

An Assessment of the Potential Impact of Laurel Wilt on Clonal Populations of *Lindera melissifolia* (Pondberry)

Authors: G. Susan Best, and Stephen W. Fraedrich

Source: Southeastern Naturalist, 17(4) : 616-628

Published By: Eagle Hill Institute

URL: <https://doi.org/10.1656/058.017.0409>

BioOne Complete (complete.BioOne.org) is a full-text database of 200 subscribed and open-access titles in the biological, ecological, and environmental sciences published by nonprofit societies, associations, museums, institutions, and presses.

Your use of this PDF, the BioOne Complete website, and all posted and associated content indicates your acceptance of BioOne's Terms of Use, available at www.bioone.org/terms-of-use.

Usage of BioOne Complete content is strictly limited to personal, educational, and non-commercial use. Commercial inquiries or rights and permissions requests should be directed to the individual publisher as copyright holder.

BioOne sees sustainable scholarly publishing as an inherently collaborative enterprise connecting authors, nonprofit publishers, academic institutions, research libraries, and research funders in the common goal of maximizing access to critical research.

An Assessment of the Potential Impact of Laurel Wilt on Clonal Populations of *Lindera melissifolia* (Pondberry)

G. Susan Best¹ and Stephen W. Fraedrich^{1,*}

Abstract - *Lindera melissifolia* (Pondberry) is a federally endangered shrub that reproduces primarily by ramets from rhizomes and occurs in scattered clonal populations in bottomland forests of the southeastern US. Like other members of the Lauraceae indigenous to the US, Pondberry is susceptible to laurel wilt, a lethal disease caused by the fungal pathogen, *Raffaelea lauricola*. We conducted studies to determine the impact of laurel wilt on Pondberry colonies. We grew Pondberry in pots and in raised beds for 2–5 y; during this time, multiple ramets developed around the original plants. We subsequently inoculated single Pondberry stems in colonies with *R. lauricola*, or mock-inoculated stems with sterile deionized water. Stems inoculated with *R. lauricola* began to show symptoms within 2 weeks and completely wilted in ~4 weeks. In pot studies, *R. lauricola* spread through rhizomes and caused wilt in an average of 77% of ramets in one experiment and 59% in another. The wilt also spread rapidly through connecting rhizomes in field experiments, killing as many as 59 ramets at distances of up to 4 m from the inoculated stems. Although laurel wilt has been rarely documented in Pondberry, our study demonstrates that when infections by *R. lauricola* occur, they can have detrimental effects to Pondberry colonies.

Introduction

Lindera melissifolia (Walt) Blume (Pondberry) is a deciduous, rhizomatous shrub in the Lauraceae that occurs as isolated populations in forests of the southeastern US, typically near the edges of ponds, depressions that are seasonally wet, sinks, and bottomland hardwood forests (Devall 2013, Hawkins et al. 2010). The species is rare and listed as federally endangered (USFWS 1993), and scattered populations are found in Alabama, Arkansas, Georgia, Mississippi, Missouri, North Carolina, and South Carolina (Devall 2013, Hawkins et al. 2010, USFWS 1993). Reproduction within Pondberry populations is primarily vegetative with ramets developing as sprouts from rhizomes; seedlings are rarely observed (Duvall 2013, Gustafson et al. 2013, Wright 1990). Thus, Pondberry populations consist of 1 or more genets (Gustafson et al. 2013), which could also be termed clonal colonies. The species has a shallow root system with rhizomes growing horizontally from stems, and these mainly occur in the upper few centimeters of soil (Wright 1990). Populations of Pondberry have been impacted by habitat loss, animal damage, and some insect and disease problems (Devall 2013, USFWS 1993, Wilson et al. 2005).

Like other members of the Lauraceae that are indigenous to the US, Pondberry is highly susceptible to laurel wilt (Fraedrich et al. 2011). Laurel wilt is caused by *Raffaelea lauricola* T.C. Harr., Fraedrich and Aghayeva, a fungal symbiont of

¹US Forest Service, Southern Research Station, 320 Green Street, Athens, GA 30602. *Corresponding author - sfracdri@fs.fed.us.

Manuscript Editor: Richard Baird

Xyleborus glabratus Eichhoff (Redbay Ambrosia Beetle). The beetle and fungus were introduced from Asia into the US near Savannah, GA, around 2002 (Fraedrich et al. 2008; Harrington et al. 2008, 2011; Rabaglia et al. 2006). Since 2002, laurel wilt has decimated *Persea borbonia* (L.) Spreng (Redbay) populations in forests across the southeastern US (Fraedrich et al. 2008, Wuest et al. 2017); other species such as *Sassafras albidum* (Nuttall) Nees (Sassafras) and *Litsea aestivalis* (L.) Fern. (Pondspice) have also been affected by the disease (Fraedrich et al. 2011, 2015; Olatinwo et al. 2016). Trees and shrubs become infected with *R. lauricola* when Redbay Ambrosia Beetles attack the stems of healthy plants. The fungus becomes established in the sapwood of plants and moves rapidly throughout the vascular system, causing obstructions in vessels that impede water flow, which causes wilt and plant death. Wilt typically occurs within 4–8 weeks after infection, and the sapwood of infected plants develops a black discoloration (Fraedrich et al. 2008, Mayfield et al. 2008). The dead trees are used for brood production by Redbay Ambrosia Beetles, and populations of the beetle increase greatly in areas where Redbay trees have succumbed to the wilt (Maner et al. 2014).

Wilt diseases are known to spread through roots and infect other members of clonal populations that have developed from root sprouts (Cameron et al. 2015, Crandall and Baker 1950, O’Neal and Davis 2015). Less appears to be known about the effect of wilt diseases on woody plant species that reproduce vegetatively by rhizomes, which are underground stems that grow horizontally and produce new roots and shoots at nodes. Documented instances of laurel wilt in Pondberry have thus far been rare (Fraedrich et al. 2011), and how the disease might progress in populations has not been studied. We conducted the present study in Athens, GA, outside the natural range of Pondberry, where we established plants from seeds in pots and raised planting beds. The primary objective of this study was to determine how an infection by *R. lauricola* in a member of a Pondberry colony could affect other members in that colony.

Methods

Pot experiments

We conducted 2 inoculation experiments in pots to determine if *R. lauricola* could move systemically through rhizomes from an infected stem to other ramets. We produced Pondberry seedlings from seeds originally obtained from plants in Craighead County, AR. We stratified seeds in moist, coarse sand at 5 °C for 30 d, prior to germinating them in a 1:4 coarse sand and peat mixture. We transplanted each of the 6 Pondberry seedlings into a 38-L plastic planting pots. We placed the pots outdoors in a lathe house and grew the seedlings for 2 y, during which time an average of 17 ramets were produced in the pots. On 1 July 2013, we inoculated the original Pondberry stem in each of 4 pots with an isolate of *R. lauricola* that we acquired from a laurel wilt-infected Redbay tree at Hilton Head Island, SC. We grew the *R. lauricola* isolate on malt-extract agar for 14 d at 25 °C, and extracted and quantified spores as previously described by Fraedrich and co-workers (2008). At the time of inoculation, the heights of stems in pots varied from 20 cm to 175 cm,

and stem diameters at ground level varied from 2.0 to 11.8 mm. The average diameter of the original stems was 10 mm (min–max = 8.5–11.6 mm). We wounded the original stems by drilling a 2.25-mm diameter hole just above the groundline to a 4–6-mm depth and inoculated the stem with 0.1 ml of a conidial suspension (1.5×10^6 spores/ml) of *R. lauricola*. We wounded the original stem in 2 other pots in the same manner and mock-inoculated them with 0.1 ml of sterile deionized water. We wrapped all wounds in Parafilm M (Pechiney Plastic Packaging, Menasha, WI). We watered all pots as needed and monitored plants for the presence of disease symptoms in the inoculated stems and ramets at 1–3-d intervals. After 10 weeks, we made a final observation of the number of wilted and healthy stems (i.e., original inoculated stem and ramets) in each pot. We removed the plants from pots and washed the soil from the root systems, revealing all connecting rhizomes between stems within pots. We examined wilted and healthy stems and their connecting rhizomes for evidence of xylem discoloration. We surface-sterilized with 95% ethanol and flamed pieces of discolored xylem from the stems and rhizomes, then plated them on cycloheximide-streptomycin malt agar (CSMA; Harrington 1981, 1992), a medium selective for ophiostomatoid fungi such as *Raffaelea* spp. We incubated at 25 °C for 7–10 d and then assessed plates for the presence of *R. lauricola*.

We repeated the experiment on 30 August 2013 using the same techniques described in the first experiment. The heights of stems varied from 30 cm to 175 cm, and stem diameters at ground level varied from 3.4 mm to 18.7 mm. The average diameter of the inoculated stems was 13.3 mm (min–max = 8.5–18.7 mm). The spore concentration of *R. lauricola* used in this experiment was 3.8×10^6 spores/cm³. We watered the plants as needed and monitored disease symptoms in the original stems and ramets for 11 weeks, at which time we made a final assessment of disease symptoms and evaluated the presence of *R. lauricola*, as previously described.

Field experiments

We undertook 2 larger-scale field experiments to assess the movement of *R. lauricola* through rhizomes and spread of laurel wilt among ramets in clonal Pondberry colonies. We conducted the experiments in raised planting beds where rhizome development was less restricted compared to pots.

We raised Pondberry seedlings from seeds as previously described. After germination, we transplanted seedlings to 3.8-L pots and grew them in a greenhouse for 16 months. In June 2011, we transplanted plants to raised planting beds at the Whitehall Experimental Forest in Athens, GA. We used 4 raised beds that were each 15.2 m long and 1.2 m wide. Soil in the beds was a sandy loam. In each bed, we established 10 Pondberry plants at a distance of ~1.25 m from one another. Beds were shaded with screen cloth that was supported by PVC hoop-frames and routinely irrigated during the growing season. We grew the plants in beds for ~4–5 y, and during this time, multiple ramets were produced around the original Pondberry stems. At the time that experiments were conducted, stem diameters at ground level varied from 2 mm to 22 mm, and stem heights varied from several centimeters to over a meter.

On 29 July 2015, we selected 2 of the 10 colonies in each of 4 beds to be used in the experiment and assigned the colonies to 1 of 2 treatments: (1) inoculation of the original stem in 1 colony in each bed with *R. lauricola* and (2) mock inoculation of the original stem in 1 colony in each bed. Plants had grown freely in beds and it was suspected that rhizomes and ramets from clonal colonies had intermingled with other nearby clonal colonies. For this reason, the colonies selected for the treatments within beds were widely separated from one another. We wounded all inoculated stems by drilling a hole into the stem at 1–2 cm above the groundline with a 2.25-mm diameter drill bit to a 4–6 mm depth. Inoculum of *R. lauricola* was produced as previously described. The spore concentration was 3.0×10^6 spores/ml, and *R. lauricola*-inoculated plants received 0.1 ml of a conidial suspension; we mock inoculated control plants with 0.1 ml of sterile deionized water and wrapped all wounds in Parafilm M. We watered the beds at 1–3-d intervals with drip irrigation hoses, and conducted observations at these times for disease symptoms in inoculated and control stems, and adjacent ramets. We collected samples from inoculated stems and ramets when they died, and marked the locations of the dead ramets with flagging. We examined the samples for xylem discoloration, and surface-sterilized pieces of stems, which we plated on selective agar media and assessed for *R. lauricola* as previously described.

We repeated the experiment on 15 June 2016 using the same treatments as previously described, and used 2 of the colonies in each of the 4 beds where the original stems had not been previously inoculated. Inoculum of *R. lauricola* for this experiment was produced as previously described and the concentration of the inoculum was 2.1×10^6 spores/ml. After 7 weeks, 3 *R. lauricola*-inoculated plants failed to show any symptoms of laurel wilt, and we reinoculated these plants on 9 August 2016. The spore concentration used to reinoculate these plants was 1.8×10^6 spore/ml. We monitored colonies for disease symptoms and obtained samples for assessment of *R. lauricola* as previously described.

We conducted a statistical summarization of data that included means and standard errors of the means in SYSTAT 13 (SYSTAT Software, Inc., Chicago, IL). Mock-inoculated controls plants were employed in the experiments to monitor the health of uninfected plants and determine if mortality unrelated to *R. lauricola* infection occurred during the experiments. Thus, we conducted no statistical comparisons between treatment means. We determined confidence intervals (CI; 95%) for the mean number of dead ramets following inoculation of stems with *R. lauricola* in pot and field experiments. We listed a zero value for instances when the lower limit of the confidence interval was negative.

Results

Pot experiments

The mock-inoculated Pondberry stems and other ramets in control pots remained healthy throughout the 2 experiments (Fig.1A). In both experiments, Pondberry stems inoculated with *R. lauricola* began to show symptoms of wilt within 2 weeks after inoculation, and symptoms in the ramets followed within a

week. The leaves of infected stems initially became chlorotic, began to droop, and turned brown as they died (Fig. 1B). An average of 15.8 (CI = 5.8–25.8) stems/pot died in the first experiment following inoculation with *R. lauricola*, and 7.8 (CI = 5.0–10.5) in the second experiment (Table 1), which equated to average mortality rates of 77% (min–max = 41–91%) and 59% (min–max = 37–80%) of the ramets in pots in the first and second experiments, respectively. Ramets that died from laurel wilt had rhizome connections to the inoculated stems or to other ramets that had died from the disease (Fig. 2A). The xylem of the inoculated stem, the wilted ramets and their connected rhizomes had a dark brown discoloration (Fig. 2B), and we routinely isolated *R. lauricola* from the infected xylem tissues of stems and rhizomes (Table 1). The mean distance that *R. lauricola* moved through rhizomes from inoculated stems to the outermost symptomatic ramets was 21 cm in the first experiment and 18 cm in the second experiment. Ramets that remained healthy in pots with *R. lauricola*-inoculated plants frequently appeared to have no viable connecting rhizomes to the inoculated stem or other infected ramets. If rhizome connections were present, they were often in an advanced stage of deterioration and were apparently nonfunctional.

Field experiments

Two of the *R. lauricola*-inoculated stems failed to develop any symptoms of disease in the first experiment, and other ramets around these inoculated stems also



Figure 1. (A) Healthy, uninoculated Pondberry plant with multiple ramets, and (B) wilted inoculated stem and ramets 10 weeks after inoculation with *Raffaelea lauricola*.

failed to develop symptoms. We recorded these 2 inoculations as failed inoculation attempts and did not include them in the analyses. In the other 2 stems inoculated with *R. lauricola*, symptoms began to develop within 2 weeks after inoculation

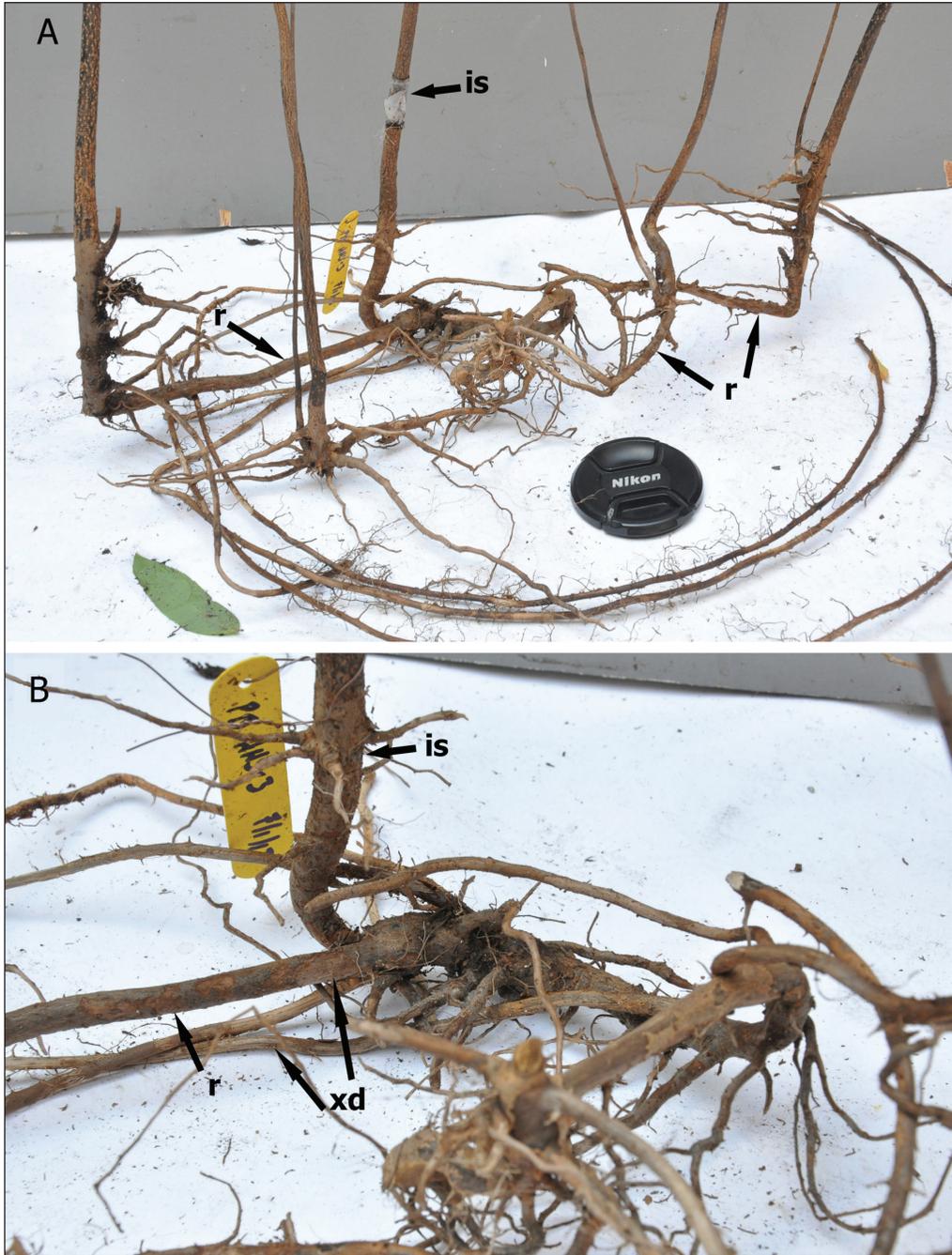


Figure 2. (A) Root system of wilted Pondberry colony grown in pots. Inoculated stem is noted as "is". Connecting rhizomes are labeled at "r". (B). Xylem discoloration (xd) in rhizome infected with *Raffaelea lauricola*.

and symptoms in adjacent ramets quickly followed (Table 2). Leaf chlorosis was again the first symptom observed and it intensified over time to a bright yellow (Fig 3A); chlorosis was followed by wilting (i.e., drooping leaves and then browning of foliage; Fig 3B). Symptom development in ramets was identical to those in the inoculated stems. In the 2 Pondberry stems which we successfully inoculated with *R. lauricola*, an average of 41 (CI = 0–270) ramets died around the inoculated stems during the remainder of the growing season. After 12 weeks, 58 stems in 1 colony located at distances up to 172 cm from the original inoculated plant had died, and in the other colony, 22 ramets died at distances up to 337 cm (Table 2). Stems of dead ramets exhibited xylem discoloration and we isolated *R. lauricola* from 84% of the wilted ramets. Pondberry plants mock-inoculated with sterile deionized water and ramets adjacent to these plants remained healthy throughout the growing season.

In the second experiment, an average of 13.2 (CI = 0–27) ramets died following inoculation with *R. lauricola* (Table 2), and symptom development in ramets was similar to that observed in the first field experiment. The maximum distances that the disease spread within colonies varied from 74 cm to 403 cm from the inoculated stems. We isolated *R. lauricola* from 95% of the wilted ramets. We observed no mortality in Pondberry plants that were mock-inoculated with sterile deionized water.

Table 1. Pot experiments. Mortality of Pondberry stems (i.e., inoculated stem and ramets) following inoculation with *Raffaelea lauricola* and recovery of the pathogen from wilted stems and their rhizomes. Treatments: I = stem inoculated with *R. lauricola*; NI = stems mock-inoculated.

Exp	Trt	Pots (<i>n</i>)	Mean # (SE) stems/pot ^A	Mean. # (SE) dead stems/ pot ^A	<i>R. lauricola</i> positive (%)		Maximum distances of dead ramets from inoculated stem (cm)
					Stems	Rhizomes	
1	I	4	21.5 (3.8)	15.8 (3.1)	100	100	17–26
	NI	2	14.5 (4.5)	0.0	NA ^B	NA	NA
2	I	4	15.2 (7.8)	7.8 (0.8)	95	95	15–22
	NI	2	20.5 (12.5)	0.0	NA ^B	NA	NA

^AIncludes ramets and stem that was inoculated.

^BNot assessed due to the lack of dead stems.

Table 2. Field experiments. Mortality of Pondberry stems (i.e., inoculated stem and ramets) in raised beds following inoculation with *Raffaelea lauricola*, and recovery of the pathogen from wilted stems. Treatments: I = stem inoculated with *R. lauricola*; NI = stems mock-inoculated. Mean # (SE) dead stems/bed includes the inoculated stem and ramets.

Experiment	Trt	Beds (<i>n</i>)	Mean # (SE) dead stems/bed	Mean % (SE) of stems with <i>R. lauricola</i>	Maximum distances of dead ramets from inoculated stems (cm)
	NI	4	0.0	NA ^A	0
2	I	4	13.2 (4.4)	95 (3.0)	74–403
	NI	4	0.0	NA ^A	0

^ANot assessed because plants were not inoculated with *R. lauricola* and no mortality was observed.

Discussion

This study confirms that Pondberry is highly susceptible to laurel wilt and demonstrates that the disease can spread rapidly through rhizomes to ramets within a colony for distances as great as 4 m from infected stems. Although Pondberry is a small shrub that is not often attacked by the Redbay Ambrosia Beetle, when infections occur they can be detrimental to Pondberry colonies. Movement of *R. lauricola* through rhizomes is rapid; thus, it is likely that when a Pondberry stem is attacked by the Redbay Ambrosia Beetle, multiple ramets could be affected by the disease.



Figure 3. (A) Chlorosis (chl) developing in Pondberry ramets following inoculation of a single stem with *Raffaelea lauricola*. Inoculated stem denoted by "is"; (B) Wilted (w) Pondberry ramets 8 weeks after inoculation of one stem with *R. lauricola*. The location of the inoculated stem is noted at "is".

Naturally occurring Pondberry mortality caused by laurel wilt has been documented at only 1 location where multiple stems were affected (Fraedrich et al. 2011). Pondberry is an understory shrub in forests and may not be prone to attack by Redbay Ambrosia Beetles due to the small stem diameter of this species. In Redbay, larger-diameter trees tend to die first from laurel wilt, and only a small percentage of plants less than 2.5 cm diameter are attacked by Redbay Ambrosia Beetles and succumb to the disease (Fraedrich et al. 2008). Studies by Mayfield and Brownie (2013) found that the Redbay Ambrosia Beetle responds to visual cues and favors larger-diameter plants; the probability of attack by this beetle decreases as plant diameter decreases. However, the attraction of Redbay Ambrosia Beetles to various plant species is not strictly dependent on stem diameter alone, but also depends upon the chemical cues that are produced by plants (Hanula and Sullivan 2008, Kendra et al. 2014). At this time, the attraction of the Redbay Ambrosia Beetle to aromatic chemicals produced by Pondberry is unknown. Nonetheless, even when attacked, it is unlikely that Pondberry would serve as a good brood host for the Redbay Ambrosia Beetle, and populations of the beetle could probably not be sustained on this species due to its smaller size. In Redbay, Redbay Ambrosia Beetles did not produce brood in stems and branches below 1.6 cm diameter, and brood production in stems below 2.5 cm diameter was generally poor (Maner et al. 2014). Stem diameters in Pondberry are commonly less than 1.5 cm, although in the present study the diameter of some stems was as great as 2.2 cm. Redbay Ambrosia Beetle populations can increase very rapidly in areas where large-diameter hosts exist (Maner et al. 2014). At the 1 site where Pondberry mortality occurred naturally, numerous large-diameter Redbay were also dying from laurel wilt, and thus, beetle populations were probably high, increasing the probability of attack on Pondberry stems (Fraedrich et al. 2011). Routine monitoring of Pondberry populations in areas where laurel wilt is prevalent on larger diameter Redbay and Sassafras trees would be essential for management and control efforts in Pondberry.

The failure to induce wilt in 2 stem inoculations in the first field experiment, and 3 of the initial stem inoculations in the second field experiment was unusual compared to previous inoculations with *R. lauricola*. Wilt has been induced consistently in artificial inoculations of Redbay and other hosts including Pondberry, but these tests were often conducted under controlled conditions or on larger-diameter plants (Fraedrich et al. 2008, 2011). In the present study, we conducted the inoculations of Pondberry stems in an open field during spring and summer, when temperatures exceeded 32 °C; this and other factors such as the small stem diameters and exposure of some stems to direct sunlight may have adversely affected inoculation success. Likewise, the lower recovery of *R. lauricola* from Pondberry stems in 1 of the field experiments compared to recovery in pot experiments was probably related to environmental factors. Pondberry stems in the raised beds were exposed to more direct sunlight and higher temperatures, which probably caused rapid drying of stem tissues and mortality of *R. lauricola* after the ramets had died from the wilt.

In Pondberry stems that died following inoculation with *R. lauricola*, the disease always spread to multiple ramets around the inoculated stem, although the

number of ramets that died from laurel wilt was highly variable. This variability was particularly evident in the field experiments. Many factors probably accounted for the variation, including the numbers of ramets that grew from the original Pondberry stems, the number of rhizome connections that were viable among ramets at the time of infection, and limitations on the number of replications for experiments. Furthermore, these studies had to be conducted in pots or field beds where growth of colonies was limited. Individual clonal colonies can spread over much larger areas (Gustafson et al. 2013) and grow for much longer time periods in their natural habitats, and thus, how far the disease could spread within clonal colonies under natural conditions remains largely unknown. It is notable that infections during 1 season did not continue to spread through the rhizomes to additional ramets at the beginning of the following season. This result may have been due to the rapid spread of the disease to all ramets of a colony during the year that the infection occurred, or that all rhizomes associated with the outermost dead ramets became nonfunctional during the dormant season and were subsequently unable to move spores through the vascular system to additional healthy ramets during the next year. In the pot study, it appeared that some connecting rhizomes between the original planted Pondberry and other ramets had become nonfunctional and rhizomes were deteriorating. We did not examine the prevalence of this phenomenon under field conditions.

Other insects and fungal pathogens can also cause damage and mortality in Pondberry, and some of these pest problems are likely to be confused with laurel wilt without proper diagnostic assessments. Another Asian ambrosia beetle, *Xylosandrus compactus* Eichhoff (Black Twig Borer), has a wide host range (Chong et al. 2009, Ngoan et al. 1976) that includes Pondberry (Fraedrich et al. 2011, Wilson et al. 2005). Attacks by Black Twig Borer on the stems of Pondberry cause dieback that appears much like laurel wilt (Fraedrich et al. 2011); however, the Black Twig Borer does not carry a vascular pathogen, and thus attacks by this beetle affect single stems which may resprout after dieback. Larvae of the weevil, *Heilipus apiatus* Oliv., feed on stems and roots of Pondberry and other members of the Lauraceae (Hoffman 2003, Wolfenbarger 1948, Woodruff 1963), causing damage that can also result in wilt-like symptoms (Fraedrich et al. 2011). Likewise, fungi such as *Botryosphaeria ribis* Grossenb. and Duggar (Wilson et al. 2004, 2005) and a *Phomopsis* sp. (USFWS 1993) also have been associated with dieback in Pondberry, but these fungi and others are not systemic pathogens and the dieback they may cause would be restricted to single stems.

In areas where laurel wilt is present in larger-diameter hosts and Redbay Ambrosia Beetle populations are high, frequent monitoring of Pondberry populations for wilt would need to be conducted for any pest management program. Unfortunately, practices to control the disease in Pondberry are largely untested. Most rhizomes and roots of Pondberry are thought to occur in the upper 20 cm of a soil profile (Devall 2013, Wright 1989) and severing rhizomes around infected stems could be an effective method to halt the spread of the wilt to other members of a colony if the infections can be detected early. In a small trial, severing rhizomes to a 20-cm

depth around infected stems appeared to prevent the spread of the disease in most instances to adjacent members of colonies (G.S. Best and S.W. Fraedrich, unpubl. data), but clearly this practice requires additional testing before it could be relied upon operationally. Likewise, the systemic fungicide, propiconazole, has been effective to protect Redbay and *Persea americana* Mill. (Avocado) from infection by *R. lauricola* (Mayfield et al. 2008, Ploetz et al. 2011), and use of this fungicide may be feasible for protection of Pondberry populations at risk for wilt. Additional research on the use of this fungicide is necessary to determine if it would be effective for control, and that its use would not be detrimental to Pondberry. However, Pondberry is an endangered and protected species, and conducting research studies to develop disease-control practices presents some unique challenges. The development of such practices under natural conditions would not be feasible, and growing small Pondberry colonies in pots and field beds for studies is time-consuming and expensive, making this research extremely difficult to pursue.

Acknowledgments

We greatly appreciate the helpful comments and suggestions of Theodore Leininger, Tracy Hawkins, Brian Roy Lockhart, Emile Gardiner and Leah Roberts on an earlier draft of this manuscript. The authors also greatly appreciate the helpful discussions with Stanley Zarnoch on presentation of the data. A portion of this study was conducted by Susan Best to fulfill requirements for her Master of Science degree in Agronomy at Iowa State University.

Literature cited

- Cameron, R.S., J.L. Hanula, S.W. Fraedrich, and C. Bates. 2015. Progression and impact of Laurel Wilt Disease within Redbay and Sassafras populations in Southeast Georgia. *Southeastern Naturalist* 14:650–674.
- Chong, J.H., L. Reid, and M. Williamson. 2009. Distribution, host plants, and damage of the Black Twig Borer, *Xylosandrus compactus* (Eichhoff), in South Carolina. *Journal of Agricultural and Urban Entomology* 26:199–208.
- Crandall, B.S., and W.L. Baker. 1950. The wilt disease of American Persimmon caused by *Cephalosporium diospyri*. *Phytopathology* 40:307–325.
- Devall, M.S. 2013. The endangered Pondberry (*Lindera melissifolia* [Walter] Blume, Lauraceae). *Natural Areas Journal* 33:455–465.
- Fraedrich, S.W., T.C. Harrington, R.J. Rabaglia, M.D. Ulyshen, A.E. Mayfield, J.L. Hanula, J.M. Eickwort, and D.R. Miller. 2008. A fungal symbiont of the Redbay Ambrosia Beetle causes a lethal wilt in Redbay and other Lauraceae in the Southeastern United States. *Plant Disease* 92:215–224.
- Fraedrich, S.W., T.C. Harrington, C. Bates, J. Johnson, L. Reid, G.S. Best, T.S. Leininger, and T.D. Hawkins. 2011. Susceptibility to laurel wilt and disease incidence in two rare plant species, Pondberry and Pondspice. *Plant Disease* 95:1056–1062.
- Fraedrich, S.W., C.W. Johnson, R.D. Menard, T.C. Harrington, R. Olatinwo, and G.S. Best. 2015. First report of *Xyleborus glabratus* (Coleoptera: Curculionidae: Scolytinae) and laurel wilt in Louisiana, USA: The disease continues westward on Sassafras. *Florida Entomologist* 98:1266–1268.
- Gustafson, D.J., A.P. Giunta, and C.S. Echt. 2013. Extensive clonal growth and biased sex ratios of an endangered dioecious shrub, *Lindera melissifolia* (Walt) Blume (Lauraceae). *Journal of the Torrey Botanical Society* 140:133–144.

- Hanula, J.L., and B. Sullivan. 2008. Manuka oil and phoebe oil are attractive baits for *Xyleborus glabratus* (Coleoptera Scolytinae), the vector of laurel wilt. *Environmental Entomology* 37:1403–1409.
- Harrington, T.C. 1981. Cycloheximide sensitivity as a taxonomic character in *Ceratocystis*. *Mycologia* 73:1123–1129.
- Harrington, T.C. 1992. *Leptographium*. Pp. 129–133, In L.L. Singleton, J.D. Mihail, and C.M. Rush (Eds). *Methods for Research on Soilborne Phytopathogenic Fungi*. American Phytopathological Society. St. Paul, MN. 265 pp.
- Harrington, T.C., S.W. Fraedrich, and D.N. Aghayeva. 2008. *Raffaelea lauricola*, a new ambrosia beetle symbiont and pathogen on the Lauraceae. *Mycotaxon* 104:399–404.
- Harrington, T.C., H.Y. Yun, S.S. Lu, H. Goto, D.N. Aghayeva, and S.W. Fraedrich. 2011. Isolations from the Redbay Ambrosia Beetle, *Xyleborus glabratus*, confirm that the laurel wilt pathogen, *Raffaelea lauricola*, originated in Asia. *Mycologia* 103:1028–1036.
- Hawkins, T.S., D.A. Skojac, Jr., N.M. Schiff, and T.D. Leininger. 2010. Floristic composition and potential competitors in *Lindera melissifolia* (Lauraceae) colonies in Mississippi with reference to hydrologic regime. *Journal of the Botanical Research Institute of Texas* 4:381–390.
- Hoffman, R.L. 2003. *Heilipus apiatus*, a striking large weevil new to the Virginia fauna (Coleoptera: Curculionidae). *Banisteria* 22:58–59.
- Kendra, P.E., W.S. Montgomery, J. Niogret, G.E. Pruett, A.E. Mayfield, M. Mackenzie, M.A. Deyrup, G.R. Bauchan, R.C. Ploetz, and N.D. Epsky. 2014. North American Lauraceae: Terpenoid emissions, relative attraction and boring preferences of Redbay Ambrosia Beetle, *Xyleborus glabratus* (Coleoptera: Curculionidae: Scolytinae). *PLoS ONE* 9(7):e102086. DOI:10.1371/journal.pone.
- Maner, M.L., J.L. Hanula, and S. Horn. 2014. Population trends of the Redbay Ambrosia Beetle (Coleoptera: Curculionidae: Scolytinae): Does utilization of small diameter Redbay trees allow populations to persist? *Florida Entomologist* 97:208–216.
- Mayfield, A.E., and C. Brownie. 2013. The Redbay Ambrosia Beetle (Coleoptera: Curculionidae: Scolytinae) uses stem-silhouette diameter as a visual host-finding cue. *Environmental Entomology* 42:743–750.
- Mayfield, A.E., III, E.L. Barnard, J.A. Smith, S.C. Bernick, J.M. Eickwort, and T.J. Dreaden. 2008. Effect of propiconazole on laurel wilt disease development in Redbay trees and on the pathogen in vitro. *Arboriculture and Urban Forestry* 34:317–324.
- Ngoan, N.D., R.C. Wilkinson, D.E. Short, C.S. Moses, and J.R. Mangold. 1976. Biology of an introduced ambrosia beetle, *Xylosandrus compactus*, (Coleoptera-Scolytidae) in Florida. *Annals of the Entomological Society of America* 69:872–876.
- Olatinwo, R., C. Barton, S.W. Fraedrich, W. Johnson, and W.J. Hwang. 2016. First report of laurel wilt, caused by *Raffaelea lauricola*, on Sassafras (*Sassafras albidum*) in Arkansas. *Plant Disease* 100:2331–2332.
- O’Neal, E.S., and D.D. Davis. 2015. Intraspecific root grafts and clonal growth within *Ailanthus altissima* stands influence *Verticillium nonalfalfae* transmission. *Plant Disease*. 99:1070–1077.
- Ploetz, R.C., J.M. Perez-Martinez, E.A. Evans, and S.A. Inch. 2011. Toward fungicidal management of laurel wilt of Avocado. *Plant Disease* 95:977–982.
- Rabaglia, R.J., S.A. Dole, and A.I. Cognato. 2006. Review of American Xyleborina (Coleoptera: Curculionidae: Scolytinae) occurring north of Mexico, with an illustrated key. *Annals of the Entomological Society of America* 99:1034–1056.
- US Fish and Wildlife Service (USFWS). 1993. Recovery Plan for Pondberry (*Lindera melissifolia*). Atlanta, GA. 56 pp.

- Wilson, A.D., N.M. Schiff, M.S. Devall, K.F. Connor, P.B. Hamel, E.S. Gardiner, and T.D. Leininger. 2004. Incidence and severity of botryosphaeria stem canker and dieback of Pondberry (*Lindera melissifolia*) in Mississippi. *Phytopathology* 94:S110.
- Wilson, A.D., N.M. Schiff, T.D. Leininger, P.B. Hamel, E.S. Gardiner, K.F. Connor, and M.S. Devall. 2005. Black Twig Borer exacerbates damage caused by botryosphaeria stem canker and dieback of Pondberry in the Delta National Forest of Mississippi. *Phytopathology* 95:S112.
- Wolfenbarger, D.O. 1948. *Heilipus squamosus* Lec, A new enemy of the Avocado. *Proceedings of the Florida State Horticultural Society* 61:260–268.
- Woodruff, R.E. 1963. An Avocado weevil (*Heilipus apiatus* Oliv.). Florida Department of Agriculture and Consumer Services, Division of Plant Industries, Entomological Circular 11. Gainesville, FL. 1 p.
- Wright, R.D. 1990. Species biology of *Lindera melissifolia* (Walt.) Blume in northeast Arkansas. Pp. 176–179, *In* R.S. Mitchell, C.J. Sheviak, and D.L. Leopold (Eds). *Ecosystem Management: Rare Species and Significant Habitats*. *Proceedings 15th Natural Areas Association Conference, Bulletin 471*, New York State Museum, Albany, NY.
- Wuest, C.E., T.C. Harrington, S.W. Fraedrich, H.Y. Yun, and S.S. Lu. 2017. Genetic variation in native populations of the laurel wilt pathogen, *Raffaelea lauricola*, in Taiwan and Japan and the introduced population in the United States. *Plant Disease* 101:619–628.