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# Efficacy of Propiconazole for Prevention of Sassafras Mortality from Laurel Wilt Disease Using a Tree Micro-Injection and Micro-Infusion Delivery System

**By C.W. Johnson, R.O. Olatinwo, J. Hwang, and C. Brownie**

**Abstract.** Laurel wilt is a lethal disease of American Lauraceae caused by *Harringtonia lauricola.* Propiconazole is a systemic fungicide which arrests fungal growth among a variety of plant hosts. Propiconazole as a preventive treatment against laurel wilt in sassafras (*Sassafras albidum*) has not been evaluated. We treated sassafras trees with propiconazole using the Arborjet QUIK-jet® Micro-Injection™ and TREE I.V. Micro-Infusion™ systems (Arborjet, Inc., Woburn, MA, USA) and challenged trees by inoculating them with *H. lauricola*. Out of 7 trees treated using the QUIK-jet Micro-Injection system, 6 (86%) survived 52 or more weeks following inoculation with *H. lauricola*, while only 11% of inoculated control trees (1 of 9) survived over this period. All trees not damaged by hurricanes ($n$ = 13) treated with propiconazole using the TREE I.V. Micro-Infusion system survived significantly longer than untreated control trees after inoculation with *H. lauricola*; 10 of 13 trees (77%) survived with < 50% crown loss, and 8 of 13 trees (62%) appeared entirely healthy 54 weeks post-inoculation. In the TREE I.V. Micro-Infusion system trial, 15 of 19 control trees (79%) had either died or lost ≥ 50% of living crown 54 weeks post-inoculation with *H. lauricola*. Results indicate sassafras trees treated with propiconazole using the Arborjet QUIK-jet Micro-Injection and TREE I.V. Micro-Infusion systems are significantly less likely to die within one year of infection with *H. lauricola*; however some trees may exhibit significant crown decline (≥ 50%) over this period.

**Keywords.** Ambrosia Beetle; Bioassay; Fungicide Infusion; Fungicide Injection; *Harringtonia lauricola*; Propiconazole; *Raffaelea lauricola*; Redbay; *Sassafras albidum*; *Xyleborus glabratus*.

## INTRODUCTION

Laurel wilt disease (LWD) is a vascular disease caused by *Harringtonia lauricola* ([T.C. Harr., Fraedrich & Aghayeva] Z.W. de Beer & M. Procter.; Ophiostomatales), a fungal symbiont of the redbay ambrosia beetle (RAB), *Xyleborus glabratus* (Eichhoff; Coleoptera: Curculionidae: Scolytinae)(Harrington et al. 2008; Harrington et al. 2011; de Beer et al. 2022). After locating their host, female RABs oviposit eggs at the termini of multiple branching galleries within the xylem (Brar et al. 2013), and developing brood feed on the mycelia of *H. lauricola* introduced during gallery construction from female mandibular mycangia (Fraedrich et al. 2008). In its native range of southeast Asia, *X. glabratus* exhibits a strong preference for and primarily colonizes dead and moribund members of the Lauraceae family (Hulcr and Lou 2013).

In 2002 a single *X. glabratus* was collected near Port Wentworth, Georgia, USA (Rabaglia et al. 2006), and in 2003 widespread, unexplained mortality of the North American Lauraceae species redbay (*Persea borbonia* [L.] 'Spreng') was reported in the area (Fraedrich et al. 2008). Numerous dead redbay trees were observed, and declining trees were characterized by brown, wilted leaves and black staining of the xylem tissue in the tree bole and branches (Fraedrich et al. 2008). It is now known that *X. glabratus* will attack and inoculate healthy North American Lauraceae with *H. lauricola*, causing vascular wilting and relatively rapid mortality. The precise manner by which Lauraceae hosts die is likely similar to that described for avocado, in which a rapid decrease in hydraulic conductivity follows infection (Inch and Ploetz 2011) due to the occlusion of xylem vessels by

tyloses and gel formation in response to infection (Inch and Ploetz 2011; Inch et al. 2012).

Laurel wilt occurs across the southeastern USA, extending from Virginia west to Kentucky, south to Arkansas and Texas, and east to Florida. Mortality has been reported in multiple Lauraceae hosts occurring in North America, including *Persea palustris* (swamp bay)(Fraedrich et al. 2008; Olatinwo et al. 2019); *Litsea aestivalis* L. 'Fern' (pondspice)(Hughes et al. 2011); *Lindera benzoin* L. 'Blume' (spicebush) (Fraedrich et al. 2016; Olatinwo et al. 2021); *Sassafras albidum* Nutt. (sassafras)(Smith et al. 2009; Riggins et al. 2011; Bates et al. 2013; Cameron et al. 2015; Fraedrich et al. 2015; Mayfield et al. 2019); *Cinnamomum camphora* (camphor tree)(Smith et al. 2009); and the economically important *Persea americana* Mill (avocado)(Mayfield et al. 2008b; Ploetz et al. 2017).

Sassafras is a desirable native horticultural and urban tree, being tolerant of poor soils and having a restricted growth form and outstanding fall colors. It is widely distributed across the eastern USA, occurring from Maine to Michigan and Texas to Florida (Griggs 1990), and is most abundant in the Ozark regions of Missouri and Arkansas, the Ohio River Valley, and the Appalachian Mountains (Randolph 2017). Monitoring studies where LWD is established have found mortality rates among sassafras populations approaching 80% (Cameron et al. 2015), and surveillance of sassafras in Louisiana where LWD is present has indicated similar rates of mortality (authors unpublished). Formby et al. (2017) predicted that 48% of the US sassafras population will not experience sufficient cold to restrict LWD. Thus, it is expected that urban areas and forests with high sassafras abundance will experience significant rates of mortality. The removal of sassafras from forests will also be a significant economic, ecological, and cultural loss. Sassafras has some commercial value in southern forests (Forest Products Laboratory 1999), is a host plant of multiple native pollinator larvae (Randolph 2017), and provides forage for wildlife (Griggs 1990; Immel 2016). Sassafras also has a unique cultural history and value, as Native Americans and early settlers sought it out for its medicinal properties and as a food additive (Immel 2016).

Trunk injection and infusion systems are a relatively safe and effective means of delivering systemic pesticides for therapeutic and preventive treatments while significantly reducing nontarget risks. Simple, pressurized systems used to assist in the uptake of products, including propiconazole (Propizol®; Arborjet, Inc., Woburn, MA, USA), through shallowly drilled holes have effectively prevented (or have had therapeutic effects against) oak wilt disease in live oak (*Quercus virginiana*)(Appel and Kurdyla 1992; Wilson and Lester 1996), various *Quercus* sp. in both the red- and white-oak groups (Eggers et al. 2005), and northern red oak (*Q. rubra*)(Ward et al. 2004; Blaedow 2009), and have prevented LWD in redbay (Mayfield et al. 2008a). The delivery systems of products and the terminology used to describe such technology varies. Here we present findings based on the use of the TREE I.V. Micro-Infusion™ (Arborjet, Inc., Woburn, MA, USA), where micro-infusion refers to the application of a diluted product (and larger total volume of solution) under low pressure (35 PSI [241 kPa]) in delivery sites spaced 7.6 to 10.2 cm apart; and the use of the QUIK-jet® Micro-Injection™ system, which requires the applicator to manually inject the undiluted product (relatively small volumes) to delivery sites spaced 7.6 to 10.2 cm apart. One major difference between the 2 systems is the application time. The depth of delivery holes is insufficient in either case to accept the total volume of product applied; in both cases, the surrounding xylem tissue absorbs the product. Thus, trees will require more time to absorb and translocate the larger volume of solution applied using the TREE I.V. Micro-Infusion™ system. The micro-infusion method in some cases requires multiple hours per tree, depending on factors such as time of day and health of the tree, while the delivery of undiluted product using the QUIK-jet® Micro-Injection™ system can be completed in minutes. This micro-injection system has been effectively used to deliver both propiconazole as well as the insecticide emamectin benzoate as a preventive and therapeutic treatment of California sycamore (*Platanus racemosa* Nutt.) prior to or following attack by an invasive ambrosia beetle and its associated pathogenic, symbiotic fungi (Grosman et al. 2019). Although the micro-injection system affords for more rapid application, it may not be more efficacious in delivering the product throughout the tree. Propiconazole offers effective prevention of Dutch elm disease in *Ulmus* sp. when using infusion methods, but it may be less efficacious when using micro-injection techniques (Haugen and Stennes 1999; Stipes 2017). Stipes (2017) contends that in treating elms for Dutch elm disease, the larger volume of diluted propiconazole delivered using an

infusion system results in better translocation of the product than the concentrated treatments delivered using injection systems. While propiconazole is known to protect redbay against laurel wilt for at least 30 weeks (where trees were inoculated with *H. lauricola* and injected with propiconazole in April)(Mayfield et al. 2008a) using a micro-infusion system, wider applications among Lauraceae species, such as high-value sassafras, and alternative injection systems, such as micro-injection, have not been evaluated. Thus, our primary objective was to evaluate the use of propiconazole as a preventive treatment against LWD in sassafras using 2 methods of product delivery: the QUIK-jet® Micro-Injection™ and TREE I.V. Micro-Infusion™ systems, testing the null hypothesis that the time (weeks) elapsed to expression of greater than 50% crown wilt, branch mortality, and/or tree mortality between the propiconazole-injected and *H. lauricola*-inoculated trees did not significantly differ from trees in the untreated control group.

## MATERIALS AND METHODS

### Evaluation of QUIK-jet® Micro-Injection™ System (2019–2020)

To evaluate the use of propiconazole as a preventive treatment against laurel wilt using the QUIK-jet® Micro-Injection™ system, we selected 34 sassafras trees primarily located in mature oak-pine-hickory forests within Kisatchie National Forest (KNF), Grant and Rapides Parishes, LA, USA. Due to the difficulty of locating readily accessible sassafras trees in the area, a wide range of diameters (12.7- to 40.6-cm diameter at breast height)(DBH) was allowed in the study. The treatments below were randomly assigned with the restriction that treatments were spread evenly across the range of tree diameters.

PZOL + HL: tree injected with propiconazole and inoculated with *H. lauricola* ($n = 9$)
HL: tree inoculated with *H. lauricola* and not injected with propiconazole ($n = 9$)
AG: tree inoculated with sterile agar and not injected with propiconazole ($n = 8$)
PZOL + AG: tree injected with propiconazole and inoculated with sterile agar ($n = 8$)

Treated trees received propiconazole (Propizol®; Arborjet, Inc., Woburn, MA, USA) formulated at a rate of 10 mL/in DBH (equaling 3.9 mL [0.61 g] per cm; the recommended rate for prevention of LWD per label instructions) in holes drilled using a 3/8-in high helical drill bit 5 cm deep at a 45° downward angle every 10.2 cm of circumference at the base of trees. All holes were drilled within 12 in (30.5 cm) of the base of trees, within root flares where visible. Injections were made through #4 Arborplugs® inserted at drill sites such that the Arborplug® barbs created a seal between the inner bark and xylem tissue. Trees in the PZOL treatment group were injected in late March 2019, prior to leaf flush, and after allowing time for product translocation, HL inoculations were conducted 2 months later (late May 2019). In a few cases it was suspected during drilling that the 5-cm-deep treatment holes terminated or bisected decayed xylem. Given the difficulty in locating accessible trees in the project area, alternative trees were not chosen. However, all propiconazole-injected trees were felled following death or in December 2020 at the cessation of the QUIK-jet® Micro-Injection™ system trial to determine what effect any undetected internal wood decay may have had on treatment efficacy. Trees were cut at the plane of injection to expose drill sites and then sanded with increasingly finer grit (80 to 220) with an orbital hand-sander and aged under magnification using a Nikon SMZ445 (8 to 35× magnification)(Nikon Metrology, Inc., Brighton, Michigan, USA). All trees where greater than 50% of treatment holes bisected decayed wood were excluded from further analyses. Sound wood surrounded all treatment holes within 3 trees, however decayed wood was bisected by a single hole in 3 trees and by 2 holes in 2 trees. Because this did not constitute more than 25% of total treatment sites per tree, we retained these trees for analyses. In one case, each of the 5 total treatment holes in 1 tree penetrated decayed wood, and thus this tree was excluded from analyses. One additional tree in the PZOL + HL treatment was excluded after it was discovered to be naturally infected with laurel wilt at the time of field inoculation (HL) based on crown wilting and vascular staining in outer sapwood.

### Evaluation of TREE I.V. Micro-Infusion™ System (2020–2021)

To evaluate the use of propiconazole as a preventive treatment against laurel wilt using the TREE I.V. Micro-Infusion™ delivery system, 42 wild sassafras trees (12.7- to 48.3-cm DBH) were selected in forests within the KNF, LA, USA, as described in the previous trial above, and randomly assigned to either the

PZOL or HL group described below. We observed no phytotoxic effects of the PZOL treatment in the 2019–2020 trial, therefore the AG and PZOL + AG groups were excluded in favor of greater replication in the remaining treatment groups. Hurricanes Laura and Delta severely damaged (> 50% crown loss) or felled 10 study trees in late 2020, resulting in the unbalanced replication listed below.

PZOL + HL: tree injected with propiconazole and inoculated with *H. lauricola* ($n$ = 13 trees)
HL: tree inoculated with *H. lauricola* only ($n$ = 19 trees)

Trees were injected at a rate of 10 mL/in DBH (equaling 3.9 mL [0.61 g] per cm; the recommended rate for prevention of LWD) and diluted with a 2 to 1 by volume ratio of distilled water. To improve product uptake, infusion sites were located at the root flare or in the primary roots themselves, when visible. Treatment holes were created with a high helix 3/8-in drill bit sized for the #4 Arborplug® (seated as described previously), maintaining a distance of at least 7.6 cm between plugs, and drilled to a depth of 3.8 cm (these treatment holes were drilled more shallowly than those in 2019 to decrease the probability of intersecting internal rot). The TREE I.V. system requires that the treatment solution be drawn from 1-L supply bottles pressurized (using a hand-operated pressure pump) to 35 PSI (241 kPa). Due to the local COVID-19 pandemic and associated work restrictions, PZOL tree injections were conducted later than those in 2019, occurring after leaf flush (May 28 to June 5, 2020). After allowing time for treatment translocation, all HL inoculations were conducted on June 30, 2020.

## *Harringtonia lauricola* Inoculations

In each trial, after allowing at least 30 days for translocation of product within propiconazole-injected trees, where required, both propiconazole-injected and control trees were inoculated with 3-week-old laboratory cultures of local isolates of *H. lauricola* on potato dextrose agar (PDA). Agar plugs were placed in 2 separate holes 5-mm (0.2-in) in diameter punched through the bark with a cork-borer (Figure 1) as described by Mayfield et al. (2008a). Treatments not requiring *H. lauricola* inoculation (2019–2020 trial only) received sterile agar plugs only.

## Tree Health Assessments

Tree health was assessed beginning 4 weeks post-inoculation and continued biweekly (in the 2019–2020 QUIK-jet® Micro-Injection™ system trial) and monthly (in the 2020–2021 TREE I.V. Micro-Infusion™ system trial). After budbreak in succeeding years, assessments resumed monthly for trees which remained alive (here defined as turgid, green foliage present somewhere within the tree) until the end of the leaf-fall in late October 2020 (for the QUIK-jet® Micro-Injection™ system trial) or 2021 (for the TREE I.V. Micro-Infusion™ system trial). Trees were categorized as follows: (1) healthy (no symptoms of LWD), less than 50% of tree crown wilted; (2) more than 50% of tree crown wilted (or if the entire crown was wilted, healthy epicormic shoots were present along the tree bole and vascular staining present in xylem of tree bole in spot check at breast height); or (3) dead (tree devoid of all green foliage in the crown or along the bole [epicormic shoots] and vascular staining evident in xylem of tree bole).

In addition to monitoring for symptoms consistent with those produced by LWD, we also examined foliage for any evidence of a phytotoxic response, such as leaf margin or interveinal discoloration or necrosis. Xylem samples were collected from trees declared dead or those with significant crown wilt symptoms (≥ 50% crown wilting or only epicormic shoots along bole) at the cessation of the study to confirm presence/absence of *H. lauricola* in all trees.

## Bioassays

To determine if tissue samples from propiconazole-injected trees (using both delivery systems) exhibited fungistatic activity, all living trees were sampled at 3 months and 12 months post-inoculation (additionally, the 4 surviving PZOL + HL and lone surviving PZOL + AG trees treated with the QUIK-jet® Micro-Injection™ delivery system were sampled at 15 months post-inoculation for this purpose). To determine if *H. lauricola* inoculations were successful, trees were sampled at death or 12 months post-inoculation (for the 2019–2020 QUIK-jet® Micro-Injection™ system trial; however, in error the 4 surviving PZOL + HL inoculated trees were not sampled for this purpose) (Table 1). Similarly, trees were sampled at 3 months and 12 months post-inoculation (whether dead or alive) in the 2020–2021 TREE I.V. Micro-Infusion™ trial. Wood tissue samples (punch discs) were collected

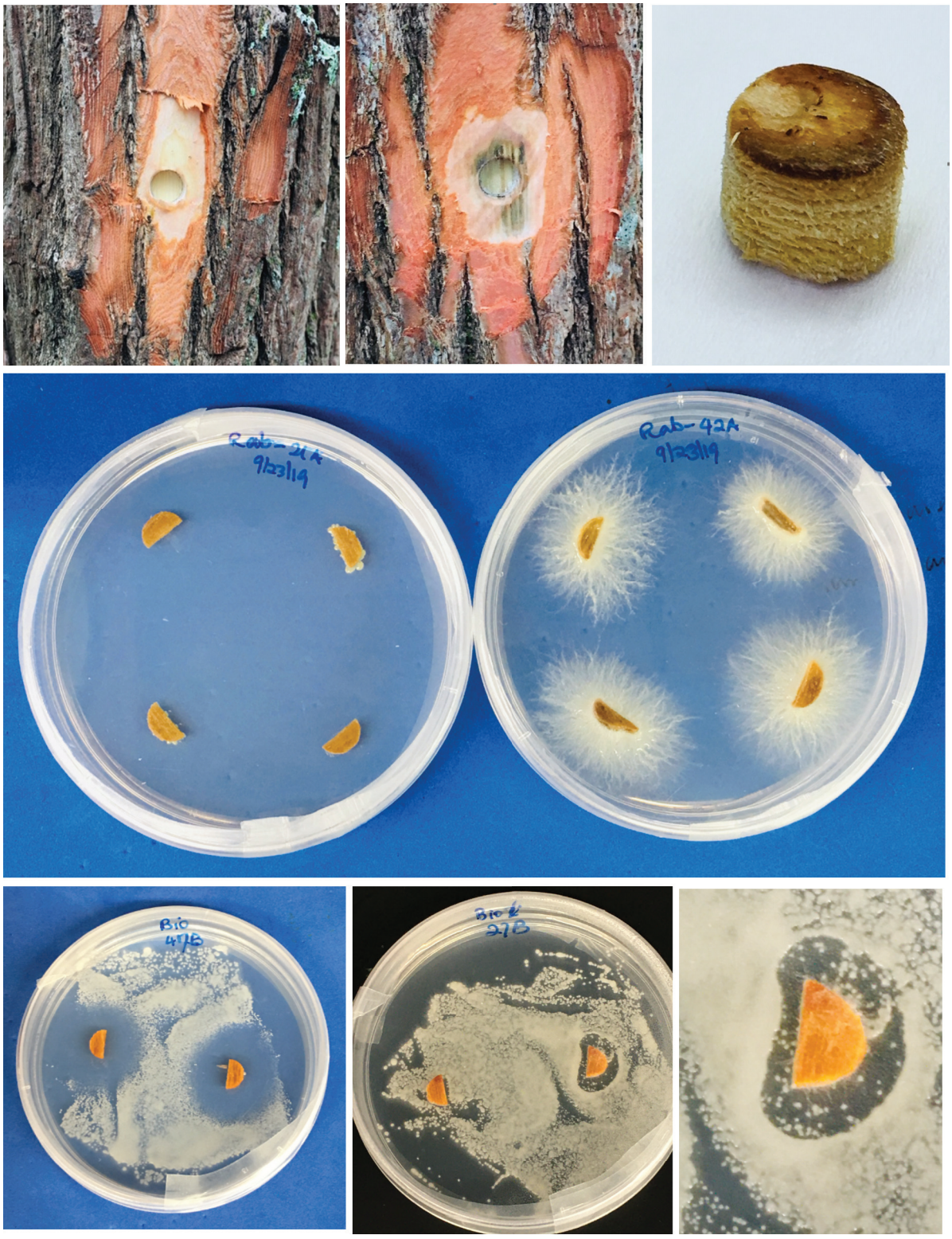


**Figure 1. Tissue-sample discs collected from inoculated and noninoculated sassafras trees (top); tissue discs plated on potato dextrose agar (PDA) for reisolation of *H. lauricola* (middle); and inhibition zones from the evaluation of the movement and retention of propiconazole in sassafras tissue samples on PDA pre-inoculated with spores of *H. lauricola* (bottom).**

from the outer xylem tissue of propiconazole-injected trees after the outer bark was removed (Figure 1). The discs were obtained at 2 levels: 30 cm directly above and below the *H. lauricola* inoculation point, subdivided into 2 pieces. The 4 pieces were plated on PDA that was pre-inoculated with approximately $1 \times 10^5$ *H. lauricola* spores evenly spread with a sterilized L-shaped glass rod on the agar. The plates were incubated in the laboratory at an ambient temperature of approximately 25 °C and visually evaluated under a dissecting microscope for signs of fungal inhibition zones (Figure 1). The number of discs exhibiting inhibition of *H. lauricola* growth was recorded after 2 weeks and categorically scored as positive (YES) or negative (NO) according to the inhibition zones. A positive score indicated complete inhibition of growth on the disc, where visible inhibition zones surrounded the disc.

### Statistical Analyses

To reflect the rate of crown decline observed and time to death or survival in the QUIK-jet® Micro-Injection™ system trial, the tree health scores were used to calculate 2 "time to event" variables. These "time to event" variables consisted of: (1) "weeks to ≥ 50% crown death," defined as the number of weeks from inoculation of the tree with laurel wilt to the observation of crown wilting or branch death within ≥ 50% of the tree crown, or crown entirely dead but epicormic sprouts present on bole; and (2) "weeks of survival," defined as the number of weeks from inoculation of the tree with HL to either tree death (absence of any green, turgid foliage within the crown or along the tree bole) or survival at the end of the evaluation period.

### QUIK-jet® Micro-Injection™ System Analyses (2019–2020)

The QUIK-jet® Micro-Injection™ system trial data were subjected to a 2 × 2 factorial analysis of variance (ANOVA) on the 2 "time to event" variables. The treatment factors PZOL (PZOL vs. no PZOL) and HL (HL vs. AG) showed strong interaction and variance heterogeneity that was not corrected by transformation. A one-way ANOVA was therefore carried out using PROC MIXED with the REPEATED statement and GROUP option (SAS v.9.4, Cary, NC, USA) to allow for variance heterogeneity as follows. For the variable "weeks to mortality," the error variance for the treatment HL was allowed to differ from that for the other 3 treatments. For the variable "time to ≥ 50% death," the error variance was assumed to be the same for the 4 treatments. Pairwise comparisons of least squares means among the 4 treatments were carried out using the Tukey-Kramer method in PROC MIXED. In cases where trees within the treatment groups survived beyond the 62-week evaluation period, the trees were assumed to survive at least 63 weeks to allow for mean (± standard error)(SE) calculations and analyses but are presented as > 62 weeks in the results and all tables and figures.

### TREE I.V. Micro-Infusion™ Delivery System Analyses (2020–2021)

Preliminary analysis of covariance and graphs indicated that DBH was not a useful predictor of the rate of decline in tree health. Also, loss of trees due to hurricanes left many pairs incomplete; thus pairing and DBH were ignored in further analyses. Data were highly discrete and censored, and residual plots indicated that ANOVA methods were not appropriate. Therefore, the "weeks to ≥ 50% crown death" and "weeks to tree death" variable data were grouped into 3 categories: (1) dead at week 16; (2) alive at week 16 but dead by week 63; and (3) alive at week 63. A 2 × 3 contingency table, with rows and columns corresponding to treatments and time-to-event categories, respectively, was constructed for each response, and the Cochran-Mantel-Haenszel (CMH) test for equality of row scores was used to test for differences between treatments (Cochran 1954). Analyses were performed using PROC FREQ of SAS (v.9.4) with OPTION CMH and CMH statistic 2. As the "time to event" data were categorized prior to analyses, no rounding of weeks was required to produce discrete values (as in the QUIK-jet® Micro-Injection™ system analyses).

## RESULTS AND DISCUSSION

### QUIK-jet® Micro-Injection™ System Analyses (2019–2020)

PZOL + HL injected trees ($n = 7$) using the QUIK-jet® Micro-Injection™ system expressed LWD crown symptoms in ≥ 50% of the crown significantly later ($t_{28} = -15.49$, $P < 0.001$) and survived significantly longer ($t_{28} = -5.14$; $P = 0.001$) than HL trees ($n = 9$; Table 1). Out of 7 PZOL + HL trees, 6 (86%) survived at least 52 weeks following inoculation, while 6 out of 9 (67%) HL trees died within the first 20 weeks of inoculation (Table 1). Out of 7 PZOL + HL trees, 4 (57%) persisted from inoculation to leaf fall

**Table 1. The 2019–2020 QUIK-jet® Micro-Injection™ system trial. Least squares mean (± 1 SE) for weeks to development of ≥ 50% crown decline and tree mortality among treatments using the Arborjet QUIK-jet® Micro-Injection™ system following inoculation with *Harringtonia lauricola* or sterile agar. Within a column, differing letters denote significant difference (Tukey-Kramer test; $\alpha = 0.05$). Note: where survival exceeded 62 weeks, values were rounded to 63 for mean calculation and analyses. Standard error (SE); Propiconazole (PZOL); *Harringtonia lauricola* (HL); agar (AG); standard error of the mean (SEM).**

| | Tree (*n*) | Weeks to ≥ 50% crown mortality | Weeks of tree survival | Crown score at final evaluation | HL reisolated at tree death or 12 months |
|---|---|---|---|---|---|
| PZOL + HL[a] | 1 | > 62 | > 62 | 2 | - |
| | 2 | 49 | 62 | Dead | Y |
| | 3 | 49 | 49 | Dead | Y |
| | 4 | > 62 | > 62 | 1 | - |
| | 5 | > 62 | > 62 | 1 | - |
| | 6 | 49 | 60 | Dead | Y |
| | 7 | > 62 | > 62 | 1 | - |
| | **Mean (± 1 SEM)** | 57.0 ± 2.3 *b* | 60.4 ± 1.4 *b* | - | - |
| HL[b] | 1 | 18 | 49 | Dead | Y |
| | 2 | 4 | 10 | Dead | Y |
| | 3 | 4 | 14 | Dead | Y |
| | 4 | 8 | 14 | Dead | Y |
| | 5 | 4 | 20 | Dead | Y |
| | 6 | 10 | 56 | Dead | Y |
| | 7 | 18 | 49 | Dead | Y |
| | 8 | 4 | 6 | Dead | Y |
| | 9 | 8 | 10 | Dead | Y |
| | **Mean (± 1 SEM)** | 8.7 ± 2.1 *a* | 24.9 ± 6.8 *a* | - | - |
| AG[c] | 1 | 49 | > 62 | 2 | Y |
| | 2 | 49 | > 62 | 2 | Y |
| | 3 | 49 | > 62 | 2 | Y |
| | 4 | 49 | > 62 | 2 | Y |
| | 5 | 60 | > 62 | 1 | N |
| | 6 | 56 | > 62 | 2 | Y |
| | 7 | 60 | > 62 | 1 | N |
| | 8 | 60 | > 62 | 1 | Y |
| | **Mean (± 1 SEM)** | 54.4 ± 2.2 *b* | 61.3 ± 1.4 *b* | - | - |
| PZOL + AG[d] | 1 | > 62 | > 62 | 1 | Y |
| | 2 | 49 | > 62 | 2 | Y |
| | 3 | 49 | 49 | Dead | N |
| | 4 | 60 | > 62 | 2 | Y |
| | 5 | 56 | > 62 | 2 | Y |
| | 6 | 49 | 60 | Dead | Y |
| | 7 | 60 | 60 | Dead | N |
| | 8 | 56 | 60 | Dead | Y |
| | **Mean (± 1 SEM)** | 55.2 ± 2.2 *b* | 59.5 ± 1.4 *b* | - | - |

[a] Trees injected with propiconazole and inoculated with *Harringtonia lauricola*
[b] Trees not injected with propiconazole and inoculated with *Harringtonia lauricola*
[c] Trees not injected with propiconazole and inoculated with agar
[d] Trees injected with propiconazole and inoculated with agar

in late October 2019 with no symptoms; at 62 weeks post-inoculation, 2 such trees expressed minor symptoms (< 50% of the crown affected) possibly associated with LWD, and 2 trees appeared entirely healthy. Although no PZOL + HL trees expressed LWD symptoms in ≥ 50% of the crown within the first 16 weeks, 3 of 7 trees (43%) did express some LWD symptoms in 1 to 2 branches beginning within 6 to 10 weeks post-inoculation. However, these symptoms could possibly have been due to causes other than LWD. *Harringtonia lauricola* was recovered at the time of death from each of the 3 PZOL + HL trees which died prior to the end of the study and from all trees in the HL treatment group (Table 1).

We observed no evidence of leaf margin or interveinal discoloration or necrosis that might have been attributed to phytotoxic effects of propiconazole (Propizol®; Arborjet, Inc., Woburn, MA, USA), and PZOL + AG trees ($n$ = 8) did not differ significantly from AG ($n$ = 8) with respect to weeks to ≥ 50% crown decline or survival from trees inoculated with AG only (Table 1). Although all AG and PZOL + AG ($n$ = 8) survived at least 49 weeks post-inoculation, the majority (75%) of trees within the 2 treatments expressed laurel wilt symptoms in ≥ 50% of the crown or were dead by week 62 post-inoculation (Table 1), and *H. lauricola* was recovered from 81% of these trees (Table 1). These trees likely became infected with wild LWD, as the disease was progressing through the study area during the period.

Inhibition of *H. lauricola* growth was observed in only 1 of 7 (14%) sassafras in the PZOL + HL group in samples collected both 3 and 12 months post-*Harringtonia lauricola* inoculation (5 and 14 months post-propiconazole injection)(Table 2). No inhibition was observed among trees in the HL treatment group ($n$ = 9). However, inhibition was observed among 6 of 8 (75%) trees 3 months post-inoculation and in 6 of 7 (86%) of surviving propiconazole-injected and agar-inoculated trees, suggesting propiconazole distribution and retention was greater in those trees not inoculated with *H. lauricola*. This result is unexpected given that

**Table 2. The 2019–2020 QUIK-jet® Micro-Injection™ and 2020–2021 TREE I.V. Micro-Infusion™ system trials. The proportion of propiconazole-injected sassafras trees (and percent of total per treatment) exhibiting *Harringtonia lauricola* inhibition in wood-tissue discs obtained 30 cm above or below inoculation points 3, 12, and 15 months post-inoculation with *H. lauricola* using the Arborjet QUIK-jet® Micro-Injection™ and TREE I.V. Micro-Infusion™ delivery systems. Propiconazole (PZOL); *Harringtonia lauricola* (HL); agar (AG).**

| | | 3 months | | 12 months | | 15 months | |
|---|---|---|---|---|---|---|---|
| **Injection system** | **Treatment** | **Above** | **Below** | **Above** | **Below** | **Above** | **Below** |
| **QUIK-jet® Micro-Injection™ (2019–2020)** | PZOL + HL[a] | 1/7 (14%) | 1/7 (14%) | 1/7 (14%) | 0/7 (0%) | 3/4 (75%) | 3/4 (75%) |
| | PZOL + AG[b] | 5/8 (63%) | 6/8 (75%) | 1/7 (14%) | 5/7 (71%) | 1/1 (100%) | 1/1 (100%) |
| **TREE I.V. Micro-Infusion™ (2020–2021)** | PZOL + HL[a] | 5/13 (38%) | 6/13 (46%) | 10/13 (77%) | 8/13 (62%) | N/A | N/A |

[a] Trees injected with propiconazole and inoculated with *Harringtonia lauricola*
[b] Trees injected with propiconazole and inoculated with agar

4 of 7 (57%) PZOL + HL trees had no visible symptoms of LWD within the crown at the time of sampling, and these trees did not express symptoms for the 2 months remaining prior to leaf fall. The remaining 3 of 7 (43%) PZOL + HL trees expressed LWD-like symptoms in < 50% of crown, however these symptoms could have been unrelated to LWD. Stipes (2017) reported a preventive effect against Dutch elm disease symptoms in *Ulmus* sp. after propiconazole was no longer detected in samples, but that was based on a longer time post-injection (> 52 weeks). Stipes (2017) hypothesized that propiconazole injection may induce a phytoalexin-like response, lending disease resistance. One possible explanation of these results might be that while fungicide concentrations were high enough to maintain a degree of xylem function (and crown health), the production of tyloses and gums during the trees' response to *H. lauricola* inoculation (Inch and Ploetz 2011; Inch et al. 2012) slowed movement of the fungicide, resulting in lower concentrations of propiconazole at bioassay sampling points. Interestingly, samples collected from each of the 4 surviving PZOL + HL and single surviving propiconazole-injected and agar-inoculated tree at 15 months post-*Harringtonia lauricola* inoculation (17 months post-injection with propiconazole) all exhibited some degree of fungal inhibition, suggesting continued upward movement and perhaps increased concentration of propiconazole at bioassay sampling points.

## TREE I.V. Micro-Infusion™ Delivery System Analyses (2020–2021)

HL trees expressed moderate to severe symptoms (weeks to ≥ 50% crown mortality) significantly sooner than did PZOL + HL trees ($\chi^2 = 12.12$, $df = 1$, $P < 0.001$) and were significantly more likely to die within the first year of inoculation (within 63 weeks; $\chi^2 = 5.37$, $df = 1$, $P < 0.021$). Thirteen PZOL + HL trees survived the hurricanes, each of which survived at least sixteen weeks post-inoculation, until leaf fall in late October 2020 temporarily halted evaluations (although two trees did lose ≥ 50% of living crown due to LWD by this point)(Figure 2). At 63 weeks post-inoculation, 10 of 13 (77%) of PZOL + HL trees ($n = 13$) remained alive with < 50% crown loss. In contrast, 15 of 19 (79%) HL trees had either died or lost ≥ 50% of living crown by this time (Figure 3). Control trees inoculated with *H. lauricola* in the 2019–2020 trial rapidly declined and died. However, in the 2020–2021 trial we observed that while the terminal branches and fine twigs of multiple HL trees died soon after inoculation, epicormic shoots along larger branches were noted in the crown or along the main bole of multiple trees on subsequent evaluations, and 8 of 19 (42%) HL trees remained alive (though all symptomatic) at 63 weeks post-inoculation. The crowns of 4 of these surviving HL trees were entirely dead, and these trees were considered alive only due to epicormic branching along the main bole. Also, in contrast to

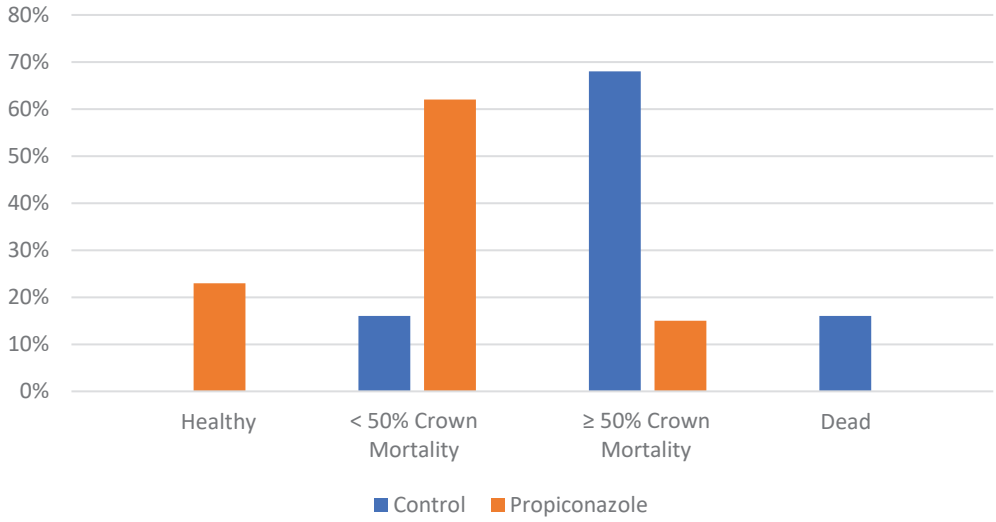


**Figure 2. Proportion of trees by treatment 16 weeks post-inoculation with *H. lauricola* (20 weeks post-injection with propiconazole) using the TREE I.V. Micro-Infusion™ delivery system.**

the 2019–2020 trial, it is worth noting that in the 2020–2021 trial, 61% of PZOL + HL trees expressed a possible phytotoxic response following injection. Soon after propiconazole injections, which preceded HL inoculation by only 1 month (as opposed to earlier PZOL injection and a longer interval prior to HL inoculation in the 2019–2020 trial) and occurred after budbreak, we observed browned margins and some interveinal discoloration among the crowns in 9 of the original 20 propiconazole-injected trees (45%). Trees recovered in the weeks following treatment, and we observed no correlation between trees affected and mortality. Phytotoxic response to propiconazole is reportedly a risk when administered for protection against Dutch elm disease using infusion methods in smaller-diameter elms early in the growing season (Haugen and Stennes 1999). The expression of such symptoms in our study were most apparent in smaller-diameter trees (< 20.3-cm DBH) but did occur to a lesser degree in trees as large as 30.5-cm DBH.

*Harringtonia lauricola* was reisolated from the stems in 16 of 19 (84%) of the HL trees and none of the PZOL + HL trees ($n = 13$) at 3 months post-inoculation with *H. lauricola* (4 months post-injection with propiconazole). No inhibition was observed in samples among the HL trees, nor in 6 of 13 (46%) PZOL + HL trees at 3 months post-inoculation (Table 2). Inhibition of *H. lauricola* growth was similar in proximity to inoculation, whether obtained below the inoculation point (46%) or above (38%). At 12 months post-inoculation, *H. lauricola* was reisolated from 13 of 14 (93%) HL trees. As reported in the 2019–2020 trial, bioassays conducted 12 months post-inoculation indicated improved retention of propiconazole (compared to samples collected within 16 weeks of inoculation), with inhibition observed in one or both sapwood samples taken from at least 10 of 13 (77%) PZOL + HL trees (Table 2). A slightly higher proportion of samples obtained above the inoculation point (77%) inhibited *H. lauricola* growth than samples collected below the inoculation point (62%).

## CONCLUSIONS

Our field inoculation trials are the first such efforts to indicate propiconazole (Propizol®; Arborjet, Inc., Woburn, MA, USA), applied using both the QUIK-jet® Micro-Injection™ and TREE I.V. Micro-Infusion™ delivery systems at a rate of 10 mL/in DBH, is an effective fungicidal product for preventing mortality and dieback in ≥ 50% of the crown of sassafras infected with LWD for the first 16 weeks following infection (where trees treated in spring persist until leaf fall of the same year), and in some cases may protect trees up to 1 year following treatments administered in spring to early summer. Mayfield et al. (2008a) reported injecting redbay trees with

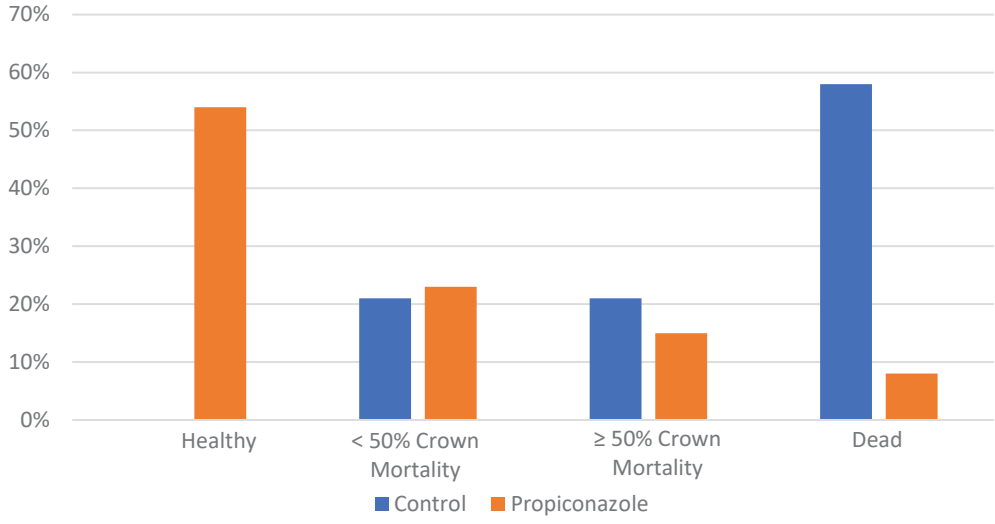


**Figure 3. Proportion of trees by treatment 63 weeks post-inoculation with *H. lauricola* (67 weeks post-injection with propiconazole) using the TREE I.V. Micro-Infusion™ delivery system.**

propiconazole (using Alamo® [Syngenta International AG, Basel, Switzerland], at the recommended high rate of 1.2-g active ingredient per cm DBH; a rate twice that recommended on the Propizol® label used in this evaluation for LWD prevention) prevented LWD symptoms in more than one-third of the crown for at least 30 weeks post-inoculation with *H. lauricola* but did not monitor treated trees beyond this point. Protection of *Quercus* sp. and *Ulmus* sp. with propiconazole may last 2 years or more against oak wilt (Wilson and Lester 1996; Ward et al. 2004; Eggers et al. 2005) and possibly up to 2 years against Dutch elm disease (Haugen and Stennes 1999; but see Ploetz et al. 2011). In each of our trials, results suggest propiconazole at the treatment rate of 0.61 g/cm DBH may prevent expression of disease symptoms in ≥ 50% of the crown beyond 16 weeks in some trees, but further study is necessary to determine when such results can be consistently expected. Our results suggest the use of micro-infusion techniques may provide more consistent results beyond this point. The higher rates of *H. lauricola* inhibition observed in bioassays and higher/longer survival times of PZOL + HL trees in the 2020–2021 TREE I.V. Micro-Infusion™ system trial may be due to better product translocation by trees using this treatment method. Micro-infusion of propiconazole in *Ulmus* sp. results in better translocation and distribution of propiconazole than that observed following micro-injection (Haugen and Stennes 1999; Stipes 2017). It is also likely this method resulted in less product lost to compromised xylem within the study trees used in the micro-infusion trial, since the treatment holes drilled for this method were not as deep as those used in trees treated using the QUIK-jet® Micro-Injection™ system. If this method is used and internal decay is suspected, we recommend care in placing product injection sites at the tree base or by increasing the number of treatment holes (but decreasing the depth) to avoid internal decay. Another advantage to delivering the required dose in a shallower hole is that product is more likely to be located in more physiologically active layers of the xylem. In examinations of sanded cross sections of PZOL + HL trees treated with the micro-injection system in 2019–2020, the smallest tree in this group included in the analyses (DBH 14.9 cm) was 46 years old (at injection plane 15 to 30 cm above ground) and had 16 growth rings in the outer 2.5 cm of xylem. It is likely that a lower rate of translocation of propiconazole in the deeper xylem coupled with relatively lower rates of growth and transpiration in such a tree would result in poor distribution of the fungicide within the tree.

Despite the encouraging results of field inoculation trials, the findings in both trials that bioassays of samples collected within the first 16 weeks of inoculation from PZOL + HL trees were less likely to inhibit *H. lauricola* growth than those collected more than 1 year later is difficult to explain. Mayfield et al. (2008a) reported very little change in retention within stem samples over time following propiconazole injections in redbay, with *H. lauricola* inhibition observed in 100% of samples ($n = 12$) collected at 4.5 months, and in 92% of samples collected at 7.5 months post-inoculation with *H. lauricola*. It is unknown whether our results were related to slowed movement of the fungicide due to the trees' response (creation of tyloses, etc.) to *H. lauricola*, an artifact of sampling intensity and location, or some other unknown factors.

These vagaries reinforce the guidance that preventive treatments should be applied in advance of the spring dispersal flights of redbay ambrosia beetles to allow for sufficient product distribution within trees, and trees should be retreated annually to ensure adequate protection in sassafras against LWD. Future research should be conducted on the value and potential for therapeutic treatment of LWD-infected sassafras, sampling intensity and methods for the purpose of product retention analyses, and on the duration of biologically active propiconazole concentrations in sassafras as it relates to tree protection.

## LITERATURE CITED

Appel DN, Kurdyla T. 1992. Intravascular injection with propiconazole in live oak for oak wilt control. *Plant Disease*. 76:1120-1124. https://doi.org/10.1094/PD-76-1120

Bates CA, Fraedrich SW, Harrington TC, Cameron RS, Menard RD, Best GS. 2013. First report of laurel wilt, caused by *Raffaelea lauricola*, on sassafras (*Sassafras albidum*) in Alabama. *Plant Disease*. 97(5):688. https://doi.org/10.1094/PDIS-09-12-0866-PDN

Blaedow RA. 2009. Use of the systemic fungicide propiconazole for oak wilt management: An assessment of uncharacterized host-pathogen-fungicide interactions [PhD dissertation]. Saint Paul (MN, USA): University of Minnesota. 131 p. https://hdl.handle.net/11299/50778

Brar GS, Capinera JL, Kendra PE, McLean S, Peña JE. 2013. Life cycle, development, and culture of *Xyleborus glabratus* (Coleoptera: Curculionidae: Scolytinae). *Florida Entomologist.* 96(3):1158-1167. https://doi.org/10.1653/024.096.0357

Cameron RS, Hanula J, Fraedrich SW, Bates C. 2015. Progression and impact of laurel wilt disease within redbay and sassafras

populations in southeast Georgia. *Southeast Naturalist.* 14(4): 650-674. https://doi.org/10.1656/058.014.0408

Cochran WG. 1954. Some methods for strengthening the common x2 tests. *Biometrics*. 10(4):417-451. https://doi.org/10.2307/3001616

de Beer ZW, Procter M, Wingfield MJ, Marincowitz S, Duong TA. 2022. Generic boundaries in the *Ophiostomatales* reconsidered and revised. *Studies in Mycology.* 101(1):57-120. https://doi.org/10.3114/sim.2022.101.02

Eggers J, Juzwik J, Bernick S, Morduant L. 2005. Evaluation of propiconazole operational treatments of oaks for oak wilt control. Saint Paul (MN, USA): USDA Forest Service, North Central Research Station. Research Note (RN) NC-390. 6 p. https://doi.org/10.2737/NC-RN-390

Forest Products Laboratory. 1999. Wood handbook: Wood as an engineering material. Madison (WI, USA): USDA Forest Service, Forest Products Laboratory. General technical report FPL; GTR-113. 463 p. https://doi.org/10.2737/FPL-GTR-113

Formby JP, Rodgers JC, Koch FH, Krishnan N, Duerr DA, Riggins JJ. 2017. Cold tolerance and invasive potential of the redbay ambrosia beetle (*Xyleborus glabratus*) in the eastern United States. *Biological Invasions*. 20:995-1007. https://doi.org/10.1007/s10530-017-1606-y

Fraedrich SW, Harrington TC, Rabaglia RJ, Ulyshen MD, Mayfield AE III, Hanula JL, Eickwort JM, Miller DR. 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(2):215-224. https://doi.org/10.1094/pdis-92-2-0215

Fraedrich SW, Johnson CW, Menard RD, Harrington TC, Olatinwo R, Best GS. 2015. First report of *Xyleborus glabratus* (Coleoptera: Curculionidae: Scolytinae) and laurel wilt in Louisiana, USA: The disease continues westward on sassafras. *Florida Entomologist*. 98(4):1266-1268. https://doi.org/10.1653/024.098.0445

Fraedrich SW, Harrington TC, McDaniel BA, Best GS. 2016. First report of laurel wilt, caused by *Raffaelea lauricola*, on spicebush (*Lindera benzoin*) in South Carolina. *Plant Disease.* 100(11):2330. https://doi.org/10.1094/PDIS-05-16-0674-PDN

Griggs MM. 1990. *Sassafras albidum* (Nutt.) Nees. In: Burns RM, Honkala BH, technical coordinators. *Silvics of North America: Volume 2, hardwoods*. Agriculture handbook 654. Washington (DC, USA): USDA Forest Service. p. 773-777. https://www.srs.fs.usda.gov/pubs/misc/ag_654_vol2.pdf

Grosman DM, Eskalen A, Brownie C. 2019. Evaluation of emamectin benzoate and propiconazole for management of a new invasive shot hole borer (*Euwallacea* nr. *fornicatus*, Coleoptera: Curculionidae) and symbiotic fungi in California sycamores. *Journal of Economic Entomology*. 112(3):1267-1273. https://doi.org/10.1093/jee/toy423

Harrington TC, Fraedrich SW, Aghayeva DN. 2008. *Raffaelea lauricola*, a new ambrosia beetle symbiont and pathogen on the *Lauraceae*. *Mycotaxon*. 104:399-404. https://www.srs.fs.usda.gov/pubs/ja/ja_harrington008.pdf

Harrington TC, Yun HY, Lu S-S, Goto H, Aghayeva DN, Fraedrich SW. 2011. Isolations from the redbay ambrosia beetle, *Xyleborus glabratus*, confirm that the laurel wilt pathogen, *Raffaelea lauricola*, originated in Asia. *Mycologia*. 103(5): 1028-1036. https://doi.org/10.3852/10-417

Haugen L, Stennes M. 1999. Fungicide injection to control Dutch elm disease: Understanding the options. *Plant Diagnosticians Quarterly*. 20(2):29-38.

Hughes M, Smith JA, Mayfield AE III, Minno MC, Shin K. 2011. First report of laurel wilt disease caused by *Raffaelea lauricola* on pondspice in Florida. *Plant Disease*. 95(12):1588. https://doi.org/10.1094/PDIS-06-11-0528

Hulcr J, Lou Q-Z. 2013. The redbay ambrosia beetle (Coleoptera: Curculionidae) prefers *Lauraceae* in its native range: Records from the Chinese National Insect Collection. *Florida Entomologist*. 96(4):1595-1596. https://doi.org/10.1653/024.096.0444

Immel DL. 2016. Plant guide: Sassafras: *Sassafras albidum* (Nutt.) Nees. Lincoln (NE, USA): USDA, NRCS National Plant Data Center. [Accessed 2022 June 2]. https://plants.usda.gov/DocumentLibrary/plantguide/pdf/cs_saal5.pdf

Inch SA, Ploetz RC. 2011. Impact of laurel wilt, caused by *Raffaelea lauricola*, on xylem function in avocado, *Persea americana*. *Forest Pathology*. 42(3):239-245. https://doi.org/10.1111/j.1439-0329.2011.00749.x

Inch S, Ploetz R, Held B, Blanchette R. 2012. Histological and anatomical responses in avocado, *Persea americana*, induced by the vascular wilt pathogen, *Raffaelea lauricola*. *Botany*. 90(7):627-635. https://doi.org/10.1139/b2012-015

Mayfield AE III, Barnard EL, Smith JA, Bernick SC, Eickwort JM, Dreaden TJ. 2008a. Effect of propiconazole on laurel wilt disease development in redbay trees and on the pathogen in vitro. *Arboriculture & Urban Forestry*. 34(5):317-324. https://doi.org/10.48044/jauf.2008.043

Mayfield AE III, Smith JA, Hughes M, Dreaden TJ. 2008b. First report of laurel wilt disease caused by a *Raffaelea* sp. on avocado in Florida. *Plant Disease*. 92(6):976. https://doi.org/10.1094/PDIS-92-6-0976A

Mayfield AE, Villari C, Hamilton JL, Slye J, Langston W, Oten K, Fraedrich SW. 2019. First report of laurel wilt disease caused by *Raffaelea lauricola* on sassafras in North Carolina. *Plant Disease*. 103(1):155. https://doi.org/10.1094/PDIS-05-18-0871-PDN

Olatinwo R, Hwang J, Johnson W, Fraedrich SW. 2019. First report of laurel wilt disease caused by *Raffaelea lauricola* on swamp bay in Louisiana. *Plant Disease*. 103(7):1777. https://doi.org/10.1094/PDIS-02-19-0284-PDN

Olatinwo R, Hwang J, Johnson W. 2021. First report of laurel wilt disease caused by *Raffaelea lauricola* on spicebush in Louisiana. *Plant Disease*. 105(8):2250. https://doi.org/10.1094/PDIS-11-20-2511-PDN

Ploetz RC, Pérez-Martínez JM, Evans EA, Inch SA. 2011. Toward fungicidal management of laurel wilt of avocado. *Plant Disease*. 95(8):977-982. https://doi.org/10.1094/pdis-08-10-0595

Ploetz RC, Hughes MA, Kendra PE, Fraedrich SW, Carrillo D, Stelinski LL, Hulcr J, Mayfield AE III, Dreaden T, Crane JH, Evans EA, Schaffer BA, Rollins J. 2017. Recovery plan for laurel wilt of avocado, caused by *Raffaelea lauricola*. *Plant Health Progress*. 18(2):51-77. https://doi.org/10.1094/PHP-12-16-0070-RP

Rabaglia RJ, Dole SA, Cognato AI. 2006. Review of American Xyleborina (Coleoptera: Curculionidae: Scolytinae) occurring north of Mexico, with an illustrated key. *Annals of the*

*Entomological Society of America*. 99(6):1034-1056. https://doi.org/10.1603/0013-8746(2006)99[1034:ROAXCC]2.0.CO;2

Randolph KC. 2017. Status of *Sassafras albidum* (Nutt.) Nees in the presence of laurel wilt disease and throughout the eastern United States. *Southeastern Naturalist*. 16(1):37-58. https://doi.org/10.1656/058.016.0104

Riggins JJ, Fraedrich SW, Harrington TC. 2011. First report of laurel wilt caused by *Raffaelea lauricola* on sassafras in Mississippi. *Plant Disease*. 95(11):1479. https://doi.org/10.1094/pdis-05-11-0446

Smith JA, Mount L, Mayfield AE III, Bates CA, Lamborn WA, Fraedrich SW. 2009. First report of laurel wilt disease caused by *Raffaelea lauricola* on camphor in Florida and Georgia. *Plant Disease*. 93(2):198. https://doi.org/10.1094/PDIS-93-2-0198B

Stipes RJ. 2017. Dutch elm disease: An overview of the biology and management regimens. In: Pinchot CC, Knight KS, Haugen LM, Flower CE, Slavicek JM, editors. *Proceedings of the American elm restoration workshop 2016*; 2016 October 25–27; Lewis Center, OH, USA. Newtown Square (PA, USA): USDA Forest Service, Northern Research Station. General technical report NRS-P-174. p. 21-23. https://www.fs.usda.gov/nrs/pubs/gtr/gtr-nrs-p-174papers/03stipes-gtr-p-174.pdf

Ward K, Juzwik J, Bernick S. 2004. Efficacy of Alamo for prophylactic and therapeutic treatment of oak wilt in red oaks, 2004. *Fungicide and Nematicide Tests*. 60:OT018. https://www.nrs.fs.usda.gov/pubs/jrnl/2004/nc_2004_ward_001.pdf

Wilson AD, Lester DG. 1996. Evaluation of propiconazole application methods for control of oak wilt in Texas live oaks. *Fungicide and Nematicide Tests*. 51:389. https://www.srs.fs.usda.gov/pubs/ja/ja_wilson007.pdf

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*C.W. Johnson (corresponding author)*
*Entomologist*
*US Forest Service*
*Forest Health Protection*
*Alexandria Forestry Center*
*2500 Shreveport HWY*
*Pineville, LA, USA*
*318-473-7170*
*wood.johnson@usda.gov*

*R.O. Olatinwo*
*Research Plant Pathologist*
*US Forest Service*
*Southern Research Station*

*J. Hwang*
*Plant Pathologist*
*US Forest Service*
*Forest Health Protection*

*C. Brownie*
*Professor Emerita*
*North Carolina State University*
*Department of Statistics*

**Conflicts of Interest:**
The authors reported no conflicts of interest.

**Résumé.** Le flétrissement du laurier est une maladie mortelle des lauracées américaines causée par *Harringtonia lauricola*. Le propiconazole est un fongicide systémique qui arrête la croissance du champignon chez une diversité de plantes hôtes. Le propiconazole utilisé en tant que traitement préventif contre le flétrissement du laurier chez le sassafras (*Sassafras albidum*) n'a pas été évalué. Nous avons traité les sassafras avec du propiconazole en utilisant les systèmes Arborjet QUIK-jet® Micro-Injection™ et TREE I.V. Micro-Infusion™ (Arborjet, Inc., Woburn, MA, USA) et nous avons éprouvé les arbres en les inoculant avec *H. lauricola*. Parmi les 7 arbres traités à l'aide du système QUIK-jet Micro- Injection, 6 (86%) ont survécu 52 semaines ou plus après l'inoculation avec *H. lauricola*, alors que seulement 11% des arbres témoins inoculés (1 sur 9) ont survécu pendant la même période. Tous les arbres non endommagés par les ouragans ($n$ = 13) traités avec du propiconazole en recourant au système TREE I.V. Micro-Infusion ont survécu significativement plus longtemps que les arbres témoins non traités après inoculation avec *H. lauricola*; 10 des 13 arbres (77%) ont survécu avec une perte de houppier inférieure à 50% tandis que 8 des 13 arbres (62%) étaient apparemment entièrement sains 54 semaines après l'inoculation. Dans l'essai du système TREE I.V. Micro-Infusion, 15 des 19 arbres témoins (79%) étaient soit morts ou avaient perdu 50% ou plus de leur houppier vivant 54 semaines après l'inoculation avec *H. lauricola*. Les résultats indiquent que les sassafras traités au propiconazole en utilisant les systèmes Arborjet QUIK-jet Micro-Injection et TREE I.V. Micro-Infusion sont significativement moins susceptibles de mourir dans l'année qui suit l'infection par *H. lauricola*; cependant, certains arbres peuvent présenter un déclin significatif de la couronne supérieur ou égal à 50% au cours de cette période.

**Zusammenfassung.** Die Lorbeerwelke ist eine tödliche Krankheit der amerikanischen Lauraceae, die durch *Harringtonia lauricola* verursacht wird. Propiconazol ist ein systemisches Fungizid, das das Pilzwachstum bei einer Vielzahl von Pflanzenwirten hemmt. Propiconazol als vorbeugende Behandlung gegen die Lorbeerwelke bei Sassafras (*Sassafras albidum*) wurde noch nicht untersucht. Wir haben Sassafrasbäume mit dem Arborjet QUIK-jet® Micro-Injection™ und TREE I.V. Micro-Infusion™ System (Arborjet, Inc., Woburn, MA, USA) mit Propiconazol

behandelt und die Bäume mit *H. lauricola* inokuliert. Von 7 Bäumen, die mit dem QUIK-jet Mikroinjektionssystem behandelt wurden, überlebten 6 (86%) 52 oder mehr Wochen nach der Inokulation mit *H. lauricola*, während nur 11% der geimpften Kontrollbäume (1 von 9) über diesen Zeitraum überlebten. Alle nicht durch Wirbelstürme geschädigten Bäume ($n$ = 13), die mit dem TREE I.V. Micro-Infusion System mit Propiconazol behandelt wurden, überlebten nach der Inokulation mit *H. lauricola* deutlich länger als unbehandelte Kontrollbäume; 10 von 13 Bäumen (77%) überlebten mit einem Kronenverlust von < 50%, und 8 von 13 Bäumen (62%) erschienen 54 Wochen nach der Inokulation völlig gesund. Im Versuch mit dem TREE I.V. Mikroinfusionssystem waren 15 von 19 Kontrollbäumen (79%) 54 Wochen nach der Inokulation mit *H. lauricola* entweder abgestorben oder hatten ≥ 50% der lebenden Krone verloren. Die Ergebnisse deuten darauf hin, dass Sassafrasbäume, die mit dem Arborjet QUIK-jet Mikroinjektionssystem und dem TREE I.V. Mikroinfusionssystem mit Propiconazol behandelt wurden, innerhalb eines Jahres nach der Infektion mit *H. lauricola* deutlich seltener absterben; bei einigen Bäumen kann es jedoch in diesem Zeitraum zu einem erheblichen Kronenrückgang (≥ 50%) kommen.

**Resumen.** El marchitamiento del laurel es una enfermedad letal de las Lauraceae americanas causada por *Harringtonia lauricola*. El propiconazol es un fungicida sistémico que detiene el crecimiento de hongos en una variedad de plantas huéspedes. No se ha evaluado el propiconazol como tratamiento preventivo contra el marchitamiento del laurel en sasafrás (*Sassafras albidum*). Tratamos los árboles de sasafrás con propiconazol utilizando Arborjet QUIK-jet® Micro-Injection™ y TREE I.V. Micro-Infusion™ Systems (Arborjet, Inc., Woburn, MA, USA) inoculando los árboles con *H. lauricola*. De los 7 árboles tratados con el sistema de microinyección QUIK-jet, 6 (86%) sobrevivieron 52 o más semanas después de la inoculación con *H. lauricola*, mientras que solo el 11% de los árboles de control inoculados (1 de 9) sobrevivieron durante este período. Todos los árboles no dañados por huracanes ($n$ = 13) tratados con propiconazol utilizando el sistema TREE I.V. Micro-Infusion sobrevivieron significativamente más tiempo que los árboles de control no tratados después de la inoculación con *H. lauricola*; 10 de 13 árboles (77%) sobrevivieron con < 50% de pérdida de copa, y 8 de 13 árboles (62%) parecían completamente sanos 54 semanas después de la inoculación. En el ensayo del sistema de microinfusión TREE I.V., 15 de los 19 árboles de control (79%) habían muerto o perdido ≥ el 50% de la corona viva 54 semanas después de la inoculación con *H. lauricola*. Los resultados indican que los árboles de sasafrás tratados con propiconazol utilizando los sistemas Arborjet QUIK-jet Micro-Injection y TREE I.V. Micro-Infusion tienen significativamente menos probabilidades de morir dentro de un año de la infección con *H. lauricola*; sin embargo, algunos árboles pueden exhibir una disminución significativa de la copa (≥ 50%) durante este período.

## *Arboriculture & Urban Forestry* Quiz Questions

To complete this quiz, go to the ISA website, log into your MyISA account, and make your way to the page for *Arboriculture & Urban Forestry* CEU Quizzes (www.isa-arbor.com/store/ceuquizzes/113).

Add the quiz to your cart, proceed through checkout, and look for the content to appear on your personal dashboard under the header, "My Quizzes." If you need a username and password, send us an e-mail (isa@isa-arbor.com).

A passing score for this quiz requires sixteen correct answers. Quiz results will display immediately upon quiz completion. CEU(s) are processed immediately. You may take the quiz as often as is necessary to pass.