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Fastidious Prokaryotes and Plant Health

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ABSTRACT

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The prokaryotes are almost everywhere or we can phrase like this “prokaryotes are wherever there is life”. They were the earliest organisms on earth. Today, they still dominant the biosphere for the following two facts: 1) their collective biomass outweighs all eukaryotes combined at least tenfold, and 2) more prokaryotes inhabit a handful of fertile soil or the mouth or skin of a human than the total number of people who have ever lived. They thrive in habitats that are too cold, too hot, too salty, too acidic, or too alkaline for any eukaryote because they display diverse adaptations that allow them to inhabit many environments and they have great genetic diversity. Phytopathogenic fastidious prokaryotes are plant pathogens that either resist to grow in any available bacterial culture media or require specific or enriched media to grow. They include *Xylella fastidiosa*, *Leifsonia xyli* subsp. *xyli*, *L. xyli* subsp. *cynodontis* and *Clavibacter michiganensis* subsp. *sepedonicus* and *C. michiganensis* subsp. *michiganensis* that reside in xylem and spiroplasmas, phytoplasmas and *Candidatus* Liberibacter spp. that reside in phloem. The impacts that fastidious prokaryotes have on plant health have been enormous. Two major maladies caused by *X. fastidiosa* resulted in serious economic loss on wine and citrus juice industry. Three plant diseases, namely citrus stubborn, corn stunt and periwinkle diseases are caused by spiroplasmas. Over 500 plant diseases were reportedly the results of phytoplasmal infestation [22]. *Ca*. Liberibacter spp., are the causal agents of decades-long citrus disease called Huanglongbing or citrus greening and the recent-emerged zebra chip disease of potato and others. Pierce’s disease is the limiting factor for the establishment of wine industry for the entire southeastern United States from Texas to the Carolinas along the gulf coast of Mexico. Recent introduction of the glassy-winged sharpshooter leafhoppers in California has threatened the winery industry of California. The successful isolation of *X. fastidiosa* from the tissues with citrus variegated chlorosis (CVC) symptoms followed by the identification of the major insect vectors provided crucial information for citrus growers and citrus juice industry to deal with the CVC crisis in Brazil. The successful isolation of *X. fastidiosa* from blueberry tissues with leaf scorch symptoms followed by the identification of the susceptibility/resistance of various blueberry cultivars provided significant information for the blueberry industry which has recently become the number one fruit commodity in Georgia.

Keywords: fastidious prokaryotes, *Xylella fastidiosa*, *Ca*. Liberibacter spp., spiroplasmas, phytoplasmas, Huanglongbing, Hemiptera, glassy-winged sharpshooter, Pierce's disease of grape, citrus variegated chlorosis, bacterial leaf scorch of blueberry

INTRODUCTION

In the Kingdom Prokaryotae, there are two domains, Archaea and Bacteria which differ in structure, physiology, and biochemistry [25]. Archaea like bacteria but are thought to be more closely related to eukaryotes than to bacteria. The prokaryotes are almost everywhere or we can phrase like this "prokaryotes are wherever there is life". They were the earliest organisms on earth. Today, they still dominant the biosphere for the following two facts: 1) their collective biomass outweighs all eukaryotes combined at least tenfold, and 2) more prokaryotes inhabit a handful of fertile soil or the mouth or skin of a human than the total number of people who have ever lived. They thrive in habitats that are too cold, too hot, too salty, too acidic, or too alkaline for any eukaryote because they display diverse adaptations that allow them to inhabit many environments and they have great genetic diversity. [http://www.course-notes.org/Biology/Outlines/Chapter_27_Prokaryotes].

The prokaryotes are small and most are unicellular with the cell sizes ranging from 1 μm to 10 μm, but they can vary in size from 0.2μm to 750μm. Being so small, they have both harmful and beneficial impacts on humans and plants. Human life is only possible due to the action of prokaryotic microbes, both those in the environment and those species that call us home. Internally, they help us digest our food, produce crucial nutrients for us, protect us from pathogenic microbes, and help train our immune systems to function correctly. However on the harmful side, though pathogenic prokaryotes represent only a small fraction of prokaryotes species, yet they cause about half of human diseases. For example, there are between 2 and 3 million people a year die of the lung disease tuberculosis, caused by the bacillus *Mycobacterium tuberculosis*.

The prokaryotes that cause plant diseases belong in the Bacteria Domain. In the Division Gracilicutes, the Gram-negative bacteria, under the Class Proteobacteria, prokaryotes that cause plant diseases belong in three known Families and one unnamed Family. In Family Enterobacteriaceae, there are four Genera: *Erwinia, Pantoea, Serratia,* and *Sphingomonas*. In Family Pseudomonadaceae, there are seven Genera: *Acidovorax, Pseudomonas, Ralstonia, Rhizobacter, Rhizomons, Xanthomonas,* and *Xylophilus*. In Family Rhizobiaceae, there are two Genera: *Agrobacterium* and *Rhizobium*. In a still unnamed Family, there are two Genera: *Xylella* [24] and *Candidatus* Liberibacter. In the Division Firmicutes, the Gram-positive bacteria, under the Class Firmibacteria, there are two Genera: *Bacillus* and *Clostridium* whereas under the Class Thallobacteria, there are six Genera: *Arthrobacter, Clavibacter, Curtobacterium, Leifsonia, Rhodococcus,* and *Streptomyces.* In the Division Tenericutes, under the Class Mollicutes, prokaryotes that cause plant diseases belong in two Families. In the Family Spiroplasmataceae, there is one Genus, *Spiroplasma* and in the Family Acholeplasmataceae, there is one Genus, *Candidatus* Phytoplasma [1].

Fastidious prokaryotes are those that either resist to grow in any available media, such as phytoplasmas, *Ca*. Liberibacter spp., and *Ca*. Phlomobacter fragariae or those that require specific and enriched media, such as spiroplasmas, *X. fastidiosa*, *Leifsonia xyli* subsp. *xyli, L. xyli* subsp. *cynodontis* and *Clavibacter michiganensis* subsp. *sepedonicus*. Based on the inhabitant, *X. fastidiosa, Leifsonia* spp., and *C. michiganensis* subsp. *sepedonicus* are xylem-inhabiting while spiroplasmas, phytoplasmas, *Ca*. Liberibacter spp., and *Ca*. Phlomobacter fragariae are phloem-inhabiting prokaryotes.

Xylem-limited bacterial plant pathogens and plant health

Based on the diseases reported around the world, *X. fastidiosa* causes diseases in the America Continent including North and South America. In the US, they occur in the whole southeastern States along the Gulf coast of Mexico, and California. In the southern hemisphere, the diseases occur in Brazil, Argentina, and Paraguay. In Asia, the pear leaf scorch [15] and PD of grapes [23] were reported in Taiwan. In Europe there was a report describing PD of grapes in Kosovo [3], former Yugoslavia which sits in southern Europe. The *X. fastidiosa*-induced diseases seemed to occur in the region between 15-45 degrees latitude of both north and south of Equator. It is interesting to note that Taiwan sits at the Tropic of Cancer where the pear leaf scorch disease and PD occur and that Sao Paulo in Brazil sits at the Tropic of Capricorn where the severe citrus variegated chlorosis (CVC) [8,12] and coffee leaf scorch occur. Kosovo sits at about 45 degree North of Equator.

There are 19 diseases that were confirmed to be caused by *X. fastidiosa.* They are Pierce's disease of grape, alfalfa dwarf, phony peach (PP), plum leaf scald, CVC, periwinkle wilt, ragweed stunt, and leaf scorch of almond, elm, mulberry, oak, sycamore, pecan, maple, oleander, blueberry, coffee, pear, and Chitalpa [6, 8, 12, 13, 15, 17, 19, 20, 21]. The common symptoms induced by *X. fastidiosa* include marginal leaf necrosis, scorching or scalding of leaves, early leaf fall, dieback of branches, and wilting to death. The specific symptoms vary among different hosts. Symptoms of Pierce's disease of grapes usually start with marginal leaf necrosis to chlorosis; normally a yellow band would form between the green and necrotic tissues for white wine grapes and a purple band for red wine grapes. The following unique symptoms will follow: petioles remain attached to the canes, green island formation due to irregular maturing process of barks, dried up raisins, and eventual dying and dead vines occurs in 2-4 years after initial infection in GA. The specific symptoms on peach of phony disease include darker green leaves and extremely shortened terminal growth which resulted in a shape of an umbrella canopy.

CVC was first observed in 1987 on sweet orange trees in the southwestern part of Minas Gerais, Brazil. Since then, the disease has been observed in the neighboring State of San Paulo and other citrus producing states [8]. Rossetti et al. [18] were the first to show by electron microscopy that a xylem-limited bacterium, probably a strain of *X. fastidiosa*, was present in all symptomatic leaves and fruits tested but not in similar tissues from symptomless trees.

CVC causes severe leaf chlorosis between veins when young trees are infected. Symptomatic leaves exhibit brown gummy lesions on the lower side in corresponding to the chlorotic yellow areas on the upper leaf surface. Reduced growth vigor and abnormal flowering and fruit set occur in infected trees. Fruits from affected trees are often small and hard with high acidity which is not fitting for juice making and no fresh market value [10, 12]. A bacterium was consistently cultured from plant tissues from CVC twigs of sweet orange trees but not from tissues of healthy trees on several cell-free media known to support the growth of *X. fastidiosa*. Bacterial colonies typical of *X. fastidiosa* became visible on PW (Fig. 1), CS20 and PD2 agar media after 5 and 7-10 days of incubation, respectively. The cells of the CVC bacterium were rod-shaped, 1.4-3 μm in length, and 0.2-0.4 μm in diameter, with rippled walls. An antiserum against an isolate (8.1.b) of the bacterium gave strong positive reactions to double-antibody-sandwich (DAS), enzyme-linked immunosorbent assay (ELISA) with other cultured isolates from CVC citrus, as well as with several type strains of *X. fastidiosa* (Table 1) [11]. Sweet orange seedlings inoculated with a pure culture of the CVC bacterium supported multiplication of the bacterium, which became systemic within 6 months after inoculation and could be re-isolated from the inoculated seedlings. Symptoms characteristic of CVC developed 9 months post inoculation. *X. fastidiosa* can infect most of the citrus cultivars, species and hybrids, yet the severity of symptoms varies. Sweet oranges are the most susceptible. Grapefruit, mandarins, mandarin hybrids, lemons, limes, kumquat and trifoliate orange are moderately susceptible, showing less severe symptoms. Rangpur lime, citron, and pummelo are less susceptible.

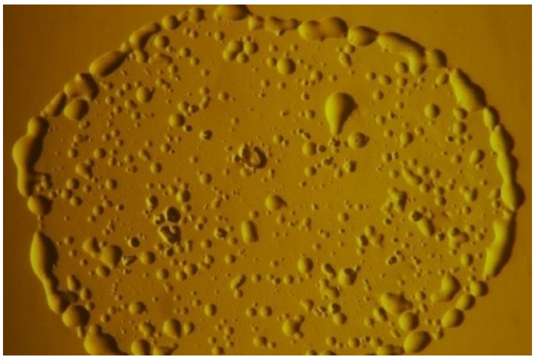

Fig. 1. Colonies of the CVC-bacterium obtained from a drop of tissue homogenate on PW 9 days after inoculation (x 16). (photo by Chung-Jan Chang).

Relative to total sales, blueberries are the number one fruit commodity in the state of Georgia, surpassing even peaches. Production is concentrated in the southern coastal flatwoods. Rabbiteye blueberry (*Vaccinium virgatum* Aiton), a native species, has long been the predominant blueberry species cultivated in Georgia. More recently, however, growers have increased the production of the southern highbush cultivars (*V. corymbosum* interspecific hybrids) as a result of a very favorable market window. Growers and scientists started to observe a new disorder affecting the southern highbush selection FL 86-19 in the Georgia blueberry production region. An initial symptom was marginal leaf scorch (burn) of the older leaves which is very distinct and is surrounded by a dark line of demarcation between green and dead tissue, similar to that observed with extreme drought or fertilizer salt burn. New developing shoots were usually abnormally thin with a reduced number of flower buds. Leaf drop eventually occurred with young twigs or stems of the southern highbush selection FL 86-19 developing a yellow, “skeleton-like” appearance which was why “yellow stem” or “yellow twig” was often used to describe the disorder. At this stage, the root system still appeared healthy, except for the possible loss of fine new roots. Whole plants or individual canes showed symptoms. The plant eventually died after leaf drop, typically during the second year of observation [9].

Table 1. Serological relatedness between the CVC-bacterium and strains of *Xylella fastidiosa*

		OD[a] at 405 nm	
Antigens Tested	ATCC Number	15 min	30 min
CVC-bacterium, isolate 8.1.b	--	0.860	>2
Xylella fastidiosa from			
Mulberry leaf scorch	35868	0.022	0.057
Mulberry leaf scorch	35869	0	0.032
Oak leaf scorch	35874	0.014	0.045
Ragweed stunt	35876	0.071	>2
Periwinkle wilt	35878	0	0.07
Almond leaf scorch	35870	0.743	>2
Pierce's disease of grape	35879	0.852	>2
Pierce's disease of grape	35881	0.815	>2
Pierce's disease of grape			
Georgia isolate Chateau 3C	--	0.613	>2
Georgia isolate 112.V1	--	0.814	>2
Georgia isolate 116.V6	--	0.737	>2
Georgia isolate 116.V11	--	0.601	>2
Georgia isolate MS7	--	0.654	>2
E. coli	--	0.059	0.083

[a]ELISA conducted using antiserum prepared against isolate 8.1.b of the CVC bacterium.

This prompted the enzyme-linked immunosorbent assay (ELISA) tests and isolations of *X. fastidiosa*. A single diseased blueberry bush of the selection FL 89-16 was excavated from a blueberry farm in South Georgia on 2 Feb. 2006. The bush was subsequently stored under cold room conditions (5 °C), in a plastic trash bag to prevent moisture loss, until attempted detection of *X. fastidiosa* using direct isolation and ELISA tests (Agdia, Inc., Elkhart, IN). From this initial plant, two leaf and two root tissue samples were collected for isolation and ELISA testing on 2 Mar. 2006. The diseased blueberry bush was then moved to a greenhouse and planted in a 30.5-cm diameter pot. This original diseased plant was used to monitor the survival of the bacterium and symptom development on new growth after being stored for 48 d at 5 °C. ELISA results indicated all four tissues tested positive for the bacterial pathogen, *X. fastidiosa*, whereas only the two root tissues provided positive isolations. One leaf and one root tissue sample were later collected from each of five additional diseased plants for isolation and ELISA testing. Both isolation and ELISA testing methods obtained positive results. Cultures were multiplied to inoculate seedlings of three cultivars: 'Southern Belle' (eight plants), 'Premier' (six), and 'Powderblue' (six) on 23 May 2006 and one selection, FL 86-19 (eight), on 31 May 2006. Two FL 86-19 plants started to show symptoms of marginal necrosis 54 days postinoculation , whereas one plant each of ‘Southern Belle’ and ‘Powderblue’ started to show symptoms of marginal necrosis 63 days postinoculation and ‘Premier’ stayed symptomless. All eight culture-inoculated FL 86-19 plants (100%) showed symptoms 72 days postinoculation, but no symptoms were observed on the control plants. One hundred twenty-six days postinoculation, two ‘Powderblue’ and four ‘Southern Belle’ plants showed mild symptoms, whereas all ‘Premier’ plants were asymptomatic. Positive reisolations of the bacteria from the inoculated symptomatic plants, not from asymptomatic plants, fulfilled Koch's postulates, which confirmed *X. fastidiosa* was the causal bacterium of the new blueberry disorder, the bacterial leaf scorch of blueberry.

This original blueberry bush provided valuable information on the survivability of the *X. fastidiosa* blueberry strain. The bacterium was able to survive at 5 °C for 48 d when the bush was kept in a plastic bag before being planted in a large pot and kept in the greenhouse. On 10 July 2006, tissues from this bush were collected for isolation and ELISA and the results were positive for both methods. The blueberry industry, particularly the growers, in the southeastern United States will find this information especially important, because the research suggests that the bacteria is able to survive in the aboveground tissues through the south Georgia winter because it is unlikely for the temperature to remain at 5 °C 24 h a day for a consecutive 48 d in the winter. Furthermore, the source of inoculum for transmission would likely be available year-round [9].

By 3 months after initial inoculation, all eight *X. fastidiosa*-injected FL 86-19 plants showed symptoms, whereas all four PW medium-only-injected plants remained asymptomatic. For the other three cultivars, only two of six ‘Powderblue’ and four of eight ‘Southern Belle’ showed mild symptoms, whereas zero of six ‘Premier’ plants were symptomatic even at 4 months postinoculation. Both ELISA and direct isolations confirmed the presence of *X. fastidiosa* in symptomatic plants. Yellow stems or twigs were a strong symptomatic indicator of *X. fastidiosa* infection. There seemed to be a different degree of susceptibility among the three cultivars and one selection with selection FL 86-19 clearly being the most susceptible consistent with what had been observed in the field [9].

Further studies indicated that there is varietal resistance in some southern highbush blueberries. The FL 86-16 variety is particularly susceptible to infection. When compared with other southern highbush or rabbiteye varieties, the “V5” variety showed resistance to the bacterium (Fig.2). This is encouraging, since it indicates that breeding can be utilized to develop varieties that are highly resistant to Xylella. Likewise, surveys have shown that there are other varieties that either do not develop symptoms or that slow epidemic spread of the disease (Fig. 3). ‘FL 86-19’ is highly susceptible, as is the ‘O’Neal’ cultivar. ‘Star’ is susceptible, but it is representative of desirable cultivars that will develop the disease but still likely be economically viable; field epidemics observed in ‘Star’ and similar cultivars do not develop as rapidly, allowing adequate time to recoup investments [5].

Phloem-limited plant pathogenic prokaryotes and plant health

In Mollicutes, the cell wall-less and phloem-limited prokaryotes, there are two major plant pathogens: spiroplasmas and phytoplasmas. Spiroplasmas are cells with helical forms during log phase growth. Most spriroplasmas are cultivable in enriched media that contain supplemented sterols and other ingredients [7]. They are facultative Phytoplasmas have been associated with more than 500 plant diseases worldwide [16] ever since the historical discovery by Doi et al. [10] of then referred as mycoplasma-like organisms (MLO) found in the pholem elements of plants infected with

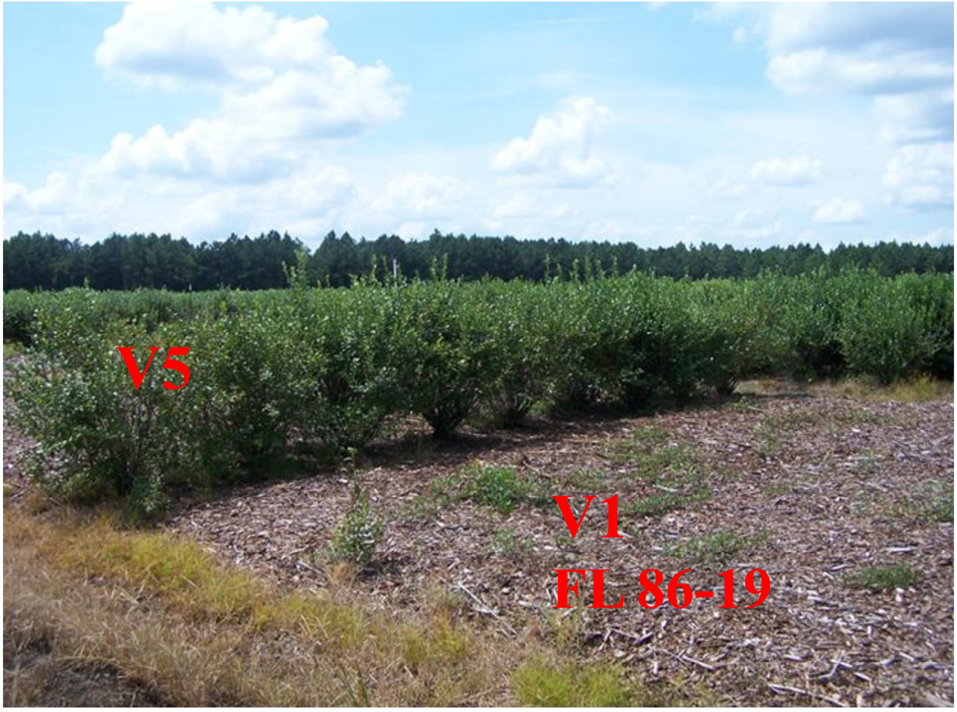

Fig. 2. Resistance. In this planting, a single row of 'V5' plants was alternately planted after 10 rows of V1 'FL86-19' plants (repeated numerous times). The surrounding 'FL86-19' plants were all infected, with significant mortality, and they have been removed at this point. The 'V5' plants consistently showed no symptoms of disease or mortality after five years at this site. This indicates field resistance in the 'V5' line. (Brannen *et al*., 2011. UGA Cooperative Extension Circular 922:1-6).

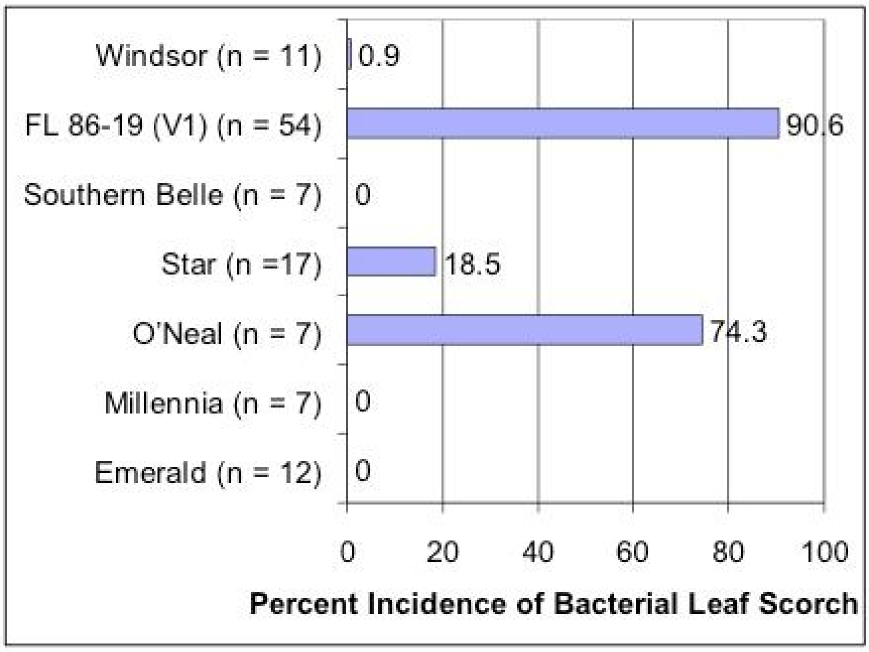

Fig. 3. Incidence (percentage of symptomatic plants) of bacterial leaf scorch by cultivar at one site. The number of rows surveyed (n) is shown in parentheses next to the cultivar name. 'FL 86-19' is highly susceptible, as is the 'O'Neal' cultivar. 'Star' is susceptible, but it is representative of desirable cultivars that will develop the disease but still likely be economically viable; field epidemics observed in 'Star' and similar cultivars do not develop as rapidly, allowing adequate time to recoup investments. (Brannen *et al*., 2011. UGA Cooperative Extension Circular 922:1-6).

mulberry dwarf, potato witches'-broom, aster yellows, or paulownia witches'-broom. Phytoplasmas are still noncultivable even though they have been classified into 30 group-subgroups and four undetermined entities based on the 16S rDNA RFLP grouping (http://plantpathology. ba. ars.usda.gov/pclass/ pclass_taxonomy.html).

There were unintentional fans of phytoplasmas for as early as 1000 years ago in Song Dynasty and as recent as nowadays. When phytoplasmas infect peonies, the plants produced flowers not in the typical red or yellow colors, but in a delicate green we call virescence. The green flower was considered so attractive and valuable about 1000 years ago in China that the Song Dynasty's imperial court received a special annual tribute composed of the blossoms. More recently, phytoplasmas have helped brighten winter holidays by transforming otherwise lanky poinsettias, with their eye-catching red leaves, into bushy ornamentals for their fans, lovers of Christmas decorations [(22)].

But most effects of phytoplasmas on plants are not that appealing. They were actually naively quite destructive and malicious. For example, they wither grapes in Europe and Australia; dwarf corn growth in South America; devastate pears and apples in the United States and Europe; destroy peanuts, sesame, and soybean in Asia; and sicken elms, coconuts, asters, and hydrangeas on multiple continents. Just one 2001 phytoplasma outbreak in apple trees caused a loss of about 25 million Euros in Germany and about 100 million Euros in Italy [(22)].

For all the destruction they inflict, you might expect that lots of big agricultural companies and respected academic labs have garnered ample amount of information about them. Well to the contrary, to this day, the inability to grow these bacteria outside plants or insects hinders efforts to get a handle on their biology and genomes despite the fact that plant pathologists had spent over half a century thinking that phytoplasmas were viruses.

Recent finding of secreted AY-WB protein 11 (SAP11) by Bai et al [(2)] and secreted protein TENGU by Hoshi et al [(14)] seemed to suggest that phytoplasmas are finally giving up some of their secrets of how they used the proteins to modify the activity of plant genes that participate in the disease development. Some scientists are already contemplating whether they can create plants with only the positive attributes of an infection. Perhaps adding a single phytoplasma gene to a plant's DNA could create bushy poinsettias or green peonies that don't carry the annoying pathogens [(22)].

Spiroplasma citri causes stubborn disease on citrus and brittle root disease on horseradish. *S. citri* is transmitted in a propagative, circulative manner by several leafhoppers including *Circulifer tenellus* and *Scaphytopius nitridus* in citrus-growing regions of California and Arizona and *C. haematoceps (syn. Neoaliturus haematoceps)* in the Mediterranean region. The pathogen multiplies in the vector but no transovarial transmission occurs. Spatial and temporal analysis of CSD incidence indicate only primary spread occurring and no or very limited secondary spread (citrus to citrus). *C. tenellus and N. haematoceps* have a wide host range which includes many natural hosts of *S. citri* but citrus is a non host of these leafhoppers. Citrus becomes infected when inoculative *Circulifer* vectors feed temporarily on citrus during migratory flights [(4)].

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摘 要

張宗仁[1,2]、石憲忠[3]、蘇秋竹[4]、詹富智[2,5]. 難培養原核生物與植物健康概述. 植病會刊 22:233-243. ([1]美國 喬治亞大學植物病理系、[2]台中市 國立中興大學植物病理系、[3]台中市 農委會農業試驗所應用動物組、[4]台中市 農委會農業毒物藥物試驗所農藥應用組、[5]聯絡作者，電子郵件：fjjan@nchu.edu.tw; 傳真:+886-4-22854145)

原核生物幾乎無所不在，或者可說是「只要有生命的地方就有原核生物」。它們是地球上最早出現的生物體。迄今，因下述二個原因，它們仍然在生物圈佔據主導的地位：(1) 原核生物總合的生物質量高於所有真核生物總生物質量達十倍之多;(2) 一把肥沃土壤或人類的口腔或皮膚上的原核生物數量比存在於地球上的人類數量還多。它們可以生存於非常寒冷、非常炎熱、高鹽性、高酸性或高鹼性的環境下，它們具有多樣的適應性，使之能在許多環境下生存，同時，它們也具有高度的遺傳多樣性。難培養的植物病原原核生物為植物的病原之一，它們無法生長于目前用於培養細菌的培養基，或是需要在特定的營養或非常營養的培養基上才可生長。此類原核生物包括侷限於植物導管內的 *Xylella fastidiosa*、*Leifsonia xyli* subsp. *xyli*、*L. xyli* subsp. *cynodontis*、*Clavibacter michiganensis* subsp. *sepedonicus* 及 *C. michiganensis* subsp. *michiganensis* 與侷限於韌皮部內的螺旋體、植物菌質體及柑橘黃龍病菌 (*Candidatus* Liberibacter spp.) 等。這些不易培養的植物病原菌對於植物的健康具有極大的影響。其中，*X. fastidiosa* 可造成製酒業及生產柑橘果汁產業的巨大經濟損失。螺旋體可造成三種植物病害:柑橘頑固病、玉米矮化病及日日春的病害。植物菌質體則可造成超過 500 種的植物病害[2]。而存在數十年之久的柑橘病害，黃龍病或稱柑橘立枯病，以及最近發生在馬鈴薯上的斑馬紋病及其他多種病害，皆由柑橘黃龍病菌 (*Ca.* Liberibacter spp.)所引起。在整個美國南部地區，由德州到卡羅來納州沿著墨西哥灣沿岸直至墨西哥製酒產業都受到皮爾氏病的影響。近來曾有報導指出，褐透翅尖頭葉蟬 (glassy-winged sharpshooter leafhoppers) 對於加州的製酒業造成危害。自呈現柑橘斑駁黃化病徵的組織上成功的分離出 *X. fastidiosa* 及鑑定出其主要的媒介昆蟲，提供在防治柑橘斑駁黃化病上最重要的資訊，使巴西種植柑橘的農民及生產柑橘果汁的業者得以克服此病害發生時的危機。而由出現葉緣焦枯病徵的藍莓組織上成功的分離及鑑定出 *X. fastidiosa*，繼而成功發展出抗病或耐病的藍莓栽培品系，使得喬治亞州的藍莓產業在近年來得以躍升為水果類商品第一名。

關鍵字: 難培養的原核生物、*Xylella fastidiosa*、黃龍病菌、螺旋體、植物菌質體、黃龍病、半翅目、褐透翅尖頭葉蟬、葡萄皮爾氏病、柑橘斑駁黃化病、藍莓細菌性葉緣焦枯病