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Research Article - forest threats

Spread, Vector Flight Behavior, and Impact of Laurel Wilt in Sassafras Beyond the Gulf-Atlantic Coastal Plain

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Abstract

Laurel wilt is a destructive vascular disease of trees in the laurel family (Lauraceae) caused by a nonnative insect/pathogen complex. This study monitored the recent spread and impact of laurel wilt in sassafras (*Sassafras albidum* [Nutt.] Nees) from the Gulf-Atlantic Coastal Plain region of the southeastern United States (US) into the adjacent Piedmont/Sandhills and Mountain regions. Laurel wilt was detected at thirteen of forty-six sassafras sites including seven outside the Coastal Plain. Compared to nondiseased sites, sassafras mortality due to laurel wilt increased rapidly from 2018 to 2020 and occurred in all diameter classes monitored (≥ 5 cm diameter at breast height, dbh). Flight trapping for the laurel wilt vector, the redbay ambrosia beetle (RAB, *Xyleborus glabratus* Eichhoff), with α-copaene lures did not enhance early detection of latent laurel wilt infections. Seasonal flight activity of the RAB in the Piedmont and Mountains suggested two generations per year with little to no flight from December through March.

Keywords: redbay ambrosia beetle, invasive species, insect trapping, tree mortality, forest monitoring

Nonnative invasive insects and pathogens cause substantial tree mortality resulting in numerous economic and ecological impacts in forests (Lovett et al. 2016, Mayfield et al. 2021). More than 40% of the current live biomass in the conterminous United States (US) is estimated to be at risk of future damage by established invasive pests (Fei et al. 2019). Among the most damaging of these current forest threats in the US is laurel wilt, a vascular disease of woody plants in the family Lauraceae. Laurel wilt is caused by the fungus *Raffaelea lauricola* Harrington, Fraedrich & Aghayeva, an ambrosial symbiont of the redbay ambrosia beetle (RAB), *Xyleborus glabratus* Eichhoff (Fraedrich et al. 2008, Harrington et al. 2008). Both the beetle and the fungal pathogen are native to Asia (Hughes et al. 2017, Wuest et al. 2017) and were introduced to North America sometime before 2002 when RAB was first detected in coastal Georgia (Rabaglia et al. 2006). Healthy plants become diseased when RAB initiates boring activity on host stems and inoculates the xylem with *R. lauricola*. Laurel wilt has killed hundreds of millions of redbay (*Persea borbonia* [L.]

Study Implications

Sassafras is the most widely-distributed lauraceous tree species in the US and is valued for its ecological functions, durable wood, and numerous cultural uses. Our results strongly suggest the potential for substantial loss of the existing sassafras resource as laurel wilt continues spreading through the eastern US. Survey traps baited with current lures for the primary insect vector are unlikely to improve early detection of new laurel wilt infections. The georeferenced network of plots established for this project provides baseline data for future monitoring efforts and could be revisited to look for evidence of sassafras resistance to the laurel wilt pathogen.

Spreng) and swamp bay (*Persea palustris* [Raf.] Sarg.) trees in the southeastern US (Hughes et al. 2017). It also causes mortality in other native and cultivated lauraceous species in North America (including avocado, *Persea americana* Mill., in commercial production (Ploetz et al. 2017a)) and represents a serious potential threat to lauraceous species in other parts of the world (Olatinwo et al. 2021).

Sassafras (*Sassafras albidum* [Nutt.] Nees) is the most widely distributed member of the Lauraceae in the US. Its range extends from Maine west to Michigan, southwest to eastern Oklahoma and Texas, and eastward to northern Florida and the Atlantic coast (Griggs 1990, Randolph 2017). It is intolerant of shade and commonly colonizes old fields, fencerows, burned areas, and other disturbed sites. Sassafras occurs on a wide variety of soils, primarily as a small to medium-sized tree, but may reach 30 m tall on good soils when not overtopped. Stems commonly occur in clusters or thickets due to vegetative reproduction via root sprouts (Griggs 1990). Like other members of the family, sassafras is rich in essential oils and various parts of the plant have been used by Native Americans and other groups in a wide variety of medicinal, culinary, and other cultural uses (Immel 2016). The wood is durable, lightweight, easily worked, and has potential for increased commercial use (Harding et al. 1997, Cassens 2007). Sassafras also has wildlife value, as the seeds are consumed by various bird species and the foliage is browsed by deer (Dills 1970, Immel 2016).

As laurel wilt began spreading in the southeastern Coastal Plain through redbay and swamp bay populations, it became evident that sassafras, although less abundant in this region, was also a susceptible host of *R. lauricola* (Cameron et al. 2008, Fraedrich et al. 2008). Subsequent observations confirmed that laurel wilt epidemics were occurring in various parts of the Coastal Plain that were geographically isolated from redbay and swamp bay trees (Riggins et al. 2011, Bates et al. 2013, Fraedrich et al. 2015, Olatinwo et al. 2016, Loyd et al. 2020). Although early research suggested that sassafras may have been a relatively inferior host for RAB (Hanula et al. 2008, Mayfield and Hanula 2012), in later studies, it was comparable with redbay and swamp bay in terms of attractiveness and brood suitability (Mayfield et al. 2013, Kendra et al. 2014, Fraedrich et al. 2018). Furthermore, using cold tolerance data for the RAB, Formby et al. (2018) projected that under a modest climate warming scenario, (+1.4°C by 2050), 91% of the current sassafras range is not cold enough to cause substantial RAB winter mortality.

In 2018, the known distribution of laurel wilt remained primarily within the Gulf-Atlantic Coastal Plain, and it was uncertain how quickly and with what impact laurel wilt would move into the Piedmont and Mountain regions of the eastern US using sassafras as a primary host. Unlike redbay and swamp bay, sassafras is deciduous and does not retain its wilted foliage long after infection (Mayfield et al. 2019), making laurel wilt potentially easier to overlook during visual surveys. Furthermore, the variability in climate and forest composition across different physiographic regions may result in differing disease dynamics (e.g., host mortality progression, vector behavior) in the Piedmont or Mountains compared to the Coastal Plain, where most of the information about laurel wilt has been collected to date.

To examine the spread and impact of laurel wilt in sassafras, a project was funded by the USDA Forest Service Evaluation Monitoring Program in 2018 to establish sentinel sassafras plots, both within the known distribution of laurel wilt in the Coastal Plain, and ahead of the known distribution in the Piedmont and Mountains. Sites near risk-of-entry points for RAB (e.g., close to existing infestations, hardwood mills, or campgrounds) were targeted. The presence of laurel wilt and the severity of sassafras mortality was monitored over a 3-year period. At a subset of sites, flight activity of the RAB was monitored and the efficacy of two different trap designs (funnel trap, panel trap) was evaluated. This article reports on the results of this project and discusses implications for the future spread, impact, and monitoring of laurel wilt in sassafras.

Methods

Site Selection

In early 2018, monitoring sites containing live sassafras trees ≥ 5 cm dbh were selected in three different physiographic regions of the southeastern US as defined by USACE (2022): the Gulf-Atlantic Coastal Plain, the Piedmont (including adjacent Sandhills) and the Central and Eastern Mountains (including the southern Appalachian Mountains, Cumberland Plateau, and Highland Rim/Central Basin regions) (Figure 1). In the Coastal Plain, sites with sassafras that were not known to be affected by laurel wilt (or only very recently affected) were targeted for monitoring. In the Piedmont/Sandhills and Mountains regions, sites with sassafras located near known laurel wilt infestations or near RAB risk of entry points were targeted. Risk of entry points included (1) mills using hardwood material or other facilities/corridors where RAB-infested wood could be passing through or stored and (2) campgrounds or RV parks into which patrons could potentially bring infested firewood (which can serve as a means of human-assisted spread of RAB). Monitoring sites included forest stands, edges/fencerows, and parks, but residential and intensively managed areas where trees were at increased risk of removal within 5 years were excluded. Forest sites were typically mixed pine/hardwood stands and occasionally hardwood-dominated river floodplain stands. A total of forty-six sites were monitored, including fifteen sites in the Coastal Plain, twelve in the Piedmont, and nineteen in the Mountains (Figure 1, Table 1). Not every site was monitored in every year due to changes in agency personnel, restricted travel/field work during the COVID-19 pandemic, or unexpected elimination of sites via human disturbance.

Vegetation Monitoring and Laurel Wilt Assessment

At each monitoring site, a target of twenty live sassafras trees (average $n = 19$, range 6–32) of ≥ 5 cm dbh were tagged for repeated annual monitoring of crown health and survival during the leaf-on season (Table 1). Trees that may have died before the initiation of the study were not captured in the monitoring sample. The crown health of each tagged sassafras tree was visually rated by estimating, to the nearest 5%, the proportion of the entire tree crown represented in the following foliage categories: (1) healthy (leaves green and full turgor), (2) wilted or discolored (not attributed to fall color change), and (3) missing (small diameter twigs are present but leaves have dropped

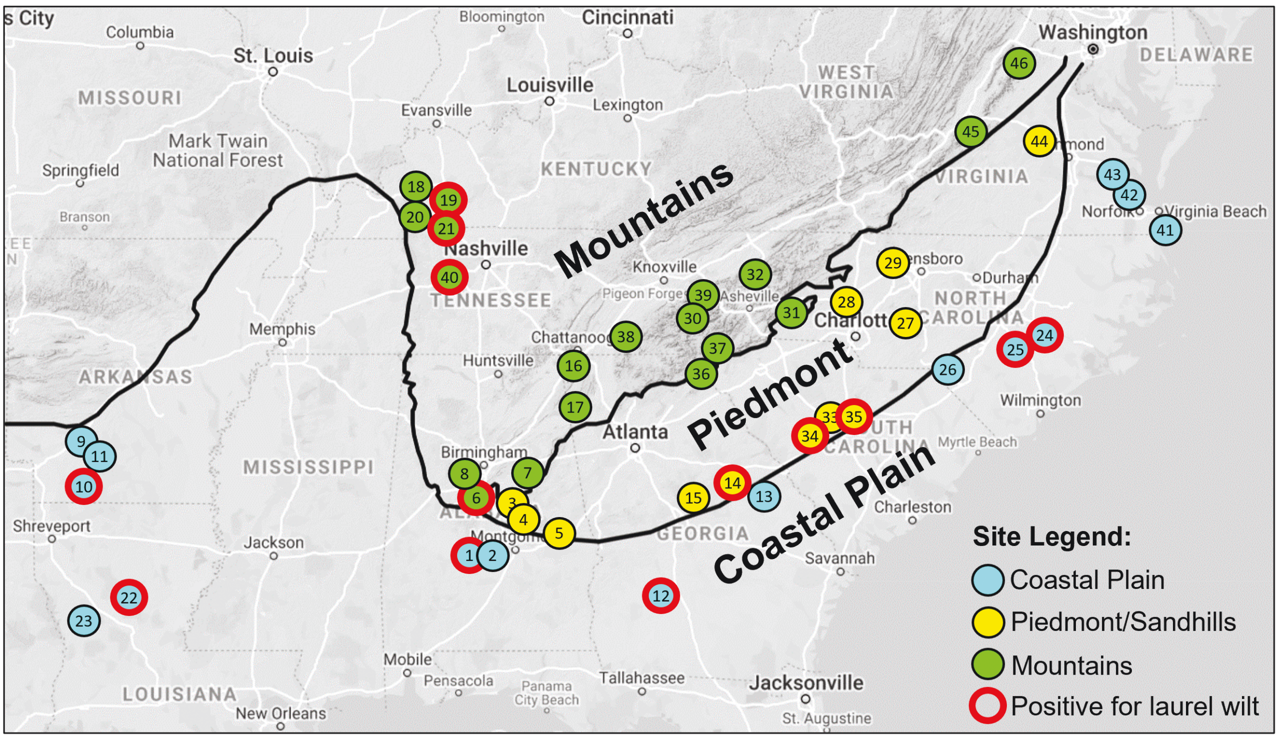

Figure 1. Locations of forty-six laurel wilt monitoring plots with sassafras in the southeastern US, color-coded by physiographic region. Red outline indicates laurel wilt pathogen detection in 2018–2020. See Tables 1 and 2 for site names and related data. Map data © 2022 Google, National Institute of Statistics and Geography (INEGI).

Table 1. Location information, stand characteristics, number of monitored sassafras trees, and redbay ambrosia beetle (RAB) trapping status for forty-six laurel wilt monitoring sites in the eastern US, 2018–2020.

				Number of trees monitored			RAB trapping site				
Site	State	Region	Stand characteristics[d]	2018	2019	2020	2018	2019	2020	Latitude	Longitude
1[a]	AL	Coast.	E,R	14	12[b]	12		X	X[c]	32.76960	-86.96354
2	AL	Coast.	E,I,R	26	26	26	X		X	32.32569	-86.68504
3	AL	Piedm.	E,R	19	19	19				32.96178	-86.36980
4	AL	Piedm.	E,I,R	12	12	12				32.89444	-86.33726
5	AL	Piedm.	P,F	21	21	11				32.58601	-85.54662
6[a]	AL	Mount.	E,I	12[b]	12	12	X[c]	X		33.03935	-86.94576
7	AL	Mount.	E,R	20	20	20				33.35687	-86.08361
8	AL	Mount.	I	30	30	30				33.25452	-87.06491
9	AR	Coast.	I	20	20	20		X	X	33.88342	-93.26756
10[a]	AR	Coast.	I,R	20	20[b]	20	X	X	X[c]	33.34587	-93.18618
11	AR	Coast.	P,I	20	20	20	X	X	X	33.63950	-93.00447
12[a]	GA	Coast.	R,F	25	25[b]	25	X	X	X	31.72397	-83.98787
13	GA	Coast.	E,R	16	-	-				33.21929	-82.39848
14[a]	GA	Piedm.	I	17	17	17[b]	X	X	X	33.20832	-82.78081
15	GA	Piedm.	I	26	26	26				33.01444	-83.43117
16	GA	Mount.	E,R	20	25	25				34.82396	-85.48026
17	GA	Mount.	E,R	20	20	20				34.27773	-85.38842
18	KY	Mount.	F,R	-	20	20		X		37.14917	-87.93250
19[a]	KY	Mount.	I	-	22[b]	22		X[c]	X	36.88223	-87.47414
20	KY	Mount.	E,R	-	24	24		X		36.84790	-87.91344
21[a]	KY	Mount.	E,I	-	20[b]	20		X[c]	X	36.67731	-87.45982
22[a]	LA	Coast.	E,R	6[b]	6	-	X[c]	X	X	31.73850	-92.54366
23	LA	Coast.	E,R	9	9	-	X	X	X	31.45139	-93.17306
24[a]	NC	Coast.	E,R	23	23[b]	23	X[c]	X	X	35.23508	-77.89440
25[a]	NC	Coast.	E,R	22[b]	-	-	X[c]			35.06107	-78.22373
26	NC	Coast.	R	25	25	-				34.75529	-79.39910
27	NC	Piedm.	E,R	22	22	22				35.39706	-79.96967
28	NC	Piedm.	E,R	24	24	24				35.69519	-80.99399
29	NC	Piedm.	E,R	27	22	28				36.13127	-80.25154
30	NC	Mount.	P,E,I	20	20	20	X	X	X	35.45909	-83.43813
31	NC	Mount.	I	25	25	-				35.50030	-81.82070
32	NC	Mount.	I	20	32	-				36.03718	-82.43048
33	SC	Piedm.	I	-	10	10		X	X	34.09370	-81.13141
34[a]	SC	Piedm.	E	9[b]	22	22		X[c]	X	33.92844	-81.49618
35[a]	SC	Piedm.	P,I	10[b]	22	22	X[c]	X	X	34.08450	-80.90921
36	SC	Mount.	I	-	16	-				34.69991	-83.29323
37	SC	Mount.	I	-	15	-				34.97554	-83.07732
38	TN	Mount.	I	-	25	-				35.23339	-84.54810
39	TN	Mount.	E	19	-	19	X	X	X	35.77259	-83.25620
40[a]	TN	Mount.	F	-	19[b]	-		X[c]		36.00043	-87.37092
41	VA	Coast.	P,E	11	11	11	X	X	X	36.62988	-75.90011
42	VA	Coast.	P,I	20	20	20	X	X	X	37.06400	-76.43180
43	VA	Coast.	P,I	10	10	10				37.31521	-76.70376
44	VA	Piedm.	E	-	10	-		X		37.74184	-77.90711
45	VA	Mount.	I	20	20	20				37.83661	-78.97310
46	VA	Mount.	I	20	20	20				38.75374	-78.23846
Total trees or sites:				680	839	672	15	23	19		

Note: Sassafras monitoring and RAB trapping were also conducted in 2021 at sites 19 and 21 only.

[a]Denotes sites at which laurel wilt was confirmed.

[b]Denotes the year (by column) in which the laurel wilt pathogen was first confirmed among the monitored sassafras trees.

[c]Denotes the year (by column) in which the RAB vector was first detected via flight trapping.

[d]Stand characteristics codes: E = forest edge, I = forest interior, F = fencerow, P = park, R = roadside.

prematurely). The sum of the three ratings totaled 100%. Trees with no healthy foliage were considered dead. Evidence for ambrosia beetle attack such as entrance holes and boring dust were noted. Trees with suspected laurel wilt infection (<70% healthy foliage or evidence of ambrosia beetle attack) were sampled by removing bark and examining the outer xylem for sapwood discoloration. Discolored sapwood was chiseled, placed in a plastic zipper-seal bag, and shipped overnight to a USDA Forest Service Southern Research Station (SRS) plant pathology lab at Athens, Georgia, or Pineville, Louisiana, for confirmation of *Raffaelea lauricola* using standard isolation techniques and morphological or genetic identification (Fraedrich et al. 2008, Harrington et al. 2008, Dreaden et al. 2014).

RAB Monitoring

Redbay ambrosia beetle flight activity was monitored at a subset of sites (Table 1) that were located (1) near existing laurel wilt infections, (2) where suspicious crown symptoms were detected during vegetation monitoring, or (3) at other areas of particular interest. At each trapping site, two traps were baited with a 50% α-copaene lure (product #3302, Synergy Semiochemical Corp., Burnaby, BC, Canada). One trap in each pair was a black 8-unit Lindgren funnel trap and the other was a black triple-vane multi-panel trap (products #4072 and #4057, respectively, Synergy Semiochemical Corp.). Traps were suspended from a tree branch or rope stretched between two trees and spaced at least 10 m apart in the vicinity of the sassafras trees being monitored. Traps were deployed and checked biweekly for a total of 8 weeks between early June and October. Marine/RV antifreeze (propylene-glycol-based) was used as a preservative in the collection cups and at each collection, insects were strained into a paint filter and antifreeze was replaced. Insects were shipped to the USDA Forest Service SRS entomology laboratory at Asheville, North Carolina, and all RAB specimens were identified, counted, and preserved in 95% ethanol.

In addition to the 8-week trapping period used at most sites, trapping for an extended duration was conducted in 2019, 2020, and 2021 at select sites in each region where laurel wilt was suspected or confirmed (sites 6, 19, 21, 22, 23, 24, 33, 34, and 35). This was to gain additional information about the seasonality of RAB flight in sassafras in these regions. At two of the northern-most sites in Kentucky (sites 19 and 21), two traps per site were monitored for 3 months (September–November 2019) and then increased to four traps per site (two funnel, two panel) from late March 2020 through September 2021. At these latter two Kentucky sites, most ambrosia beetle species in the trap catches (RAB and other associates in the Scolytinae subfamily) were identified using published keys and other diagnostic material (Rabaglia et al. 2006, Gomez et al. 2018, Smith et al. 2019).

Data Analysis

Percent mortality among the tagged sassafras trees was calculated at each site annually. A mixed-model analysis of variance (ANOVA) was conducted in SAS v. 9.2 (SAS Institute, Cary, North Carolina) to evaluate all factorial combinations of Region, Pathogen, and Year as fixed effects (with Year considered a subplot factor), and Site(Region*Pathogen) as a random effect, on percent sassafras mortality. Mortality rate (expressed as a proportion) was transformed using square root (y + 0.005) to correct variance heterogeneity and improve plots of residuals versus predicted values. P-values ≤ 0.05 in Type 3 tests of fixed effects were considered significant, and least squares means comparisons were made using Student's t-test.

For sites in which RAB flight trapping was conducted, the number of RAB per trap per week was calculated. The effect of trap type (funnel vs. panel) on mean RAB per week was evaluated using a Wilcoxon signed-rank test (nonparametric equivalent of a paired t-test) and by limiting the data set to the site × date combinations on which at least one RAB was collected in a trap of either type. At sites where RAB trapping was conducted for an extended duration, RAB flight activity was analyzed graphically over time. At Kentucky sites 19 and 21, trap catches of the three most abundant ambrosia beetle species (*Xyleborus glabratus* [RAB], *Xylosandrus crassiusculus* [Motschulsky], and *Xyleborinus saxesenii* [Ratzeburg] were graphed by collection date.

Results

Vegetation Monitoring and Laurel Wilt Assessment

Laurel wilt was detected at 28% (thirteen of forty-six) of the monitoring sites during the 3-year project period (2018–2020), including six sites in the Coastal Plain, three in the Piedmont, and four in the Mountains (Figure 1, Table 1). Four of these detections were previously-unreported county records for laurel wilt (site 1, Chilton County, Alabama; site 6, Bibb County, Alabama; site 10, Columbia County, Arkansas; site

12, Worth County, Georgia), generating new range information for the national Laurel Wilt Distribution Map maintained by the Forest Service, Forest Health Protection, Southern Region (USDA-FS FHP 2022). The laurel wilt pathogen was initially detected in at least one site during each year of the study.

There was a significant effect of pathogen presence ($F = 40.42$, df = 1, 40, $P < 0.001$), monitoring year ($F = 41.52$, df = 2, 56, $P < 0.001$), and pathogen × year ($F = 14.95$, 2, 56, $P < 0.001$) on the mean percent mortality of the monitored sassafras stems. At the start of the study in 2018, the mean percent mortality of tagged sassafras trees at all disease-free sites was < 1% and did not differ significantly from the mean mortality at sites where laurel wilt would ultimately be detected between 2018 and 2020 (11%) (Figure 2). In 2019 and 2020, however, mean sassafras mortality at diseased sites increased to 30% and 60%, respectively, whereas mortality in disease-free stands was significantly lower and remained below 5% (Figure 2). The elevated sassafras mortality in diseased stands relative to disease-free stands was evident in all diameter classes, including among the few stems that were incidentally below the target minimum dbh of 5 cm (Figure 3). Sassafras mortality was notably rapid in a number of stands, progressing to 100% within 3 years at four sites (6, 12, 21, and 35). For sites with laurel wilt, marked increases from 2018 to 2020 in mean percent sassafras mortality were also evident within each of the three physiographic regions (Table 2).

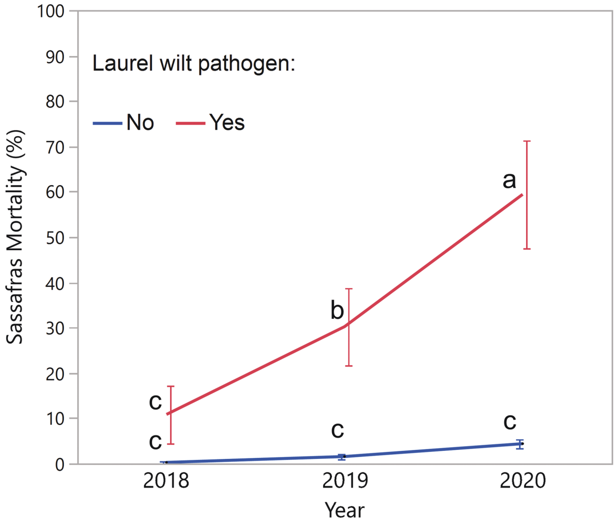

Figure 2. Mean percent mortality of sassafras trees from 2018–2020 at sites where the laurel wilt pathogen was confirmed versus sites where it was not detected. Vertical bars denote standard error of the mean. Means labeled with the same letter are not significantly different ($\alpha = 0.05$).

RAB Monitoring

Flight trapping for RAB was conducted at fifteen, twenty-three, and nineteen sites in 2018, 2019, and 2020 respectively (Table 1). RAB was detected at eleven of these sites across the 3-year project period, including five sites in the Coastal Plain, two in the Piedmont, and four in the Mountains (Table 1). Detection and confirmation of the laurel wilt pathogen through sampling of host material usually preceded vector detection (five sites) or occurred in the same year (seven sites) (Table 1). At only one site (site 24, Coastal Plain) did RAB presence in a trap (2018) precede successful isolation of the pathogen from host material (2019). At two sites in Georgia (sites 12 and 14), the laurel wilt pathogen was confirmed in symptomatic sassafras in 2019 and 2020, respectively, without capture of any

Table 2. Number of monitored sites, percentage of sites with laurel wilt, and mean (SE) percent sassafras mortality in sites with and without laurel wilt, by year and region.

Region	Year	Number of sites monitored	Percentage of sites w/laurel wilt	Mean (SE) % sassafras mortality by laurel wilt status	
				No	Yes
Coastal Plain	2018	15	46.7	0.8 (0.8)	1.3 (1.3)
	2019	14	42.9	1.3 (0.9)	26.6 (7.4)
	2020	10	40.0	4.7 (1.8)	65.0 (16.7)
Piedmont	2018	10	30.0	0.0 (0.0)	25.6 (20.8)
	2019	13	23.1	2.1 (1.5)	43.9 (22.0)
	2020	11	27.3	2.5 (1.3)	58.0 (27.6)
Mountains	2018	11	9.1	0.0 (0.0)	33.3 (0.0)
	2019	18	22.2	1.3 (0.7)	26.3 (24.6)
	2020	12	25.0	5.9 (1.8)	53.2 (27.6)

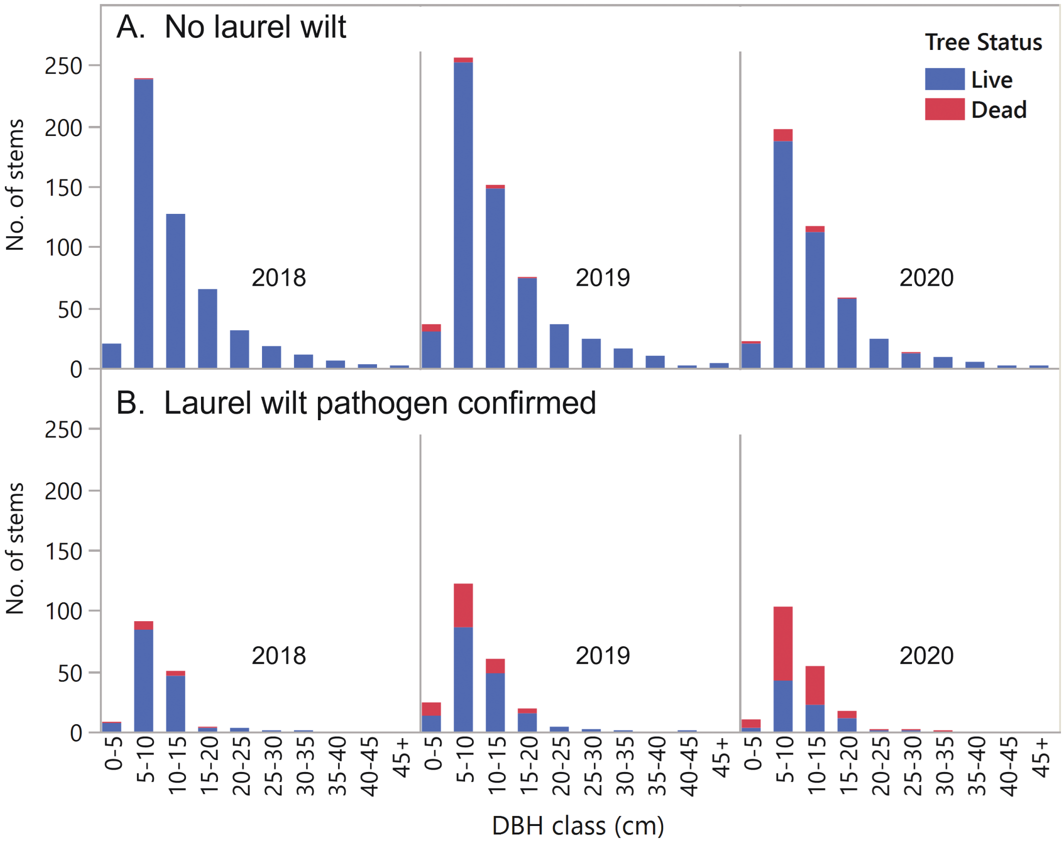

Figure 3. Diameter distribution of live and dead sassafras trees at sites where laurel wilt was not detected (panel A) and sites where the laurel wilt pathogen was confirmed (panel B), 2018–2020; dbh, diameter at breast height.

Table 3. Mean (SE) number of RAB per trap per week, and test statistics comparing trap type (funnel vs. panel) on site × date combinations when RAB was present in traps.

	RAB per trap per week			
Year	Funnel trap	Panel trap	Wilcoxon S	*P*
2018	0.07 (0.04)	0.13 (0.09)	89.5	0.089
2019	0.63 (0.15)	0.86 (0.19)	644.0	0.052
2020	0.28 (0.10)	0.20 (0.06)	-62.0	0.626

RAB in a trap before the end of the project (Table 1). When examining all site × date combinations with at least one RAB capture, there was no significant difference between the mean number of RAB per week captured in panel traps versus paired funnel traps in any year; however, the *P*-value was marginal ($P = 0.052$) in 2019, when slightly more RAB were captured in panel traps versus funnel traps (Table 3).

Flight trapping of extended duration (longer than 8 weeks) was conducted at nine sites, two of which yielded no RAB captures (sites 23 and 33, results not shown). Data from site 22 in the Coastal Plain of Louisiana revealed that RAB captures occurred throughout the calendar year (including in January) with modest peaks in late August and early December (Figure 4A). At site 24 (North Carolina, Coastal Plain) where laurel wilt was nascent, isolated captures of a single beetle per trap occurred in November, late March, and early April (Figure 4B). At two Piedmont/Sandhill sites near the edge of the Coastal Plain in South Carolina (sites 34 and 35), RAB flight began as early as February, with two periods of peak catch: the first in April and a second in late August through November (Fig. 4C, D). Similarly, at the southern-most Mountain site in Alabama (site 6), RAB was captured as early as February with peak captures in April and again in late summer (August–September) (Figure 4E).

Flight traps were monitored continuously for 2 years (September 2019–September 2021) at two Mountain region sites in Kentucky (sites 19 and 21) near the northern-most extent of laurel wilt known at the time (Figure 5). At site 21, flight activity persisted into mid-October 2019, resumed in early April 2020, and gradually increased to a peak of 56 beetles/trap/week in June 2020 (Figure 5A). RAB flight was consistently high through the summer, with a lesser peak in August

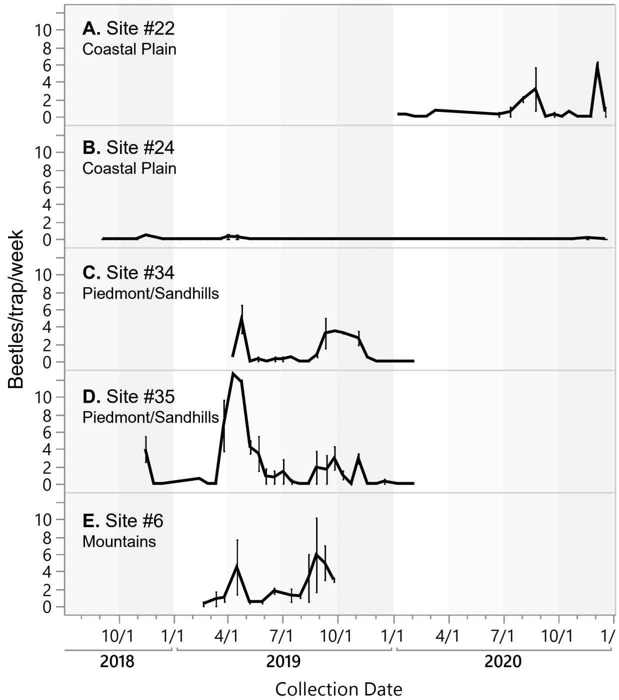

Figure 4. Number of redbay ambrosia beetles (RAB) caught per trap per week at five sites where extended-duration trapping (>8 weeks) was conducted in 2019 or 2020.

2020 and relatively low trap captures persisting until November 2020 (Figure 5A). RAB flight resumed again the following spring in early April 2021 and peaked again in June 2021, but at much lower levels than the year before (6 beetles/trap/week). This nearly ten-fold decrease in peak RAB capture from June 2020 to June 2021 corresponded with an increase in the percent mortality of tagged sassafras trees from 55% to 100% over the same time period (Figure 5D). In contrast to site 21, mean RAB trap catch at site 19 was at or near zero throughout the monitoring period from 2019 to 2021, occasionally yielding 1–3 beetles in an individual trap (Fig 5A). Percent mortality of sassafras at site 19 remained relatively low (<5%) through June 2020 but increased to 41% by June 2021 (Figure 5D).

Trap captures of the two most abundant ambrosia beetle species, *Xylosandrus crassiusculus* and *Xyleborinus saxesenii*, were compared with RAB flight activity at sites 19 and 21 (Fig. 5B, C). These insects exhibited strong peak captures in early April or May at much higher abundances (75–200 beetles/trap/week) than RAB. Relative to the spring peak, fewer *Xylosandrus crassiusculus* and *Xyleborinus saxesenii* were captured throughout the summer and fall. In contrast to the drastic reduction in RAB trap captures at site 21 between 2020 and 2021 (when all of the sassafras hosts were dead), captures of *Xylosandrus crassiusculus* and *Xyleborinus saxesenii* were similar in 2021 versus 2020 (Fig. 5B, C). *Xylosandrus crassiusculus* comprised more than 53% of the 28,023 specimens, whereas *Xyleborus glabratus* (=RAB) comprised fewer than 9% of the specimens. More than 98% of all ambrosia beetle specimens captured was comprised by eight species, all of which are nonnative to North America: *Xylosandrus crassiusculus*, *Xyleborinus saxesenii*, *Xylosandrus germanus* (Blandford), *Xyleborus glabratus*, *Cnestus mutilatus* (Blandford), *Euwallacea validus* (Eichhoff)/*E. interjectus* (Blandford), *Dryoxylon onoharaense* (Murayama), and *Cyclorhipidion pelliculosum* (Eichhoff) (Table 4).

Discussion

This project demonstrated the movement of laurel wilt beyond the Coastal Plain and into portions of the Piedmont/Sandhills and Mountain regions in Alabama, Georgia, South Carolina, Tennessee, and Kentucky. Laurel wilt in Tennessee and Kentucky was first reported by Loyd et al. (2020) and our investigations in those areas were made in response to that discovery. The movement into upland physiographic regions appears to have occurred via both short-distance expansion from adjacent infested sites in the Coastal Plain, as well as through presumed long-distance transport of the pathogen and vector into geographically disjunct areas of the Mountain region (Loyd et al. 2020). Four previously unreported county-level infestations were added to the national Laurel Wilt Distribution Map, demonstrating that supplemental evaluation monitoring projects can enhance baseline laurel wilt monitoring efforts conducted by state forestry agencies. At the time of this writing in 2022, laurel wilt has continued to spread upland and northward with new infestations in Virginia and additional counties in northern Tennessee and Kentucky (USDA-FS FHP 2022).

The impact of laurel wilt in sassafras beyond the Gulf-Atlantic Coastal Plain has been substantial and rapid. Across all sites, mean percent mortality of sassafras tagged for monitoring in sites with pathogen confirmation increased from 11% to 60% within 2 years, with certain Piedmont and Mountain sites experiencing 100% mortality of sassafras ≥ 5 cm dbh. This pattern of mortality is similar to that previously observed in redbay and sassafras populations in the Coastal Plain (Fraedrich et al. 2008, Cameron et al. 2015), where sassafras is less abundant. Because our sampling strategy targeted live sassafras trees for monitoring, our data likely underestimate the overall percent

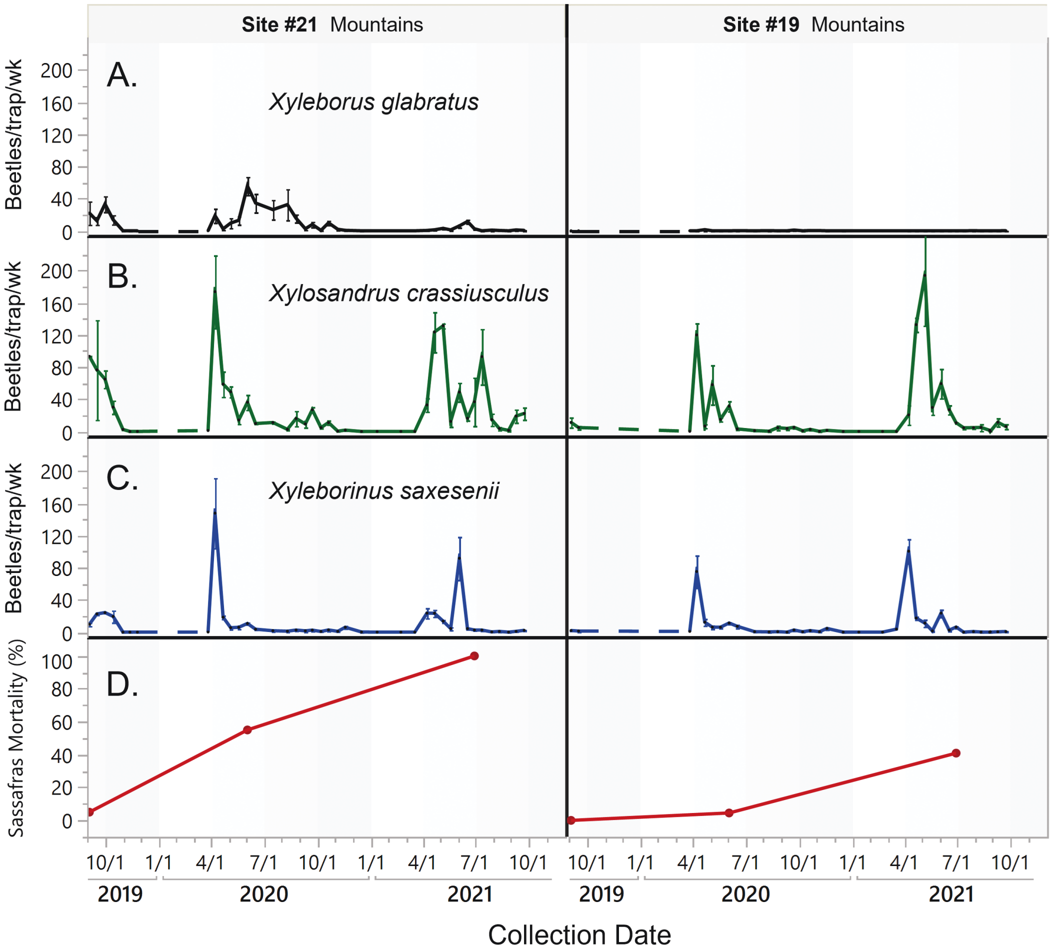

Figure 5. Mean number of ambrosia beetles caught per trap per week (three most abundant species, panels A–C) and percent sassafras mortality (panel D), at two sites in southwestern Kentucky from September 2019 to September 2021. Vertical bars denote standard error of the mean.

mortality of sassafras in stands with laurel wilt, due to exclusion of trees that may have been already killed prior to start of the study.

Recent estimates from Forest Inventory and Analysis (FIA) data suggests there are nearly two billion live sassafras stems in the US, most of which are concentrated in Midwestern and central Appalachian Mountain states (Randolph 2017). Randolph (2017) cautioned that although there was little evidence of laurel wilt–induced mortality in FIA data collected through 2014, fewer than 2% of sassafras stems occurred in areas with laurel wilt at that time, and that greater impacts should be expected as laurel wilt spreads. Past simulations of hypothetical sassafras loss have suggested that changes to forest composition, structure, and ecological interactions were likely if laurel wilt was to reach forests in central Kentucky (Nielsen and Rieske 2015). Our study, as well as recent assessments conducted at additional locations by state agencies (USDA-FS FHP 2022), indicates that laurel wilt is now well established and causing widespread mortality in this region, and studies documenting the ecological, economic, or other impacts of this invasion in the heart of the sassafras range would be timely.

Although sassafras stems < 5 cm dbh were not targeted for monitoring, mortality of stems below this diameter was documented when they were occasionally tagged by cooperating observers (Figure 3) and through other observations. For example, near site 21 in Kentucky, a wilted sassafras root sprout 1 cm in diameter growing on a forest edge was excavated, debarked, carefully examined, and found to have no ambrosia beetle entrance holes. The laurel wilt pathogen was isolated from this seedling and its root system, suggesting that the pathogen traveled to this shoot via root connection with adjacent trees. The likelihood of attack by RAB increases with stem diameter due to more apparent visual cues (Mayfield and Brownie 2013), which may allow small diameter host stems to escape inoculation by the insect. The clonal growth

Table 4. Total numbers (and percentage) of specimens of ambrosia beetle species captured in α-copaene-baited flight traps at two sites (19 and 21) in western Kentucky, September 2019–September 2021, with species nonnative to North America (per Gomez et al. 2018) noted.

Species	Count	Percent	Nonnative to North America
Ambrosiodmus rubricollis (Eichhoff)	27	0.1	X
Ambrosiodmus tachygraphus (Zimmermann)	6	< 0.1	
Ambrosiophilus atratus (Eichhoff)	24	0.1	X
Cnestus mutilatus (Blandford)	633	2.3	X
Corthylus punctatissimus (Zimmermann)	3	< 0.1	
Cyclorhipidion bodoanum (Reitter)	11	< 0.1	X
Cyclorhipidion pelliculosum (Eichhoff)	151	0.5	X
Dryoxylon onoharaense (Murayama)	179	0.6	X
Euwallacea validus (Eichhoff) *or E. interjectus* (Blandford)	465	1.7	X
Hylocurus rudis (LeConte, 1876)	68	0.2	
Hylocurus spadix Blackman	6	< 0.1	
Hypothenemus sp. Westwood	94	0.3	
Monarthrum fasciatum (Say)	66	0.2	
Monarthrum mali (Fitch)	46	0.2	
Pityophthorus sp. Eichhoff	16	0.1	
Xyleborinus saxesenii (Ratzeburg)	6,089	21.7	X
Xyleborus affinis Eichhoff	27	0.1	
Xyleborus ferrugineus (Fabricius)	41	0.1	
Xyleborus glabratus Eichhoff	2,489	8.9	X
Xylosandrus crassiusculus (Motschulsky)	14,905	53.2	X
Xylosandrus germanus (Blandford)	2,598	9.3	X
Unidentified	79	0.3	
Total	28,023	100.0	

habit of sassafras, however, provides opportunity for even small sprouts to become infected independently of vector attack. This phenomenon has the potential to accelerate sassafras mortality due to laurel wilt, even at sites where populations of the RAB vector remain low.

Our results strongly suggest that deploying α-copaene-baited flight traps in the vicinity of sassafras trees, while useful for monitoring known RAB populations, may not substantially improve early-detection efforts for laurel wilt (compared to visually monitoring for symptomatic sassafras). Detection and confirmation of the pathogen from symptomatic host material either preceded or coincided with the first trap catch of RAB at 92% (12/13) of our laurel-wilt positive sites. At the only site (site 24) where RAB presence in a trap preceded successful isolation of the pathogen, both redbay and sassafras were present in the area, and the monitored sassafras trees showed no signs laurel wilt at the start of the project (Table 1). Thus, RAB populations may have occurred first in redbay stems (which were unmonitored) and the adults dispersed from redbay into monitoring traps before sassafras became symptomatic. Furthermore, at site 19 (Kentucky, Mountains), where four traps were monitored continuously for 2 years, RAB was captured sporadically and at extremely low numbers, despite 40% mortality of the tagged sassafras trees in June 2021 and an abundance of other ambrosia beetle species in the traps (Figure 5). These results are consistent with those of Hanula et al. (2016), who provided evidence that the effective distance over which flying RAB are attracted to cubeb-oil lures (also rich in α-copaene) may be <1 m. Unless a stronger, longer-range attractant is developed, traps baited with α-copaene lures may be of limited utility as an early-detection tool for latent laurel wilt infections. Still, placing α-copaene-baited traps in stands with sassafras could help state surveyors evaluate the potential presence of RAB in certain situations, particularly if laurel wilt has been present and undetected for a number of years. These might include scenarios in which careful tree monitoring and pathogen sampling are impractical, or when targeted stands already contain sassafras mortality of an undetermined cause.

Previous studies on the seasonality of RAB flight have been conducted in stands containing redbay and swamp bay in Coastal Plain forests (Hanula et al. 2008, Brar et al. 2012). In coastal South Carolina, Hanula et al. (2008) observed RAB flight year-round, with captures beginning to increase steadily in June, peaking in September, declining to low levels by late November, and limited flight from December through March. Brar et al. (2012) observed two major peaks of RAB flight activity during February–April and September–October in Florida, with minimal flight November–January. Consistent with these observations of year-round flight in the Coastal Plain, limited RAB flight activity was also observed in this study during the colder months of December–March in the Coastal Plain of Louisiana (site 22) (Figure 4A). Similar to data reported by Brar et al. (2012), two peaks of RAB flight activity were observed in this study in the Piedmont/Sandhills of South Carolina (sites 34 and 35) and the southern limit of the Mountain region in Alabama (site 6). This suggests the presence of two generations (which peak in early spring, and then late summer/fall, respectively) in the Piedmont and lower-latitude Mountains (Fig. 4C–E). However, further north, at site 21 in Kentucky, RAB captures peaked slightly later (June), seasonal RAB flight activity was only weakly bimodal, and no flight activity was observed from December through March (Figure 5A). These differences in RAB flight seasonality may be due to the colder climate of interior Kentucky compared to warmer sites in the Piedmont and Coastal Plain. Furthermore, the notable reduction in beetle abundance in traps at site 21 between 2020 and 2021 (Figure 5A) was likely due to the elimination of fresh sassafras host material at this site (Figure 5D). Elimination of redbay and swamp bay hosts by laurel wilt in Florida has been shown to affect year-to-year variation in RAB flight patterns (Brar et al. 2012) and may also have affected the site-to-site variation observed in our study.

Although traps in this study were baited with a primary host volatile attractant of the RAB (α-copaene) and thus were intended to mimic lauraceous trees, RAB comprised fewer than 9% of the all the ambrosia beetle specimens captured at sites 19 and 21 in Kentucky (Table 4). This suggests that numerous other generalist ambrosia beetle species, many of which are nonnative to North America, may be attracted to α-copaene or are at least passively captured in traps deployed in diseased sassafras stands. The much higher capture rates of ambrosia beetle species like *Xylosandrus crassiusculus* and *Xyleborinus saxesenii*, their tendency to peak prior to RAB in the early spring, and their sustained abundance even after RAB trap captures declined (Fig. 5B, C) may indicate that these generalist ambrosia beetles are important competitors with RAB for limited host material. However, in a hanging bolt host utilization study (Mayfield et al. 2013), *Xylosandrus crassiusculus* was not recovered from containerized sassafras bolts despite an abundance of *Xylosandrus crassiusculus* that emerged from bolts of a more attractive lauraceous host (*Umbellularia californica*) that were artificially deployed in the same forest. Thus, the degree to which other ambrosia beetle species compete with RAB for potential host material may depend on the tree species composition of the forest stand and could merit additional investigation. Furthermore, ambrosia beetle species other than RAB have been known to obtain and carry *R. lauricola* (Carrillo et al. 2014, Ploetz et al. 2017b, Cruz et al. 2021); studies investigating the role of associated insect species as potential vectors could shed light on the dynamics of laurel wilt in sassafras and other natural systems.

Conclusion

Laurel wilt has moved beyond the Coastal Plain of the southeastern US and is readily spreading in the Piedmont and Mountain physiographic regions using sassafras as a primary host. Impact to sassafras populations is substantial and rapid, with the disease killing up to 100% of the sassafras stems ≥ 5 cm dbh and the pathogen spreading via root transmission. There was no substantial difference in RAB trapping efficacy between the eight-unit Lindgren funnel trap and the triple-vane multi-panel trap. Trapping with α-copaene lures in stands with sassafras was useful for monitoring known RAB populations but did not enhance early detection of latent laurel wilt infections. Seasonal flight activity of the RAB was bimodal in stands with sassafras in the Piedmont, with peak captures in April and a secondary peak between August and November. In the Mountain region in western Kentucky, RAB flight was not observed from December through March, peaked in June (with a weaker peak in late summer), and declined markedly as fresh sassafras host material was eliminated from the stand. The georeferenced network of sassafras plots established during this project provides baseline data for future monitoring efforts and could be revisited in the future for possible evidence of sassafras trees that display resistance to, or tolerance of, laurel wilt.

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