Colonization of Dodder, *Cuscuta indecora*, by ‘*Candidatus Liberibacter asiaticus*’ and ‘*Ca. L. americanus*’

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ABSTRACT

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Huanglongbing, or citrus greening, threatens the global citrus industry. The presumptive pathogens, ‘*Candidatus Liberibacter asiaticus*’ and ‘*Ca. L. americanus*’ can be transferred from citrus to more easily studied experimental hosts by using holoparasitic dodder plants. However, the interaction between ‘*Candidatus Liberibacter*’ spp. and the dodder has not been studied. We combined quantitative polymerase chain reaction with electron microscopy to show that only 65% of tendrils of *Cuscuta indecora* grown on ‘*Ca. Liberibacter*’ spp.-infected host plants had detectable levels of the pathogen. Among tendrils that were colonized by *Liberibacter* in at least one 2 cm segment, most were not colonized in all segments. Furthermore, the estimated population levels of the pathogen present in serial 2 cm segments of dodder tendrils varied widely and without any consistent pattern. Thus, there was generally not a concentration gradient of the pathogen from the source plant towards the recipient and populations of the pathogen were sometimes found in the distal segments of the dodder plant but not in the proximal or middle segments.

Populations of the pathogens ranged from 2×10^2 to 3.0×10^8 cells per 2 cm segment. On a fresh weight basis, populations as high as 1.4×10^{10} cells per g of tissue were observed demonstrating that ‘*Ca. Liberibacter*’ spp. multiplies well in *Cuscuta indecora*. However, 55% of individual stem segments did not contain detectable levels of the pathogen, consistent with a pattern of nonuniform colonization similar to that observed in the much more anatomically complex citrus tree. Colonization of dodder by the pathogen is also nonuniform at the ultrastructural level, with adjacent phloem vessel elements being completely full of the pathogen or free of the pathogen. We also observed bacteria in the phloem vessels that belonged to two distinct size classes based on the diameters of cross sections of cells. In other sections from the same tendrils we observed single bacterial cells that were apparently in the process of differentiating between the large and round forms to the long and thin forms (or vice versa). The process controlling this morphological differentiation of the pathogen is not known. The highly reduced and simplified anatomy of the dodder plant as well as its rapid growth rate compared with citrus, and the ability of the plant to support multiplication of the pathogen to high levels, makes it an interesting host plant for further studies of host-pathogen interactions.

Huanglongbing (HLB, formerly citrus greening) poses the most serious threat to the citriculture industry since the devastating outbreak of *Citrus tristeza virus* (CTV) in the 1930s to 1940s (3,4). Both pathogens are found in phloem tissue and are transmitted by phloem feeding insects or by unsuspecting plant propagators grafting infected budwood. Unlike the case of CTV, the HLB-associated pathogens are thought to be bacteria belonging to the ‘*Candidatus*’ genus *Liberibacter* (15). Different forms of the bacteria are given ‘*Candidatus*’ species status based on geographic origin: ‘*Ca. L. asiaticus*’, ‘*Ca. L. africanus*’, and ‘*Ca. L. americanus*’ (16,30). Also, unlike CTV, no source of resistance to the lethal disease is available in the genus *Citrus*. The distribution of ‘*Ca. L. asiaticus*’ has dramatically increased in the last decade, becoming established in the new world in Brazil (8,28), Florida (11,14), and the U.S. Gulf coast in recent years.

Preliminary diagnosis of HLB is based on disease symptoms in the presence of the insect vector. Confirmation of infection by ‘*Ca. Liberibacter*’ spp. is by polymerase chain reaction (PCR)-based testing of symptomatic tissues. These tests have been based on the ribosomal 16S RNA and beta operons (16) and currently quantitative and real-time variations of this approach are in wide-

spread use (20,23,32). Occasionally trees with convincing disease symptoms, including blotchy leaf mottle and lop-sided fruit, produce negative PCR results. This is attributed to an extremely non-uniform distribution of the associated bacterium, ‘*Ca. Liberibacter*’ spp., in infected trees (21,27,29). In addition, a 16Sr IX phytoplasma has been reported to produce convincing symptoms of HLB in Brazil in the apparent absence of ‘*Ca. Liberibacter*’ (31) and an aster yellows type phytoplasma (16Sr I-B) has been reported from HLB symptomatic citrus in China (5).

The dodders include several species of the holoparasitic plant genus *Cuscuta*. Bennett (1,2) and Johnson (17) were the first to demonstrate that some, but not all, plant viruses could be transmitted by several species of *Cuscuta*. Johnson was the first to transmit yellows viruses, which we now know to be phytoplasmas, by dodder (17). Kunkel emphasized the importance of the dodders as experimental tools to widen the host range of “yellows viruses” so that they could be studied in more amenable hosts, such as periwinkle (18,19). The use of dodders for this purpose has become established, for example to transmit citrus pathogens including ‘*Ca. L. asiaticus*’ to other citrus plants (26), and new hosts such as periwinkle (12) and *Murraya paniculata* (33). The details of the process by which these pathogens are transmitted have not been reported.

Unlike a sweet orange tree, *Citrus sinensis* (L.), which is anatomically very complex and large, the dodder plant is extremely simplified, being reduced to haustoria which grow into and attach to the host plant and a thin herbaceous stem without

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leaves but with flowers. In this paper, we present results of experiments to characterize the colonization of dodder by ‘*Ca. L. asiaticus*’ and ‘*Ca. L. americanus*’. Does the pathogen multiply in the dodder or is transmission simply passive, as reported for *Tobacco mosaic virus* (9,17)? If ‘*Ca. L. asiaticus*’ multiplies in the dodder, is the concentration of the pathogen uniform in this very simple experimental system, or does the distribution of the pathogen follow a gradient or is it nonuniform, as it is in citrus? Is the dodder itself diseased? We also take advantage of this experimental system to examine the complex morphologies of bacteria present in the phloem of greening infected plants (12,13,24).

MATERIALS AND METHODS

Plant and pathogen materials. ‘*Ca. L. asiaticus*’ strains B232 (Thailand), B239 (Taiwan), and B430 (Japan) and ‘*Ca. L. americanus*’ strain B427 (São Paulo, Brazil) were maintained in graft-inoculated rough lemon, *Citrus jambhiri* Lush. or sweet orange trees. Infection of these trees has been maintained by graft transmission and confirmed by the presence of symptoms of HLB and by quantitative-PCR (qPCR) testing (see below). ‘Madagascar’ periwinkle plants, *Catharanthus roseus* Don., were used as recipient plants for dodder transmission. The dodder used was identified as *Cuscuta indecora* Choisy (L. Musselman, *personal communication*). Dodder was germinated from seed placed on potting media under an intermittent mist. Newly germinated dodder was placed on healthy periwinkle plants and allowed to establish itself on these plants. Dodder was maintained on either healthy periwinkle or citrus by mass transfer of tendrils as the plants cycled in the greenhouse.

Citrus trees and periwinkle plants were greenhouse grown at the USDA-ARS in Beltsville, MD in 1 quart to 3 gallon (0.95 to 11.4 liters) pots filled with MetroMix 510 and perlite and fertilized by irrigation with “Jacks Professional water soluble fertilizer Petunia Feed plus magnesium” (21N:3P:19K) at 100 ppm nitrogen. Copper and chelated iron were added at 2 and 6 ppm, respectively. Ambient light was supplemented with 4 h of high-pressure sodium lighting from October through April. Periwinkle and citrus plants were grown from seed.

Dodder was established on infected source plants by either placing a periwinkle plant with an active dodder colony in close proximity to the source plant or by placing long detached healthy tendrils of dodder directly on the source plant. Dodder tendrils grown on infected source plants were directed towards and given the opportunity to infect periwinkle plants. To facilitate the process, periwinkle plants were placed as closely as possible to the source plants, and the tendrils were wound around the target periwinkle stems. Dodder tendrils from infected citrus were also cut off and placed onto individual periwinkle plants. Dodder was removed from periwinkle and citrus as needed if the dodder grew excessively and began to damage the host.

Sampling of dodder plants. Individual dodder tendrils varied in length and thickness and were removed intact from parasitized plants. The tendrils were photographed, oriented source to recipient, and cut into 2 cm segments from and including the source haustorium to the growing tip. A segment of 1 mm was removed from the center of each 2 cm segment and fixed for electron microscopy. The two remaining dodder stem pieces were combined and ground in a FastPrep bead mill (MP Biomedical, Solon, OH) and DNA was purified using a DNeasy procedure (Qiagen, Germantown, MD) (20,22).

Quantification of ‘*Ca. L. asiaticus*’ and ‘*Ca. L. americanus*’ in plant extracts. Q-PCR assays were performed as reported previously in a SmartCycler II (Cepheid, Sunnyvale, CA) using the target primer-probe sets HLBaspr (‘*Ca. L. asiaticus*’) or HLBampr (‘*Ca. L. americanus*’) plus a plant cytochrome oxidase (COX)-based primer-probe set (COXfpr) (20). For the purposes of standard curve development, 16S rDNA fragments were amplified

from trees confirmed to be infected with ‘*Ca. L. asiaticus*’ and ‘*Ca. L. americanus*’ with primer set OI1/OI2c and GB1/GB3 (16,28). The 16S rDNA amplicons were inserted into plasmids, designated pFL136 (20) and pLAM16S, and used to prepare standard curves of the qPCR crossing threshold (Ct) value versus log target copy number in a dilution series of qPCR targets prepared in DNA extracts obtained from dodder plants. Regression equations were calculated and used to estimate the copy number of the target molecule in a given extract. The plant mitochondrial cytochrome oxidase (COX) target was used as an internal control for DNA extract quality and reaction conditions and was included in every amplification reaction. The hydrolysis probe for the cytochrome oxidase target (COXp) was labeled with 6-carboxy-fluorescein (FAM) and Black Hole Quencher (BHQ-1). The *Ca. Liberibacter* hydrolysis probe (HLBp) was labeled with tetra-chloro-6-carboxyfluorescein (TET) and BHQ-1 (20).

Electron microscopy. Dodder segments, removed for transmission electron microscopy (TEM) were fixed for 24 h at 4°C in 3% glutaraldehyde in 0.066 M phosphate buffer pH 6.8. Glutaraldehyde was removed by three washes with phosphate buffer and the samples were post fixed in 2% osmium tetroxide for 4 h. After washing in phosphate buffer, the samples were then dehydrated in a 25 to 100% acetone series and embedded in Spurr’s resin. Ultrathin sections for TEM were stained with uranyl acetate in methanol and with Reynold’s lead citrate, viewed in a FEI Morgagni 268 TEM, and digital micrographs were recorded.

RESULTS

Interactions between dodder and host plants. Dodder grown on HLB-diseased plants initially grew exponentially, forming thick green yellow, then orange-yellow tendrils which elongated readily (Fig. 1A). As the dodder matured on a vigorous host it formed flowers then seed, and the coiled tendrils at the point of attachment thickened and turned dark orange (Fig. 1B). The dodder, when left unchecked on healthy plants smothered and eventually killed the host plant. The dodder tendrils declined with the host plant, whether the host was HLB diseased or not, and became dark orange in some cases or very thin and weak before dying off.

The initial appearance of the dodder tendrils grown on diseased plants was not consistently different from dodder grown on healthy plants. When a young dodder tendril initially coiled around citrus and began to attach, the tendril was pale yellow. As the dodder matured on infected sources the attachment sites thickened and became orange in color (Fig. 1A and B). The maturation and coloration process tended to be more rapid when the dodder was on infected host plants. Dodder grown on infected plants would grow only if the infected plant was also still growing and producing sporadic new flush. If the plant was highly symptomatic with only heavily mottled older leaves or weak yellow shoots, the dodder would establish only minimally if at all. Symptoms of ‘*Ca. L. asiaticus*’/‘*Ca. L. americanus*’ infection in periwinkle developed about 3 months following colonization of periwinkle by dodder from an infected source (Fig. 1C).

Quantification of ‘*Ca. Liberibacter*’ species in dodder. Testing by qPCR revealed that some, but not all dodder tendrils were infected by ‘*Ca. L. asiaticus*’ or ‘*Ca. L. americanus*’ (Table 1). Overall, the average rate of infection of tendrils was 65% (55/84). The highest rate of infection was by ‘*Ca. L. asiaticus*’ strain B232 (Thailand) at 78% (14/18), but it is not known if the higher rate of infection of this strain reflects a greater virulence towards dodder. Although 65% of the tendrils were colonized by ‘*Ca. Liberibacter*’ spp., the tendrils were generally not uniformly colonized (Table 1). Among the tendrils that were colonized in at least one segment, fewer than half were colonized in every segment. For example, ‘*Ca. L. asiaticus*’ strain B232 was present in every segment tested in only 10 of 18 tendrils where it was present in at

Fig. 1. Appearance of **A**, immature dodder tendrils on citrus infected with '*Candidatus Liberibacter asiaticus*' strain B232 and **B**, mature dodder tendrils on citrus infected with '*Ca. L. americanus*' strain B427 and **C**, symptoms in periwinkle by strain '*Ca. L. asiaticus*' B430 3 months after transmission by dodder.

least one segment. Similarly '*Ca. L. americanus*' strain B427 was present in every segment tested in only 4/21 tendrils where it was present in at least one segment. No consistent pattern of colonization was evident. In some tendrils there appeared to be a concentration gradient of '*Ca. Liberibacter*' spp. from the proximal segment on the source plant to the distal segment of the tendril (Fig. 2). In other tendrils, however, only the most distal segments were infected by the pathogen, or infection by '*Ca. Liberibacter*' spp. was limited to the central segments of the tendril only (Fig. 2). '*Ca. L. asiaticus*' was found in dodder segments as far as 16 to 18 cm from the source plant (Table 1).

The nonuniform colonization of linear dodder tendrils was quantified by qPCR. The concentration of '*Ca. L. asiaticus*' and '*Ca. L. americanus*', as estimated by qPCR, varied greatly from one tendril to another and from one segment within a tendril to another, with Ct values ranging from 15 to 39 (Fig. 2). The regression equations calculated from healthy dodder extracts containing a dilution series of cloned target 16S RNA target genes were $y = 13.256 - (0.336 \text{ mean Ct})$ for '*Ca. L. asiaticus*' and $y = 12.820 - (0.262 \text{ mean Ct})$ for '*Ca. L. americanus*'.

Analysis of variance on the data used to calculate the equations was significant ($P < 0.001$) and R^2 was 0.993 and 0.987, respectively. With three 16S RNA targets per '*Ca. Liberibacter asiaticus*' genome (10), the range of concentrations of '*Ca. Liberibacter*' spp. genomes present per 2 cm dodder stem segment therefore ranged from 3.0×10^8 to 2×10^2 (Fig. 2, Table 1). A 2 cm segment of dodder stem weighed on average about 21 mg. Therefore, on a fresh weight basis, the populations of '*Ca. Liberibacter*' spp. ranged from 1.4×10^{10} to 9.5×10^3 cells per g of stem tissue, within the range reported previously for citrus (21).

Observations of '*Ca. Liberibacter*' species by electron microscopy. The qPCR data on distribution of '*Ca. Liberibacter*' spp. within dodder tendrils were supported by EM observations. Many dodder tendril segments did not have any '*Ca. Liberibacter*' spp. present, but other segments had large numbers of bacteria in the phloem (Fig. 3). Bacteria were found in both longitudinal and transverse sections, and it was striking that adjacent phloem vessel elements could be either completely free or full of bacteria.

In many phloem cells, bacterial cells of different sizes and shapes were present, with both round and long forms observed (Fig. 4A). Some of the round forms measured 0.33 to 0.66 μm in diameter and were evidently transverse sections of the long forms which had lengths of 2.6 to 6.3 μm . In addition, some much larger, round bacterial cells measuring 0.86 to 1.5 μm in diameter also were present. These could not be cross sections of the long forms. In other sections (Fig. 4B) bacteria that were clearly transitioning between the two forms (round and long) were found. The

TABLE 1. Summary of quantitative polymerase chain reaction testing of '*Candidatus Liberibacter asiaticus*' and '*Ca. L. americanus*' in *Cuscuta indecora* grown on huanglongbing-affected source plants

Strain ^a	Tendrils ^b (positive/tested)	Segments ^c (positive/tested)	Lowest crossing threshold	Greatest distance ^d
B427	21/34	53/122	15.1	12 cm
V427	4/5	11/13	18.9	10 cm
B232	14/18	44/80	19.1	16 cm
V232	5/8	13/52	18.3	18 cm
B430	2/3	8/11	19.9	8 cm
V430	5/8	17/37	15.7	16 cm
B239	2/4	5/16	34.3	14 cm
V239	2/4	2/9	18.4	2 cm
Totals	55/84; 65%	153/340; 45%	15.1	16 cm

^a B or V denotes dodder grown on citrus or periwinkle, respectively. Strain 427 is '*Ca. L. americanus*'; the other strains are '*Ca. L. asiaticus*'.

^b Number of tendrils with at least one positive 2 cm segment over the number of segments tested.

^c Number of positive 2 cm segments over the number of 2 cm segments tested.

^d Greatest distance of a positive segment from the source plant.

round end of these “transitioning” bacteria were 0.86 to 1.5 µm in diameter and the elongating ends were 0.26 to 0.5 µm in width.

DISCUSSION

Early workers were successful in transmitting some viruses and phytoplasmas over distances of 10 in (25 cm) or more in dodder (17). However, common sense and anecdotal wisdom suggested that the physical separation between the source and receptor plant should be minimized, and so we placed receptor plants within the foliage of the source plant when our goal was to transmit the pathogen. In addition however, we found that *Cuscuta indecora* prefers not to colonize host plants of a different species after it is established on a host unless the original host begins to decline. The dodder may rely on volatile cues produced by the host in order to select tissues for colonization (25). We observed that after winding the dodder tendril around a recipient plant stem several times, the tendril would often return to and colonize the source plant again. On other occasions the dodder would grow through the intended recipient to another plant of the same species as the source plant, rather than establishing itself on the receptor plant. Early workers may have observed this phenomenon as well, and addressed it by cutting off dodder from the source and placing it on the receptor to force it to establish or die (17).

Our qPCR data from 84 dodder tendrils grown on HLB-symptomatic and ‘*Ca. Liberibacter*’ spp.-positive host plants showed that the colonization of *Cuscuta indecora* by ‘*Ca. Liberibacter*’ spp. is generally incomplete, and many tendrils were not infected by ‘*Ca. L. asiaticus*’ or ‘*Ca. L. americanus*’ to a level detectable by qPCR (Table 1; Fig. 2). Thus, extremely efficient transmission of the pathogen to recipient plants should not be expected. The unpredictable distribution, and frequent lack of the pathogen in the distal tips of growing dodder described in this work, suggests that multiple dodder tendrils should be used per receptor in order to get high rates of transmission. Another early approach was to perform a “dodder graft” by winding the dodder tendril around the receptor plant several times and fixing the tendrils to the receptor plant with Carbowax 4000 and string. This forced multiple

connections between source and recipient and minimized the distance between the plants (7) and thereby increased the efficiency of transmission of the virus. We observed ‘*Ca. L. asiaticus*’ as much as 18 cm from the source plant so presumably transmission could occur over this distance. However, it seems that higher concentrations of the pathogen tended to stunt the tendrils (Fig. 2, Table 1), which would explain the anecdotal wisdom of close proximity of source and receptor plants.

Cucumber mosaic virus (CMV), but not *Tobacco mosaic virus*, was shown to multiply in dodder plants in the initial experiments done on transmission of viruses by dodder, though both viruses were transmitted by dodder (2,9,17). Costa studied the interaction of CMV and dodder in some detail. CMV multiplied in *Cuscuta campestris* when grown on CMV-infected *Nicotiana tabacum* (9). This conclusion was based on results of both local lesion assays and serial propagation of dodder on nonhost plants for CMV following infection on *N. tabacum*. Costa also described symptoms of CMV infection in dodder as subtle but consistent distortions of growth. The degree of distortion was variable and the rate of growth of the tendrils was reduced. He also reported that it was more difficult to establish dodder on a CMV-infected plant than on a healthy plant (9). As Costa did with CMV, we observed growth distortions and stunting to varying degrees in *Cuscuta indecora* infected by ‘*Ca. Liberibacter*’ spp. as well as difficulty in establishing dodder on infected host plants. These distortions and stunting were not always immediately obvious but with experience became apparent. Dodder infected with ‘*Ca. Liberibacter*’ spp. also tended to develop orange coloration, mature, and set seed more rapidly than did uninfected dodder.

The distribution of ‘*Ca. L. asiaticus*’ and ‘*Ca. L. americanus*’ in infected citrus trees has been shown to be very nonuniform (21,27,29). The erratic distribution of the pathogen in citrus trees could be due to the complex architecture of the tree and random sites of inoculation with the pathogen by the insect vector. When compared to a sweet orange tree, a dodder plant is an extremely simplified and entirely linear plant, and the site of colonization of the dodder plant is limited to the proximal stem on the host that differentiates to produce haustoria. Nonetheless, the distribution of ‘*Ca. Liberibacter*’ spp. in *Cuscuta indecora* was also very non-

Fig. 2. Distribution and concentration of ‘*Candidatus Liberibacter*’ spp. in representative infected dodder plants grown on citrus or periwinkle. Graphic denotes 2 cm stem segments of dodder plants. Color scale indicates crossing threshold values following the quantitative polymerase chain reaction assay, with white indicating no ‘*Candidatus Liberibacter*’ spp. detected. From top to bottom strains B232, V430, V232, V430, B232, B427, B232, V232, B232, and V232. ‘B’ indicates sweet orange as source plant and V indicates periwinkle as source plant.

Fig. 3. '*Candidatus Liberibacter americanus*' cells filling phloem vessel elements viewed in **A**, cross section and in **B and C**, longitudinal section. Note the extreme density of '*Ca. L. americanus*' in some cells and the lack of '*Ca. L. americanus*' in other cells, as well as the pleomorphic cell morphology, particularly in **C**. Following quantitative polymerase chain reaction the crossing threshold value for this stem segment was 17.89, indicating 5.6×10^7 '*Ca. L. americanus*' per 2 cm dodder segment.

uniform. By quantifying the amount of '*Ca. Liberibacter*' spp. in consecutive segments of the dodder stem, we observed that the pathogen was in some instances uniformly present in high concentrations along the length of the stem sampled, uniformly present in low concentrations, or present only in the proximal, middle or distal segments at either high or low concentrations. Thus, for the most part there was no concentration gradient of the pathogen from source to recipient plant (Fig. 2). The concentrations of the pathogen observed ranged from 2×10^2 to 3.0×10^8 per 2 cm of dodder stem assayed. On a fresh weight basis, populations as high as 1.4×10^{10} cells per g of stem tissue were observed, consistent with population levels in citrus (21). The

Fig. 4. '*Candidatus Liberibacter asiaticus*' B232 in phloem cells. **A**, Small thin rods captured in both cross section and longitudinal section as well as much larger cells in cross section. The crossing threshold (Ct) value for this stem segment was 22.16, indicating 2.7×10^5 '*Ca. L. asiaticus*' per 2 cm stem segment. **B**, Thin rod-shaped cells captured in transition to much larger round cells. The Ct value of this segment was 20.37, indicating 1.1×10^6 '*Ca. L. asiaticus*' per 2 cm stem segment.

distribution as seen with the TEM was also variable among the phloem elements in a given section, with some elements containing very high numbers of bacteria and other adjacent elements with either low numbers of bacteria or none at all (Fig. 3). The combination of qPCR and TEM in this study allowed a complete characterization of the distribution of ‘*Ca. L. asiaticus*’ and ‘*Ca. L. americanus*’ in infected dodder at both the whole plant and ultra structural levels (Figs. 2 to 4). The distribution of ‘*Ca. L. asiaticus*’ and ‘*Ca. L. americanus*’ is as nonuniform in the very simple dodder plant as it is in the much more complex citrus tree. Some phloem stained very darkly and may have been undergoing degeneration (Fig. 3). A synonym for HLB is citrus vein phloem degeneration (26).

‘*Ca. Liberibacter*’ spp. clearly multiplied within *Cuscuta indecora* (Fig. 3). The amount of ‘*Ca. Liberibacter*’ spp. observed in individual tendrils could be effected by the concentration of the pathogen present in the phloem cells of the host that were compromised by the dodder’s haustorium, which would constitute the initial inoculum of the dodder plant. Subsequent multiplication may be controlled by the nutritional quality of the phloem sap produced by the source plant and the success of the dodder acting as a nutritional sink. Early workers demonstrated that defoliation of the receptor plant (2) and keeping the receptor plant in the dark and the source plant in the light (6) improved the efficiency of viral transmission by dodder, presumably by creating a source/sink relationship between the two plants connected by the dodder bridge.

Electron microscopy has been used several times to photograph bacteria in the phloem cells of citrus or periwinkle plants with symptoms of HLB. In such cases, two or more distinct morphologies are observed among bacteria in single tissue sections, an “elongated form” and a “round form” (12,13). Because Koch’s postulates have not been completed for ‘*Ca. Liberibacter*’ spp. and phytoplasmas have been associated with symptoms indistinguishable from those of HLB (5,31), it is important to determine if the bacteria with different morphologies present in such sections are produced by a single population of cells. We show that both the spherical and thin tubular morphologies can be present in a single bacterial cell (Fig. 4) as had been observed previously (24). Thus, ‘*Ca. Liberibacter*’ spp. is apparently pleomorphic. Final confirmation that the pleomorphic cells are in fact those of ‘*Ca. Liberibacter*’ spp. will require antibodies specific for ‘*Ca. Liberibacter*’ spp. to label the bacteria in phloem cells.

We have begun to characterize in detail the relationship between ‘*Ca. Liberibacter*’ spp. and the parasitic plant *Cuscuta indecora*. The anatomical simplicity of the dodder plant facilitates studies on the ultrastructure of the ‘*Ca. Liberibacter*’ spp./host interaction. qPCR of stem segments improved the efficiency of subsequent studies using TEM. ‘*Ca. Liberibacter*’ spp. multiplied in *Cuscuta indecora* and induced subtle symptoms of disease in infected plants. In spite of the anatomical simplification of dodder compared with citrus, the distribution of the pathogen in dodder was not uniform, as observed previously in citrus (21,27,29) nor was a concentration gradient of ‘*Ca. L. asiaticus*’/‘*Ca. L. americanus*’ from source to receptor plant observed. We are also studying the ultrastructure of the junction between the dodder haustorium and the citrus host plant infected with ‘*Ca. Liberibacter*’ spp. to study the details of the transmission process. The details of the mechanism of transmission from citrus phloem to dodder phloem and vice versa will very likely offer essential insights into the mechanisms of transmission of the pathogen between phloem cells within infected citrus.

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