

First report of *Xyleborus glabratus* (Coleoptera: Curculionidae: Scolytinae) and laurel wilt in Louisiana, USA: the disease continues westward on sassafras

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Laurel wilt, caused by *Raffaelea lauricola* Harrington, Fraedrich & Aghayeva (Ophiostomatales: Ophiostomataceae), has spread rapidly through the coastal plains forests of the southeastern United States (USA) with devastating effects on redbay (*Persea borbonia* [L.] Spreng.; Laurales: Lauraceae) populations (Fraedrich et al. 2008; Harrington et al. 2008). The pathogen that causes the disease is a fungal symbiont of the redbay ambrosia beetle, *Xyleborus glabratus* Eichhoff (Coleoptera: Curculionidae: Scolytinae), and is carried in the mandibular mycangia of the beetle (Fraedrich et al. 2008). The beetle and fungus were introduced from Asia into the USA near Savannah, Georgia, around 2002 (Fraedrich et al. 2008; Harrington et al. 2011). Other members of the Lauraceae indigenous to the USA such as sassafras (*Sassafras albidum* [Nuttall] Nees; Laurales: Lauraceae) are also highly susceptible to the disease (Fraedrich et al. 2008), but sassafras is less common than redbay in the coastal plains of the Southeast (Koch & Smith 2008). Unlike redbay, which occurs only in the coastal plains, sassafras is widespread and occurs in forests over much of the eastern half of the USA. Many questions remain about the spread of laurel wilt on sassafras in forest types that are located inland and away from the coastal plains of the Southeast.

On 2 Sep 2014, nine open-grown sassafras trees (38–61 cm diameter at breast height) were observed with wilt-like symptoms near Bernice (Union Parish), in northern Louisiana (32.814184°N, 92.671021°W). Dead foliage on the trees remained attached to branches or had abscised, and black discoloration and small tunnels were observed in the sapwood of the stems. Samples of the discolored sapwood were obtained from 2 of the trees and plated on malt extract agar (MEA) amended with cycloheximide and streptomycin as previously described (Harrington 1981; Harrington 1992; Fraedrich et al. 2008). A fungus morphologically identical to *R. lauricola* was consistently recovered from the samples, and the isolates were confirmed as *R. lauricola* using sequences of the D1/D2 region of the 28S rRNA gene (Harrington et al. 2008, 2010).

Two isolates identified as *R. lauricola* were evaluated for pathogenicity in growth chamber tests. The isolates were grown on MEA for 21 d at 25 °C, and plugs of agar with the fungus were inserted into 5 mm diameter holes in the stems of 3 redbay saplings (3 yr old; mean height = 183 cm; mean diameter at ground line = 21 mm) for each isolate, and then saplings were placed in a growth chamber with daytime and

nighttime temperatures set at 28 and 25 °C, respectively (Fraedrich et al. 2008). Three additional saplings were similarly mock-inoculated with sterile MEA plugs. The results of the tests showed that the isolates of *R. lauricola* were highly pathogenic. Symptoms of wilt were observed in the 6 saplings inoculated with *R. lauricola* within 12 d, and the saplings completely wilted within 30 d. Control saplings remained healthy with no symptoms of wilt.

Three 12-unit Lindgren funnel traps baited with manuka oil lures (Hanula & Sullivan 2008) were deployed near the symptomatic trees on 2 Sep 2014, and 157 female *X. glabratus* beetles were collected from these traps during the month of Sep. A sample of the beetles was subsequently forwarded to Steven Lingafelter (United States Department of Agriculture, Agricultural Research Service, Systematic Entomology, Riverdale, Maryland, USA), who confirmed the species as *X. glabratus*.

Additional surveys for symptomatic sassafras trees and *X. glabratus* were initiated on 29 Sep 2014 in order to better understand the extent of the infestation. Lindgren traps baited with cubeb oil lures (Hanula et al. 2013) were deployed at 13 additional sites that were located in 3 parishes (Lincoln, Claiborne, and Union) at distances of 6 to 15 km from the Bernice site. Collections were made weekly until 15 Oct 2015. In addition, informal surveys were conducted in the 3 parishes for sassafras trees with wilt-like symptoms, and sapwood samples were collected from 6 additional symptomatic trees and plated on agar media as previously described.

The survey for *X. glabratus* found the beetle in all 3 parishes at 5 of the 14 trapping sites at locations up to 10 km from the Bernice site (Table 1). Furthermore, *R. lauricola* was isolated from 5 additional symptomatic sassafras trees located in Lincoln and Claiborne Parishes, and found as far away as Ruston, Louisiana, which is 27 km south of the Bernice site.

The discovery of sassafras with laurel wilt in northern Louisiana represents a major extension of the known distribution of the disease and the first record west of the Mississippi River. The previously known westernmost locations for laurel wilt were on redbay in Harrison County, Mississippi, approximately 400 km to the southeast and on sassafras in Marengo County, Alabama, 450 km to the east. The Louisiana discovery provides additional concerns about the continued spread of the disease because this area is outside the range of the primary host,

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Table 1. Captures of *Xyleborus glabratus* in Lindgren traps baited with cubeb oil lures and diagnosis of laurel wilt symptoms in sassafras trees during surveys of 16 sites in Union, Claiborne, and Lincoln Parishes, Louisiana, from 29 Sep to 15 Oct 2014.

Site No. ^a	Presence of wilted or healthy sassafras trees	Parish	Latitude	Longitude	<i>Xyleborus glabratus</i> trap captures ^b	Isolation of <i>Raffaelea lauricola</i> ^c
0	wilted	Union	32.81423	-92.6715	7	+
1	wilted	Union	32.93963	-92.7228	0	NA
2	healthy	Union	32.92172	-92.6138	0	NA
3	healthy	Union	32.85816	-92.5440	0	NA
4	wilted	Union	32.83186	-92.5952	0	NA
5	wilted	Union	32.74704	-92.5662	0	NA
6	no sassafras trees	Claiborne	32.81854	-92.7411	6	NA
7	wilted	Claiborne	32.84037	-92.7341	15	NA
8	wilted	Claiborne	32.90194	-92.7912	NT*	NA
9	wilted	Claiborne	32.91859	-92.7743	0	+
10	healthy	Claiborne	32.78701	-92.7640	0	NA
11	healthy	Lincoln	32.73382	-92.6692	0	NA
12	wilted	Lincoln	32.74979	-92.7256	1	+
13	wilted	Lincoln	32.73045	-92.6480	1	+
14	wilted	Lincoln	32.56685	-92.6381	NT	+
15	wilted	Lincoln	32.55780	-92.6433	NT	+

^aSite "0" is the Bernice, Louisiana, site where laurel wilt was initially discovered.

^b"NT" indicates that traps were not deployed at the site and *X. glabratus* presence not assessed. "NT*" indicates that a trap was deployed but the trap was removed by an unknown person.

^c"+" indicates that *Raffaelea lauricola* was isolated from the tree; "NA" indicates that the trees were not assessed for *R. lauricola*.

redbay. No redbay or other potential hosts for laurel wilt were seen in the study area. Sassafras has been known to be highly susceptible to the disease in inoculation trials (Fraedrich et al. 2008), but there have been some doubts about the attractiveness of sassafras and its suitability as brood material for *X. glabratus*. Sassafras generally occurs at low densities (<30 trees/hectare) where it is naturally present in Louisiana (Koch & Smith 2008). The fact that laurel wilt is occurring on sassafras over a 3-parish area suggests that *X. glabratus* is capable of finding sassafras trees at relatively low densities, and *X. glabratus* populations appear to be substantial on sassafras alone.

The discovery of laurel wilt in Louisiana is not the first instance where disease has been found in sassafras trees where redbay is absent. In 2010, sassafras trees with laurel wilt were found in Marengo County, Alabama, a county in west central Alabama outside the range of redbay (Bates et al. 2013). The disease has since spread to other sassafras trees in 3 adjacent counties in Alabama (S. F., unpublished data; Bates et al. 2015). In addition, as *X. glabratus* has spread inland and away from the coastal plains in Georgia, the wilt has been observed increasingly in sassafras in areas where redbay is absent (Cameron et al. 2014).

The original models for the spread of laurel wilt predicted that the disease would not reach central Louisiana until at least 2030 (Koch & Smith 2008); however, these models could not predict anthropogenic movement of the beetle in wood. Solid wood packing material is believed to have been the pathway for the introduction of *X. glabratus* and *R. lauricola* into the USA, and the subsequent movement of infested wood has been suspected as the cause for many of the long-distance "jumps" in the distribution of the vector and pathogen as they have moved throughout the southeastern USA (Fraedrich et al. 2008; Riggins et al. 2010; Ploetz et al. 2011; Bates et al. 2013).

Based on the low density of sassafras in the southern forests, and early findings that sassafras was less suitable than redbay as a host for *X. glabratus* (Hanula et al. 2008; Mayfield & Hanula 2012), it was thought to be unlikely that *X. glabratus* would spread to interior forests (Koch & Smith 2008). Furthermore, a climate match analysis concluded that *X. glabratus* was particularly associated with plants that occur in subtropical and warm temperate areas, and thus, it was thought that

the beetle would essentially be constrained to the coastal plains of the Southeast (Koch & Smith 2008). However, recent findings suggest that sassafras may be an attractive host for *X. glabratus* (Mayfield et al. 2013), and observations in Georgia, Alabama, and Louisiana suggest that the beetle effectively locates and utilizes sassafras as a reproductive host (Bates et al. 2013; Cameron et al. 2014). In addition, a study of the cold tolerance of *X. glabratus* concluded that the beetle may be able to survive extremely low temperatures and could possibly spread as far north as southern Ontario (Formby et al. 2013), which would encompass much of the natural geographic range of sassafras (Griggs 1990).

A general concern at present is that *X. glabratus* and laurel wilt could move into the northern range of sassafras in the eastern USA. Sassafras is generally more common in northern states such as Missouri, Kentucky, and West Virginia than it is in southern states (Koch & Smith 2008). Although redbay wood has some excellent wood properties that are sought by woodworkers (Brendemuehl 1990; Ploetz et al. 2011), sassafras wood appears to be more desirable and has commercial potential (Cassen 2007; Harding et al. 1997), and thus is more likely to be utilized and transported. Another potential pathway for movement of the vector and pathogen is in wood chips collected and transported by truck or rail for pulp production. The rapid spread of laurel wilt across the southern USA is unprecedented, and it is imperative to develop a better understanding of how minor-use hardwoods are being utilized and transported within the USA in order to lessen the probabilities of spreading diseases such as laurel wilt to new areas.

Summary

Xyleborus glabratus Eichhoff (Coleoptera: Curculionidae: Scolytinae) and sassafras trees that died from laurel wilt were discovered in a 3-parish area of northern Louisiana, USA, in Sep 2014. Redbay, a species that has been severely affected by the disease in the coastal plains forests of the southeastern USA, is not found in this area of northern Louisiana, suggesting that sassafras is attractive to *X. glabratus* and an adequate reproductive host for the beetle. This is the first report of *X.*

glabratus and laurel wilt in Louisiana and the first report of the beetle and disease west of the Mississippi River.

Key Words: *Raffaelea lauricola*; redbay ambrosia beetle

Sumario

En septiembre del 2014, *Xyleborus glabratus* Eichhoff (Coleoptera: Curculionidae: Scolytinae) y árboles de sasafrás que murieron a causa de la marchitez del laurel fueron descubiertos en un área de 3 municipios del norte de Louisiana, EE.UU. El aguacatillo (*Persea borbonia*), una especie que se ha visto gravemente afectada por la enfermedad en los bosques de la llanura costero del sudeste de EE.UU., no se encuentra en esta zona del norte de Louisiana, lo que sugiere que el sasafrás es atractivo para *X. glabratus* y un hospedero reproductivo adecuado para el escarabajo. Este es el primer informe de *X. glabratus* y la marchitez del laurel en Louisiana y el primer informe del escarabajo y la enfermedad al oeste del río Mississippi.

Palabras Clave: *Raffaelea lauricola*; escarabajo ambrosia del laurel rojo

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