Research paper

Assessing the Susceptibility of Asian Species of Lauraceae to the Laurel Wilt Pathogen *Raffaelea lauricola*

Hsin-Hui Shih,[1)] Caroline E. Wuest,[2)] Stephen W. Fraedrich,[3)]
Thomas C. Harrington,[2,5)] Chi-Yu Chen[4)]

【Summary】

Laurel wilt, caused by *Raffaelea lauricola*, was discovered in the southeastern USA in 2004, and has been responsible for the death of many indigenous North American tree and shrub species in the family Lauraceae. Symptoms of the disease are typical of vascular wilts, in which woody xylem becomes discolored and nonfunctional due to infection by the fungus, and foliage subsequently dies due to a lack of water. The vector of *R. lauricola* is an ambrosia beetle, *Xyleborus glabratus*, and trees become infected when beetles bore into tree stems and branches and release fungal spores from their oral mycangia. The ambrosia beetles produce brood in the trees after wilting. Although the fungus and beetle are native to Asia, laurel wilt has not been reported on plant species native to Asia. The purpose of this study was to assess the susceptibility of Asian species in the Lauraceae to laurel wilt. Most Asian species (*Cinnamomum camphora*, *C. osmophloeum*, *Machilus zuihoensis*, *M. thunbergii*, *C. jensenianum*) developed only a light pale-gray discoloration in the sapwood following inoculation, but no other symptoms were observed. However, partial wilt of branches was observed in some inoculated plants of 2 Asian shrub species (*Lindera strychnifolia* and a *Phoebe* sp.). *Raffaelea lauricola* was isolated from discolored xylem of many of the inoculated species. Compared to North American lauraceous species such as redbay (*Persea borbonia*), the Asian species are more resistant to laurel wilt, but all inoculated host plants showed some level of susceptibility.

Key words: laurel wilt, *Raffaelea lauricola*, Asian Lauraceae, inoculation.

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1) Division of Forest Protection, Taiwan Forestry Research Institute, 53 Nanhai Rd., Taipei 10066, Taiwan. 林業試驗所森林保護組，10066台北市南海路53號。

2) Department of Plant Pathology and Microbiology, 351 Bessey Hall, Iowa State University, Ames, IA 50011, USA.

3) Southern Research Station, USDA Forest Service, Athens, GA 30602, USA.

4) National Chung Hsing University, 145 Xingda Rd., South Dist., Taichung 40227, Taiwan. 國立中興大學，40227台中市南區興大路145號。

5) Corresponding author, T. C. Harrington, e-mail:tcharrin@iastate.edu 通訊作者。

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研究報告

亞洲樟科植物
對樟科萎凋病菌*Raffaelea lauricola*之感病性評估

施欣慧[1) Caroline E. Wuest[2)] Stephen W. Fraedrich[3)]
Thomas C. Harrington[2,5)] 陳啟予[4)]

摘 要

樟科萎凋病(病原菌：*Raffaelea lauricola*)在2004年於美國東南部發現，會造成數種原生於北美地區許多種樟科喬木與灌木的嚴重危害。此病害為典型的維管束萎凋病，病原菌會使木質部變色、喪失輸送水分的功能，最後引起樹木落葉、萎凋死亡。病原菌*R. lauricola*的傳播媒介為一種菌蠹蟲—*Xyleborus glabratus*，其會鑽入樹木的枝幹中蛀蝕，並由口部的儲菌器釋放出真菌孢子。雖然前人研究顯示此病害的病原菌和媒介昆蟲皆源自於亞洲地區，但卻不曾在亞洲有嚴重危害樟科植物之報導。因此，本研究目的主要針對數種亞洲樟科植物對樟科萎凋病菌的感病性測試。接種試驗結果顯示，選定的亞洲樟科植物:樟樹(*Cinnamomum camphora*)、土肉桂(*C. osmophloeum*)、香楠(*Machilus zuihoensis*)、紅楠(*M. thunbergii*)和野黃桂(*C. jensenianum*)，接種病原菌*R. lauricola*後，於邊材組織會出現輕微淡灰色的變色，但未出現其他病徵。相反的，另兩種亞洲樟科植物，烏藥(*Lindera strychnifolia*)和一種楠屬植物(*Phoebe* sp.)，則於枝條上分別出現40%和30%的部分萎凋。多數接種植物的變色邊材組織皆可分離到病原菌。此試驗結果和北美樟科植物如：*Persea borbonia*相比，亞洲樟科植物對樟科萎凋病菌具有較高的抗病性，但不同的樹種仍有不同程度的感病性。

關鍵詞：樟科萎凋病、*Raffaelea lauricola*、亞洲樟科植物、接種。

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INTRODUCTION

Laurel wilt, caused by *Raffaelea lauricola*, is a lethal vascular wilt disease on woody North American Lauraceae species. It has been observed in South Carolina and Georgia, USA since 2004, and in Florida since 2005, and it has expanded its range to include North Carolina, Alabama, Mississippi, Louisiana, Arkansas, and Texas (Bates et al. 2015, Fraedrich et al. 2015a, Hughes et al. 2015, Olatinwo et al. 2016, Riggins et al. 2010). In the USA, the affected native hosts include redbay (*Persea borbonia* (L.) Spreng.), swampbay (*Persea palustris* (Raf.) Sarg.), sassafras (*Sassafras albidum* (Nutt.) Nees), spicebush (*Lindera benzoin* (L.) Blume), pondberry (*Lindera melissifolia*), pondspice (*Litsea aestivalis* (L.) Fernald), and cultivated avocado (*Persea americana* Mill.) (Fraedrich et al. 2008, 2011, Harrington et al. 2008).

Raffaelea lauricola is a symbiont of the redbay ambrosia beetle (RAB), *Xyleborus glabratus* Eichhoff, which was first detected in Georgia, USA in 2002. The beetle is native to Southeast Asia and has been documented in Japan, China, Taiwan, Myanmar, Bangladesh, and India on species of the Lauraceae, Dipterocarpaceae, Fagaceae, and Fabaceae (Wood and Bright 1992, Rabaglia et al. 2006, Hulcr and Lou 2013). *Raffaelea lauricola* was isolated from *X. glabratus* in Japan and Taiwan (Harrington et al. 2011), and mortality of avocado due to laurel wilt was recently reported in Myanmar (Ploetz et al. 2016). However, laurel wilt has not been recorded on native hosts in Asia.

Females of *X. glabratus* possess oral mycangia that carry conidia of *R. lauricola* (Fraedrich et al. 2008). Other *Raffaelea* species can be isolated from the mycangia of *X. glabratus*, including *R. arxii*, *R. subalba*, *R. ellipticospora*, *R. fusca*, and *R. subfusca*, but none of these other species is known to be a plant pathogen (Harrington and Fraedrich 2010, Harrington et al. 2010, Campbell et al. 2016, Dreaden et al. 2016). When female *X. glabratus* excavates brood galleries, it inoculates the tunnels with fungal spores that grow from the mycangia, and the fungi sporulate within the gallery and provide food for the beetle larvae (Batra 1967, Fraedrich et al. 2008). Female *X. glabratus* will also attack living host trees and introduce spores of *R. lauricola* into the xylem during these aborted attacks (Fraedrich et al. 2008). After introduction, systemic colonization by the pathogen and the host response lead to blockage of water transport, causing wilt and ultimately tree death, which provides brood material for later attacks by the beetle (Fraedrich et al. 2008). The host response to the systemic colonization generally includes xylem discoloration, which is gray to black in color (Fraedrich et al. 2008, Kendra et al. 2013).

Based on inoculation studies and field observations, it is evident that North American species of the Lauraceae are highly susceptible to laurel wilt (Fraedrich et al. 2008, 2011, Mayfield et al. 2008, Hughes et al. 2012, 2013, Peña et al. 2012, Ploetz et al. 2012, Ploetz and Konkol 2013). The Asian capmphortree, *Cinnamomum camphora*, may exhibit branch dieback and wilt-like symptoms in the USA when infected by *R. lauricola* (Fraedrich et al. 2015b). Inoculated camphor trees may produce a brownish discoloration of the xylem when stems are inoculated at multiple sites with *R. lauricola*, but plants developed only limited discoloration of the xylem and no foliar wilt with single-point inoculations (Fraedrich et al. 2015b).

Xyleborus glabratus and *R. lauricola* are native to Taiwan (Wood and Bright 1992, Harrington et al. 2011), where there are also numerous native plant species in the Lauraceae, but cases of laurel wilt have not been documented in Taiwan (Wuest et al. 2017). Therefore, we speculated that indigenous Asian species of Lauraceae may be more resistant to laurel wilt than are North American species. The primary objective of this study was to assess the susceptibility of several Asian species of Lauraceae to laurel wilt following inoculation by *R. lauricola*.

MATERIALS AND METHODS

Inoculation of American vs. Asian species of the Lauraceae with USA isolates

Saplings and inoculum: Plants used in the USA experiments were obtained from nurseries in Georgia and South Carolina that specialized in the production of foreign and native trees and shrub species. One American species (*P. borbonia*) and 5 Asian species (*Machilus thunbergii*, *Lindera strychnifolia*, *Cinnamomum jensenianum*, *C. camphora* and a *Phoebe* sp. were inoculated in growth chambers in Athens, GA (Fraedrich et al. 2015b). Plants were grown in 1~3-gallon containers for 2~3 yr before inoculation. Because of the limited space in the growth chamber, 4 experiments were necessary to test the 5

Asian species. In each experiment, the highly susceptible *P. borbonia* was tested to verify that the inoculum was virulent and capable of causing wilt in plants.

Each experiment used 2 isolates of *R. lauricola* from the collection at Iowa State University. The isolates were obtained from wilted redbay trees in South Carolina (C2428), Georgia (Brantley1), or Florida (C2258) and from a sassafras tree in Mississippi (C2792). Isolate C2428, not C2258 was used in each of the 4 experiments; isolate C2258 was used in experiments 1 and 3, Brantley1 in experiment 2, and isolate C2792 in experiment 4. The isolates were grown on malt extract agar (MEA; 2.5% malt extract and 2.0% agar) for 10~14 d. Conidia were collected by flooding plates with approximately 20 ml of sterile distilled water, loosening conidia with a glass rod, and passing the suspension through 3-ply sterile gauze. Spore concentrations were determined with a hemocytometer and ranged from 6.9×10^5 to 3.2×10^6 conidia mL^{-1}.

Inoculation: For most plant species and experiments, 15 plants were wounded by drilling holes (2 mm in diameter, 4~8 mm deep) into the stem at 8~12 cm above the groundline. Ten saplings of each species were inoculated with 0.2 mL of the conidial suspension, using 5 saplings for each isolate. Five saplings served as controls, in which 0.2 mL of sterile water was placed into the wound. In experiments where 15 plants were not available for a particular species, the available saplings were divided among the inoculation treatments. At least 3 saplings were available per treatment. After inoculation, the wounds were wrapped with Parafilm M (Pechiney Plastic Packaging, Menasha, WI, USA). Saplings were placed in growth chambers with a 16-h photoperiod, and temperatures were set to 25℃. Experiments were conducted for 8~10 wk. Plants were evaluated for symptoms of disease (e.g., wilt of foliage, branch dieback, and discoloration of sapwood) at the end of the experiments.

Reisolation: Pieces of stem tissue from 10~20 cm above the inoculation points were surface sterilized and plated on MEA amended with 100 ppm streptomycin and 200 ppm cycloheximide (CSMA). The plates were incubated at 25℃, and after 7~14 days, the plates were assessed for the presence of *R. lauricola*, which has a unique mucoid growth on CSMA (Fraedrich et al. 2015b).

Inoculation of Asian species of Lauraceae with Taiwan isolates

Saplings and inoculum: Saplings of 4 indigenous Lauraceae species (*Cinnamomum camphora*, *C. osmophloeum*, *Machilus zuihoensis*, and *M. thunbergii*) from nurseries in Taiwan were inoculated in a greenhouse at the Taiwan Forestry Research Institute in Lioukuei (六龜), Kaohsiung. Seedlings were grown for 2~3 yr in commercial containers. No seedlings of *C. osmophloeum* were available, so *C. osmophloeum* branches were grafted onto the rootstock of *C. burmannii*. The respective mean heights of *C. camphora, C. osmophloeum*, *M. zuihoensis*, and *M. thunbergii* were 94.5, 71.5, 109.8, and 95.4 cm, and the respective mean diameters at the groundline were 1.7, 1.6, 1.2, and 1.9 cm.

Three isolates of *R. lauricola* were isolated from 3 individual *X. glabratus* beetles obtained from bolts taken from a naturally infested tree of *C. osmophloeum* in Hsinhsien, Taiwan (Wuest et al. 2017). Isolates were cultured on CSMA (2% malt extract, 1.5% agar, 300 ppm cycloheximide, and 300 ppm streptomycin sulphate) for 7 d. The inoculum for the 3 isolates of *R. lauricola* was prepared as described above. Respective mean spore concentrations of the inoculum of isolates R1 (C3545), R2 (C3546) and R3 (C3525) were

7.3×10^6, 5.6×10^6, and 4.9×10^6 spores/ml.

Inoculation: Thirty-six trees of each species were inoculated in August 2014. There were 4 treatments (control with sterile water and *R. lauricola* isolates R1, R2, and R3) and 9 replicates for each species. The saplings were wounded by cutting stems at 7.5, 15 and 22.5 cm above the groundline at a 45º downward angle to a depth of one-third to one-half of the diameter. The 3 incisions on each sapling were spaced 90º apart from each other. A 100-μL suspension of inoculum or sterile water was inserted into each of the 3 wounds. The wound sites were wrapped with Parafilm, and the saplings were kept in the greenhouse for observation.

Two seedlings of each treatment were examined at 4 mo after inoculation to see if there was xylem discoloration around the inoculation site. The other 7 replicates were examined after 7 mo (March 2015) by peeling the bark and cutting the stems into discs. Discs were taken at the groundline (0 cm) and at 11, 19, 30, and 36 cm above the groundline. If a sapling was taller than 36 cm, then further discs were taken above 36 cm at regular intervals until the top of the sapling was reached.

Reisolation: Isolations were attempted from the 7 replicate saplings harvested after 7 mo. Attempts were made to reisolate from all the discs taken at the groundline (0 cm) and at 11, 19, 30, and 36 cm above the groundline. If there was discoloration in discs taken from above 36 cm, then reisolation was attempted from the topmost disc with xylem discoloration. The discs were soaked in commercial bleach (5~6% sodium hypochlorite) for 3 min, 70~95% ethanol for 30 sec, and then dipped in sterile water for another 30 sec. Disks were blotted dry with a sterile paper towel to remove excess moisture and placed on CSMA. Plates were observed after 4 d, and colonies were subcultured to confirm the morphology of *R. lauricola*.

RESULTS

Inoculation of American vs. Asian species of Lauraceae with USA isolates

Wilt and death of foliage developed on all inoculated *P. borbonia* plants, indicating that all isolates were virulent and capable of causing wilt. No difference was noted between the 2 isolates used in each experiment, so the data for the 2 isolates were combined (Table 1). None of the Asian species developed wilt symptoms to the extent observed in redbay. In experiment 2, initiated in July 2008, 40% of the inoculated *Lindera strychnifolia* saplings developed partial wilt, i.e., dieback of some branches while other branches remained healthy. However, no dieback was observed in *L. strychnifolia* in experiment 3 (the June 2010 inoculations). A small percentage of saplings of the unidentified *Phoebe* sp. also exhibited partial wilt.

Xylem discoloration was not observed in *M. thunbergii* or *C. camphora* following inoculation in the USA experiments. However, discoloration was observed to varying degrees in the 3 other Asian species: *L. strychnifolia*, *C. jensenianum*, and *Phoebe* sp. (Table 1). In contrast to the dark-brown to black discoloration found throughout the xylem of *P. borbonia*, the discoloration in the 3 Asian species appeared light brown or as localized streaks of darker discoloration, and discoloration was frequently inconsistent among all inoculated plants.

Raffaelea lauricola was recovered 10~20 cm above the inoculation point in some plants of every tested species, although recovery rates varied greatly among species (Table 1).

Inoculation of Asian species of Lauraceae with Taiwan isolates

Two saplings per treatment were checked

Table 1. Symptoms and reisolation of *Raffaelea lauricola* inoculated into American and Asian species of Lauraceae in four experiments conducted in the USA

Experiment no	Host species (native to America or Asia)	Number of plants inoculated[ab]	Percent with foliar wilt symptoms	Percent of plants with discolored xylem	Percent recovery of *R. lauricola from* inoculated plants
1	*Persea borbonia* (American)	10	100	100	80
	Machilus thunbergii (Asian)	10	0	0	100
	Phoebe sp. (Asian)	10	30	60	100
2	*Persea borbonia* (American)	10	100	100	100
	Lindera strychnifolia (Asian)	10	40	90	90
	Cinnamomum jensenianum (Asian)	10	0	0	90
	Cinnamomum camphora (Asian)	10	0	0	30
3	*Persea borbonia* (American)	6	100	100	100
	Lindera strychnifolia (Asian)	7	0	29	14
	Cinnamomum camphora (Asian)	10	0	0	40
4	*Persea borbonia* (American)	6	100	100	100
	Cinnamomum jensenianum (Asian)	6	0	33	50

[a] Mean heights of plants used in experiments ranged 62~153 cm, and mean diameters at the groundline ranged 8.3~26 mm.

[b] A set of 3-to-5 control plants were mock-inoculated for each species during each experiment. These control plants exhibited no signs of wilt or xylem discoloration, and *R. lauricola* was not isolated from the plants at the end of the experiment.

for symptoms in December 2014. The bark was removed from 1 sapling of each tree species, but it was difficult to assess xylem discoloration near the points of inoculation. A second replicate sapling of each species-treatment combination was cross-sectioned to determine if there was xylem discoloration, and recovery of *R. lauricola* was attempted from these discs. Small areas of xylem discoloration were observed in portions of the sectioned discs from *C. osmophloeum* and *M. zuihoensis*. *Raffaelea lauricola* was recovered from discolored discs of *M. zuihoensis*, but not from *C. osmophloeum*. No discoloration was observed in the xylem of the inoculated *C. camphora* or *M. thunbergii* saplings.

The remaining 7 replicate saplings of the inoculated trees displayed no leaf-wilting symptoms after 6~8 mo. Three saplings of *C. camphora* died from an infestation of a scale insect (*Aulacaspis yabunikkei*), which is known to cause damage to *C. camphora*, so these individuals were excluded from the experiment. At the end of the experiment, the inoculated saplings had living sapwood, but some saplings of all 4 species inoculated with *R. lauricola* appeared to have small areas of pale-gray streaking in the xylem, usually seen in cross-section as one to several minute, pale-gray areas in the xylem (indicated by red arrows, Fig. 1). Some discs from *M. thunbergii* had short lines of dark-gray discoloration within the concentric rings of the xylem, but in general, there was less xylem discoloration in *M. thunbergii* than in the other species (Fig. 2). Very few of the discs taken at the groundline showed discoloration, and the greatest discoloration was seen in discs just above the 3 inoculation points (Fig. 2). However, most of the discs from *C. osmophloeum* and *M. zuihoensis* taken at 14 cm above the highest inoculation point showed xylem discolor-

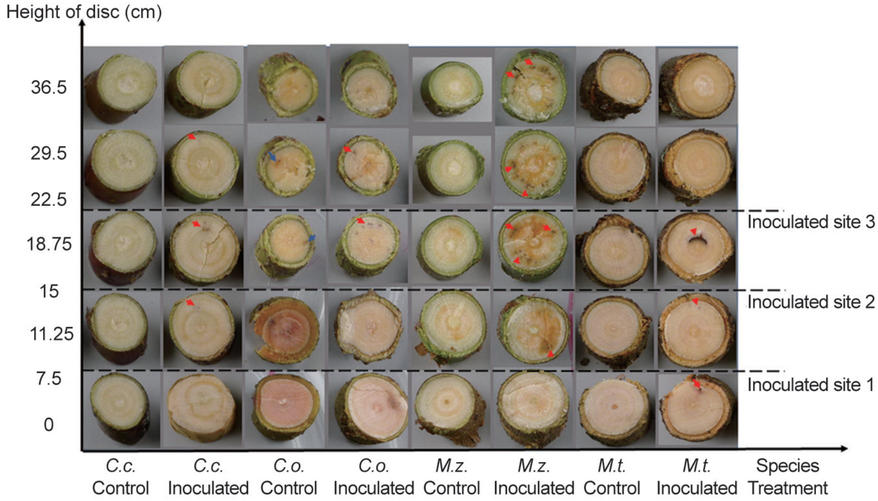

Fig. 1. Xylem discoloration in discs cut from inoculated stems of 4 Asian Lauraceae species in a greenhouse experiment. The vertical axis was the height above the groundline of inoculation sites and sectioned discs. The horizontal labels indicate control or inoculated saplings of: *C.c.* = *Cinnamomum camphora*, *C.o.* = *Cinnamomum osmophloeum*, *M.z.* = *Machilus zuihoensis*, and *M.t.* = *Machilus thunbergii*.

ation (Fig. 2). There was little variation in the amount of discoloration induced by the 3 isolates, except isolate R3 failed to induce discoloration in 3 inoculated seedlings of *C. osmophloeum* (Fig. 2).

Compared to the USA experiments, there was more discoloration in seedlings inoculated with the more-severe wounding technique used in the Taiwan experiment. However, sapwood discoloration did not always coincide with successful isolation of *R. lauricola* from the various plant species (Fig 2). In the Taiwan experiment, the frequency of reisolation of *R. lauricola* was greatest from *C. camphora* (32% of the discs; 50% of the discs with xylem discoloration and 17% of the discs with no xylem discoloration). The fungus was recovered from 31% of *M. zuihoensis* discs (44% from discolored discs and 0% from discs with no discolored xylem), including 1 discolored disc taken at 92.5 cm and another discolored disc at 99 cm above the groundline. The pathogen was recovered from 18% of *M. thunbergii* discs (57% from discolored discs and 7% from discs with no discolored xylem). Although most discs of *C. osmophloeum* showed some xylem discoloration, inoculated saplings of this species had the lowest frequency of *R. lauricola* reisolation (= 6%, 9% from discolored discs and 2% from discs with no discolored xylem), and most of these successful reisolations were from the discs between the 2 lowest inoculation points.

In the Taiwan experiment, a few of the discs of *C. camphora* and *M. thunbergii* taken at the groundline showed small points of discoloration, but *R. lauricola* was recovered from only one of those discs (Fig. 2). A few discs from control trees showed discolored xylem in the form of minute, pale-gray points (blue arrows in Fig. 2), but *R. lauricola* was not isolated from those discs.

DISCUSSION

The losses due to laurel wilt in the southeastern USA can be USA can be attributed to greater attributed to greater susceptibility in the American Lauraceae compared to relatively resistant Asian species. Laurel wilt is responsible for widespread mortality of *P. borbonia* and other native species of the Lauraceae in the southeastern USA (Fraedrich et al. 2008, 2011, Wuest et al. 2017). In North American species of Lauraceae, introduction of *R. lauricola* spores into a single inoculation point is sufficient for systemic colonization of the plants, and wilt symptoms develop within 6~12 wk

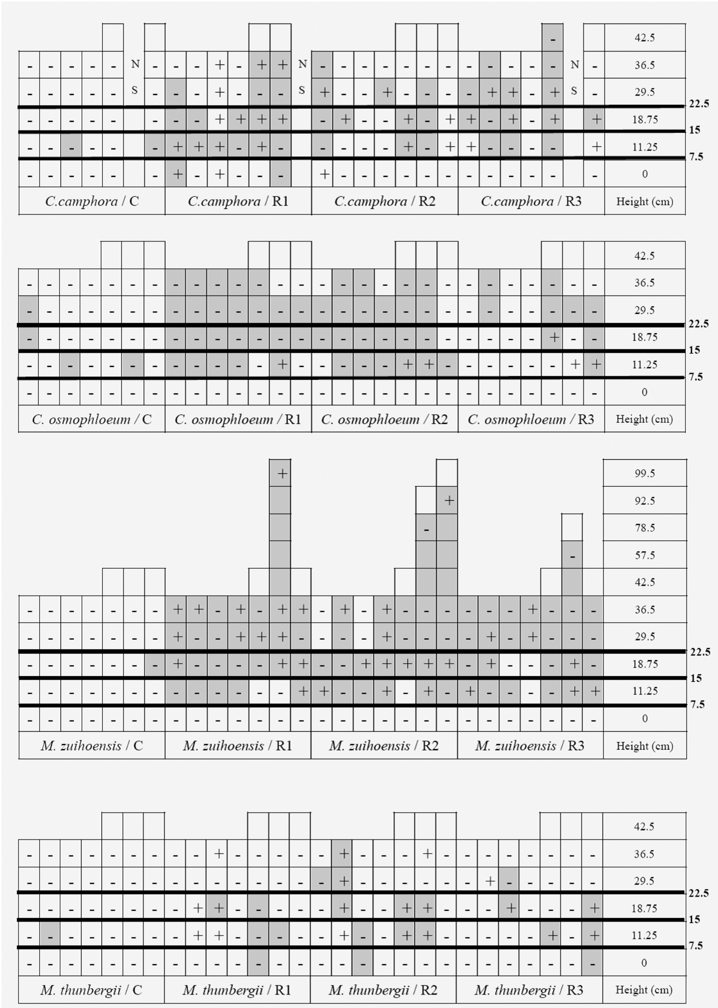

Fig. 2. Presence of xylem discoloration (gray shading) and successful recovery of *Raffaelea lauricola* (+) from 4 Asian Lauraceae species in a greenhouse inoculation experiment. There were 7 saplings for each treatment and 4 treatments: control (C) and 3 isolates of *R. lauricola* (isolates R1, R2, and R3) for each host species. The numbers on the vertical axis are the height above the groundline of sectioned discs. Three thick black lines indicate the height of the 3 inoculation points on each sapling. Discs sampled but no *R. lauricola* isolated are marked "-", and saplings not sampled (NS) died from scale insects and had to be dropped from the experiment.

(Fraedrich et al. 2008, 2011). The inoculation studies reported here show that *R. lauricola* can colonize living seedlings and saplings of Asian Lauraceae and produce limited symptoms of laurel wilt in some species, but the degree of wilting is much less in Asian species. Although the isolates used and inoculation methods varied, similar patterns of systemic colonization were seen among host genera and species in the USA and Taiwan experiments.

In seedling inoculations conducted in the USA, no wilting was seen in *C. camphora*, *C. jensenianum*, or *Machilus thunbergii*, while 100% of the inoculated *P. borbonia* wilted. Nonetheless, the pathogen was reisolated from most of the inoculated seedlings of these 3 Asian species, and xylem discoloration was seen in 2 of 16 inoculated seedlings of *C. jensenianum*. Some inoculated seedlings of the Asian *Lindera strychnifolia* and *Phoebe* sp. showed wilting, and these Asian species, which are shrubs to small trees, could be considered intermediate in susceptibility based on the USA inoculations. Because shrubs and small trees are not regularly attacked by *X. glabratus* (Fraedrich et al. 2008, Mayfield and Brownie 2013), there may be less selection pressure for disease resistance in those species.

In spite of the extensive wounding of inoculated seedlings in the greenhouse experiment in Taiwan, the Asian species proved to be relatively resistant to laurel wilt and mostly expressed only limited xylem discoloration and no foliar wilt symptoms. The xylem discoloration extended more than 14 cm above the highest inoculation point in many saplings of *C. osmophloeum*, *C. camphora*, and *M. thunbergii* in the Taiwan experiment, but there was no discoloration in the inoculated *C. camphora* or *M. thunbergii* seedlings in the USA experiments. *Machilus zuihoensis* appeared to be the most susceptible species inoculated in Taiwan. Xylem discoloration was seen at 70 and 77 cm above the highest inoculation point in 2 saplings of *M. zuihoensis*, and the pathogen was recovered from the apex of discoloration in these 2 saplings. The reisolation success of *R. lauricola* varied greatly for the other 3 inoculated species in Taiwan. Except for *C. osmophloeum*, which was not well colonized, *R. lauricola* was isolated from half of the discolored discs.

In regards to laurel wilt, *C. camphora* is the most studied Asian host. *Raffaelea lauricola* has been isolated from naturally infected *C. camphora* that exhibited dieback-like symptoms in the southeastern USA (Smith et al. 2009, Fraedrich et al. 2015b), and multiple-point inoculations caused branch dieback and wilt-like foliage symptoms, as well as sapwood discoloration, in an earlier study (Fraedrich et al. 2015b). Our inoculations of seedlings in Taiwan confirm that *C. camphora* produces xylem discoloration in response to inoculation, but the movement of *R. lauricola* is relatively limited in this host, and no discoloration was seen in seedling inoculations of *C. camphora* in the USA experiments. Based on these observations and observations in the USA, this Asian species is somewhat susceptible to laurel wilt. *Xyleborus glabratus* infestation and colonization by *R. lauricola* were associated with a *C. osmophloeum* tree with crown dieback in Taiwan (Wuest et al. 2017), but seedling inoculations indicate that *C. osmophloeum* is more resistant to systemic colonization by *R. lauricola* than is *C. camphora*.

There may be selection pressure for resistance to laurel wilt in native species of the Lauraceae in Taiwan, and this resistance may explain why clear cases of laurel wilt have not been observed in Taiwan's forests. However, it was speculated that *X. glabratus* inoculates stressed branches and stems of Lauraceous hosts during aborted attacks in Asia, as it does in the USA, and that *R. lauricola* systemically colonizes and kills the stems or branches, and thus provides brood material (Harrington et al. 2011, Fraedrich et al. 2015b). *Cinnamomum osmophloeum* is known to be a host for *R. lauricola* and *X. glabratus* in Taiwan (Harrington et al. 2011, Wuest et al. 2017). In our inoculation studies, *R. lauricola* was recovered from *C. osmophloeum* in numbers significantly lower than in the other 6 species, possibly suggesting that *C. osmophloeum* may be more resistant to *R. lauricola* than the other species tested.

In contrast, *M. zuihoensis* appeared to be the most susceptible Taiwan species in our inoculation studies. We found a living tree of *M. zuihoensis* with no outward wilt symptoms that was naturally infested by *X. glabratus* in a forest in Taiwan in March 2015. The infested tree was around 7~8 m tall and had a diameter at breast height (DBH) of approximately 15~20 cm. We observed ambrosia beetle entrance holes on the surface of the bark at about 1.5~ the 2.0 m above groundline, near a wound. When the stem was sectioned, the sapwood had a dark-gray discoloration, and *X. glabratus* adult female beetles were found in a tunnel in the discolored wood. *Raffaelea lauricola* was successfully isolated from both the *X. glabratus* females and the discolored sapwood. Therefore, we speculate that the infection of living Lauraceae by *R. lauricola* occurs in Taiwan and that *X. glabratus* can reproduce in the discolored sapwood produced in response to laurel wilt. Asian species of Lauraceae may be more susceptible to laurel wilt and conducive to *X. glabratus* reproduction if the hosts also are stressed by other insects, pathogens, or environmental factors.

CONCLUSIONS

Between the 2 studies, 7 Asian species of Lauraceae (*Cinnamomum camphora*, *C. osmophloeum*, *Machilus zuihoensis*, *M. thunbergii*, *Lindera strychnifolia*, *Cinnamomum jensenianum* and *Phoebe* sp.) were assessed for their susceptibility to laurel wilt caused by *Raffaelea lauricola*. Only the inoculated *L. strychnifolia* and *Phoebe* sp. showed wilted foliage and branch dieback. It is noteworthy that these 2 species are shrubs or small trees, and *X. glabratus* prefers to attack larger-diameter trees (Fraedrich et al. 2008, Mayfield and Brownie 2013). Perhaps these shrubby species are more susceptible to laurel wilt because of a lower selection pressure for resistance to *R. lauricola* compared to larger tree species. Nonetheless, even larger Asian species of the Lauraceae appear to be systemically colonized by *R. lauricola*, and the limited aggressiveness of *R. lauricola* in these hosts may result in killed branches or stems and provide brood material for *X. glabratus*.

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