as determined by dilution plating onto PW plates. On the basis of this standard curve, our 2-µl aliquots of extracts of seed coats or embryos from CVC-affected seed used in the Lightcycler reactions contained about 50 cells of *X. fastidiosa* (Fig. 4). Therefore, the 50-µl extracts from seed coats or embryos each contained approximately 1,250 cells of *X. fastidiosa*, and a contaminated seed would have a minimum of 2,500 *X. fastidiosa* cells. There were no significant differences in number of *X. fastidiosa* cells among the seeds from the three sweet orange cultivars evaluated (data not shown). *X. fastidiosa* was not detected in extracts of seeds prepared from apparently healthy fruit (Fig. 4).

DISCUSSION

The closest relatives of *X. fastidiosa* are in the genus *Xanthomonas* (33). Some xanthomonads are known to infect and be transmitted by infected seed (19). However, the fact that *X. fastidiosa* is limited in plants to the xylem vessel elements has persuaded many researchers that transmission of *X. fastidiosa* by seed is impossible. We detected the bacterium using PCR in the peduncle as well as in the axial, dorsal and marginal bundles, and septa of diseased fruits from the three sweet orange cultivars tested.

X. fastidiosa was also detected in the outer coat (testa) of the seeds as well as in the embryos. The rate of seed infection by *X. fastidiosa* was as great as 22.3% for 'Pera' sweet orange in our analysis. The high rate of seed infection is consistent with *X. fastidiosa* spreading through the vascular system from the infected tree to the fruit and into the seed. In the work reported here, it was not possible to completely separate the inner seed coat (tegma) from the embryos (including cotyledons) or outer seed coat (testa) so the inner seed coat was present in portions of both the outer seed coat and embryo samples. Even with this caveat, our results clearly showed infection of the seed coat (testa plus tegma) with *X. fastidiosa*.

Although the mechanism is not certain, we have demonstrated that *X. fastidiosa* can be transmitted through seed to seedlings. We isolated the bacterium from seedlings and confirmed its identity as *X. fastidiosa* by sequence analysis of the PCR product. Of the 250 seedlings evaluated that were grown from seeds from CVC-affected fruits, 59 (23.6%) were positive for *X. fastidiosa* (Table 3). Thus, the transmission is efficient, and the bacterium tends to appear first in the aerial parts of the developing seedlings, but is also detected in the developing primary root.

The results of limited quantitative real-time PCR testing showed that an infected sweet orange seed could have about 2,500 *X. fastidiosa* cells. Therefore, both standard PCR with specific primers and quantitative real-time PCR with specific hybridization probes can be used for detection of *X. fastidiosa* in citrus seed.

The vascular system and natural openings in seeds are the two most important entrances for seedborne pathogens (19). From the detailed descriptions of the sweet orange vascular system by Schneider (30), it is clear that the sweet orange seed is (i) abundantly endowed with xylem vessels which are, in turn, directly connected to the vascular system of the plant, (ii) the xylem vessels ramify throughout the seed coat, (iii) the vascular system is separated from the embryo only by a thin layer of cells in the chalaza, and (iv) the seed coat has a natural opening in it (the micropyle). Therefore, the conditions for seedborne infection by *X. fastidiosa*, a well-developed vascular system and an opening in the seed, are both met in sweet orange seeds.

There is no anatomical barrier that would prevent *X. fastidiosa* from spreading throughout the seed coat and into the chalazal end of the inner seed coat through vascular tissue. The vascular system ends at the vascular cap, where the nucellus is separated from the vascular cap only by the brown layer of chalaza, a thin layer of brown cells that also stain darkly in histological sections (30). The brown layer of the chalaza is not cutinized (7).

A great deal of ergastic materials must cross the chalaza to support the growth and development of the embryo, "…and the endosperm must act as a conductive tissue, with the seed vascular bundle, the plexus of vascular tissue in the chalaza, the brown layer in the chalaza, and the intervening nucellus figuring very importantly in the transport of food" (30). "Cells of the nucellus—especially at its base—are elongated in such a way as to be adapted to transport" (30). Perhaps *X. fastidiosa* can be carried into the developing embryo along with these ergastic materials.

Alternatively, in other instances of seed transmission of pathogens, transmission from the adhering infected seed coat to the cotyledons or to the embryos of the developing seedlings may occur during the process of germination and emergence (32). The sweet orange embryos are exposed through the natural slit-shaped openings in the integuments at the opposite end of the seed, the micropyle (30). However, this scenario does not explain how *X.*

TABLE 3. Detection of *Xylella fastidiosa* by polymerase chain reaction (PCR) and isolation in culture from sweet orange seedlings obtained from seeds extracted from citrus variegated chlorosis (CVC)-affected fruits

Cultivar	No. of plants tested	No. of positive reactions			
		Plants[z]	Primary root	Aerial parts	Root and aerial parts
Pera	87	14 (16.1%) 2	5	12	3
Natal	80	26 (32.5%) 2	6	22	2
Valencia	83	19 (22.9%) 3	3	17	1
Totals	250	59 (23.6%) 7	14 (5.6%)	51 (20.4%)	6 (10.1%)

[z] The first number is the number of positive PCR reactions, the number in parentheses is the percentage of PCR-positive seedlings, and the third number is the number of seedlings from which *X. fastidiosa* was isolated.

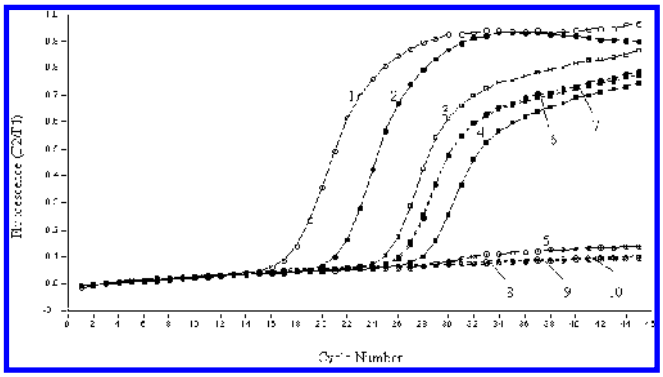

Fig. 4. Real-time amplification plot of *Xylella fastidiosa* target in infected seed parts. Curves 1 to 5, 10-fold dilution series of strain 9a5c of *X. fastidiosa* cultured in PW (5) medium; the reactions contained 4×10^4 to 4 cells, respectively. Curve 6, seed coat from seed of a citrus variegated chlorosis (CVC)-affected fruit of 'Pera' sweet orange; curve 7, embryo from seed of a CVC-affected fruit of 'Pera' sweet orange; curve 8, seed coat from seed of a healthy fruit of 'Pera' sweet orange; curve 9, embryo from seed of a healthy fruit of 'Pera' sweet orange; and curve 10, water only.

fastidiosa would gain entry into the xylem vessels. The details of this infective process deserve further study.

X. fastidiosa is a xylem-limited bacterium and, to our knowledge, this is the first report of its presence in seeds and of its transmission to seedlings through seeds. Although citrus scion cultivars are propagated by bud grafting from mother trees on rootstocks, the rootstocks are typically seedlings of specific citrus species or cultivars. Some citrus rootstocks are resistant, some are tolerant, and others are susceptible to the CVC disease; *X. fastidiosa* can survive in many commercial citrus rootstocks (14). 'Rangpur' lime is the key rootstock for citriculture in Brazil, where more than 90% of citrus trees are grafted onto this rootstock. 'Rangpur' lime rootstock mother trees may be grown in orchards in the presence of high disease and insect pressure. 'Rangpur' lime does not show foliar symptoms of the disease, and no screening of the fruit and seeds are done for *X. fastidiosa*. It may be possible for seed from asymptomatic 'Rangpur' lime trees to carry *X. fastidiosa*. Although we did not study seed of 'Rangpur' lime in this work, this should be done, and it would be wise to provide greater protection for rootstock mother trees.

The infected fruit selected for this study were grossly symptomatic for CVC and weighed 25% less than the healthy fruit used for comparisons. Thus, these fruits can be easily avoided when fruit are harvested for seed collection. Sweet orange seed did not show obvious symptoms or signs of infection by the pathogen. The possibility that seed from normal-sized fruit from infected trees may also transmit *X. fastidiosa* to seedlings should also be evaluated.

The experimental transmission efficiency of the CVC disease is low, less than 5% using sharpshooters (26) and 3% by common bud grafting with contaminated materials (13). However, disease incidence increases rapidly under field conditions (20). The possibility of transmission of *X. fastidiosa* through citrus rootstock seedlings may require a reassessment of disease management strategies.

These findings should alert researchers to the possible seed transmission of other strains of *X. fastidiosa*. We note that coffee plantations in Brazil suffer from a widespread and serious disease, coffee leaf scorch or *Requeima do café*, caused by strains of *X. fastidiosa* (6). The coffee strains of *X. fastidiosa* are closely related to the citrus strains (25,27), and indeed, the citrus strains can reproduce the coffee disease following experimental inoculations (16). In Brazil, coffee is grown either from seed or from grafted plants in which both the scion and rootstock have been grown from seed. The possibility that *X. fastidiosa* can be transmitted through coffee seed should be investigated.

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