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ORIGINAL PAPER

Cold tolerance and invasive potential of the redbay ambrosia beetle (*Xyleborus glabratus*) in the eastern United States

John P. Formby · John C. Rodgers III · Frank H. Koch · Natraj Krishnan · Donald A. Duerr · John J. Riggins

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Abstract Native Lauraceae (e.g. sassafras, redbay) in the southeastern USA are being severely impacted by laurel wilt disease, which is caused by the pathogen *Raffaelea lauricola* T. C. Harr., Fraedrich and Aghayeva, and its symbiotic vector, the redbay ambrosia beetle (*Xyleborus glabratus* Eichhoff). Cold temperatures are currently the only viable limitation to the establishment of *X. glabratus* in northern populations of sassafras. The observed lower lethal temperature of *X. glabratus* (− 10.0 °C) is warmer than its supercooling point (− 22.0 °C), indicating the beetle is a freeze intolerant and chill susceptible species. Empirically derived *X. glabratus* lower lethal temperature thresholds were combined with host distribution and microhabitat-corrected climate data to produce species distribution models for *X. glabratus* in the eastern USA. Macroclimate data (30-year mean annual minimum temperature) were corrected (− 1.2 °C) to account for thermal buffering afforded to *X. glabratus* while living inside sassafras trees. Only 0.1% of the current US sassafras spatial extent experiences sufficiently harsh winters (locales where mean annual minimum winter temperatures ≤ -6.2 °C for ≥ 12 h) to exclude *X. glabratus* establishment in our species distribution model. Minimum winter temperatures will likely cause some *X. glabratus* mortality in ∼ 52% of the current spatial extent of sassafras, although current data do not allow a quantification of *X. glabratus* mortality in this zone. Conversely, ∼ 48% of the current spatial extent of sassafras is unlikely to experience sufficiently cold winter temperatures to cause any significant impediment to *X. glabratus* spread or establishment. A modest climate change scenario (RCP4.5) of + 1.4 °C would result in 91% of the current spatial extent of sassafras in the eastern USA occurring where winter minimum temperatures are unlikely to cause any mortality to *X. glabratus*.

Keywords Cold tolerance · Distribution model · Forest invasion · Laurel wilt · Redbay ambrosia beetle · Sassafras

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Introduction

Introductions of non-native forest insects are increasing exponentially due to global trade (Liebhold et al. 1995; Haack 2006). These insects, and often the pathogens they carry, have caused or threaten the functional extinction of numerous tree species and some entire genera (e.g. *Castanea* and *Fraxinus* in North America from chestnut blight and emerald ash borer, respectively; Kuhlman 1978; Poland and McCullough 2006), and in the case of laurel wilt, threaten an entire family of plants in North America (Kendra et al. 2013). In the southern hemisphere, the Sirex woodwasp (*Sirex noctilio* Fabricius), which is native to Eurasia and northern Africa, has caused vast mortality and economic loss in pine plantations (Carnegie et al. 2005; Carnegie and Bashford 2012). These and other human-aided forest insect range expansions will continue to have immense worldwide effects on economies, ecosystem functions and services such as carbon cycles, carbon sequestration, erosion control, and water quality.

In light of these global forest health challenges, the ability to predictively model invasive forest pest distributions in introduced ranges continues to become more important. These predictive modeling tools (called species distribution models herein) are inherently based on our understanding of the mechanisms that allow insects and other organisms to tolerate and adapt to unfavorable climatic conditions, which is a central tenet of invasion biology (Lehmann et al. 2015). However, many species distribution models are built without directly investigating the ecophysiology of the invasive organism, and may trade biological relevance for speed. These “quick and dirty” models have an important place in early mitigation responses, but are often not very predictive of eventual pest distributions (Kearney et al. 2010; Formby et al. 2013).

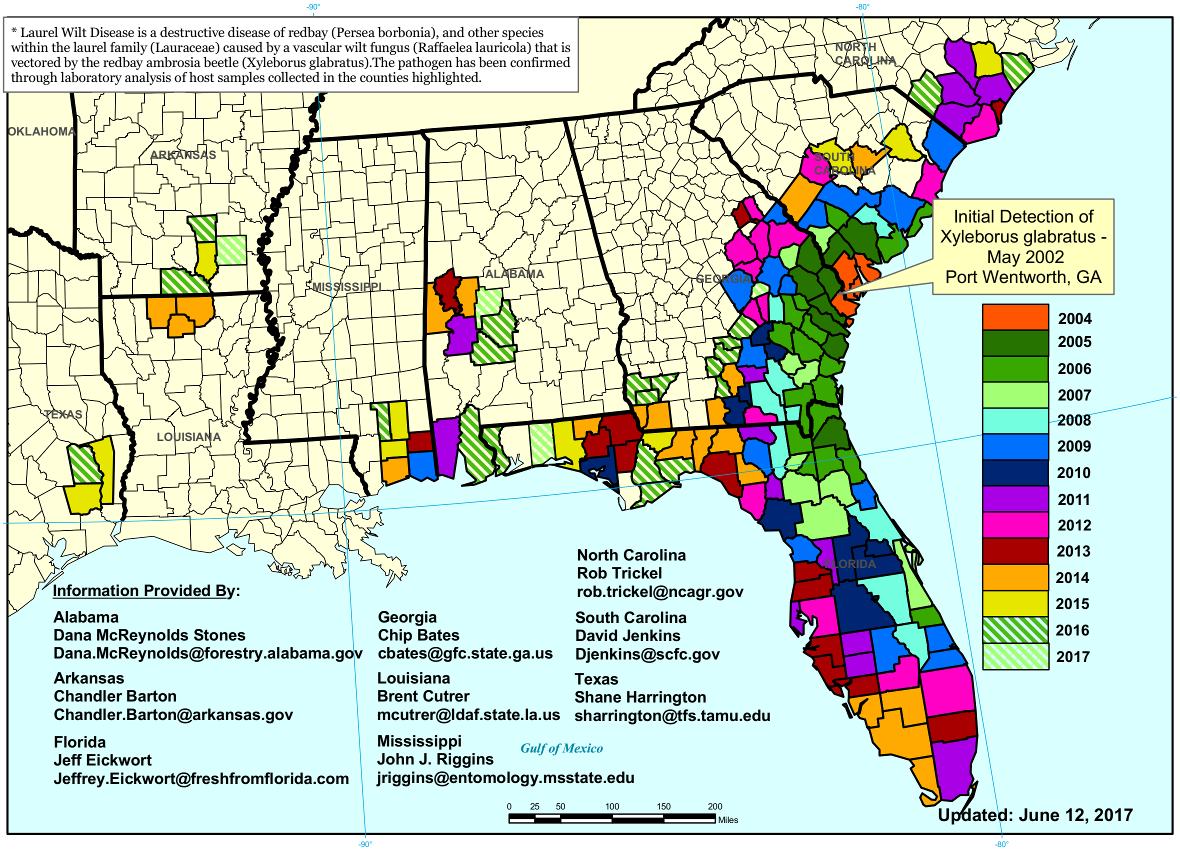
Laurel wilt, a devastating disease of North American plants within the Lauraceae, is one of the more recent non-native forest pest problems in the United States. Laurel wilt is caused by a fungal pathogen (*Raffaelea lauricola* T. C. Harr., Fraedrich and Aghayeva) carried and cultivated as food by *Xyleborus glabratus* Eichhoff (Coleoptera: Curculionidae), the redbay ambrosia beetle. *X. glabratus* is native to forests of southern Asia (Rabaglia et al. 2006), where it colonizes stressed or dying trees and is a biotic component in hardwood forest succession (Wood and Bright 1992). However, in the southeastern United States, the beetle colonizes apparently healthy trees and introduces the pathogen during tunnel and gallery excavation. The infection and symptoms rapidly spread throughout the host following inoculation, and usually result in tree mortality in as little as 4 weeks (Mayfield et al. 2008).

Laurel wilt has spread extensively throughout the southeastern United States (currently confirmed in nine states), and has reached epidemic levels (i.e. $>90\%$ mortality in redbay) in many areas following its establishment and subsequent spread from Port Wentworth, Georgia in $\sim$ 2002 (Kendra et al. 2013; Fig. 1). Laurel wilt is lethal to 11 species of North American trees and shrubs in the Lauraceae (Kendra et al. 2013), but so far has caused the greatest impact in natural redbay (*Persea borbonia* (L.) Spreng.) and swampbay (*Persea palustris* (Raf.) Sarg.) populations and agricultural avocado (*Persea americana* Mill.) groves in southern Florida. This insect–pathogen disease complex could cause the functional extinction of redbay and swampbay trees in the southeastern United States and also threatens to collapse the $\sim$ $21 million Florida avocado industry.

This invasion was initially considered to be a problem in coastal forests of the southeastern United States (Koch and Smith 2008). However, recently laurel wilt has successfully established and killed sassafras trees (*Sassafras albidum* (Nutt.) Nees) in Mississippi (Riggins et al. 2011), central Alabama (Bates et al. 2013), northern Louisiana (Fraedrich et al. 2015), and southern Arkansas (Olatinwo et al. 2016). The range of sassafras extends north into Canada and offers a potential host pathway for the spread of *X. glabratus* and *R. lauricola* into northern forests of the United States (Hughes et al. 2015).

Control options (e.g. insecticides, fungicides) have been unable to slow the spread of the beetle or the pathogen (Kendra et al. 2013); however, cold is a critical abiotic factor that determines the survival and distribution of most insect invaders (Alford et al. 2016). Therefore, low temperatures may be the only factor that can limit the expansion of *X. glabratus* in the USA. Formby et al. (2013) examined the supercooling point (point of internal water crystallization) of *X. glabratus* as a starting point for understanding the overwintering biology and the effect of low temperatures on the survival of *X. glabratus*.

Distribution of Counties with Laurel Wilt Disease* by year of Initial Detection



* Laurel Wilt Disease is a destructive disease of redbay (Persea borbonia), and other species within the laurel family (Lauraceae) caused by a vascular wilt fungus (Raffaelea lauricola) that is vectored by the redbay ambrosia beetle (Xyleborus glabratus).The pathogen has been confirmed through laboratory analysis of host samples collected in the counties highlighted.

Initial Detection of Xyleborus glabratus - May 2002 Port Wentworth, GA

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Fig. 1 County-level distribution of laurel wilt disease in the southeastern United States by initial year of detection (modified from USDA Forest Service distribution map, produced by Chip Bates). Laurel wilt confirmed counties in Arkansas, Louisiana, and central Alabama are areas where sassafras (*Sassafras albidum* (Nutt.) Nees) has been killed in the absence of redbay (*Persea borbonia* L.)

However, no other studies have investigated *X. glabratus* ecophysiology, or modeled its eventual distribution taking into account the effects of temperatures warmer than the supercooling point, or the thermal buffering provided by its woodboring microhabitat. Intermediate temperatures above the supercooling point can have dramatic sublethal or even lethal impacts on insects, depending on the cold tolerance of the insect species in question (Bale 1996). Integrating experimentally determined ecophysiology data for invasive species can increase the predictive power of species distribution models (Ungerer et al. 1999; Yoshio and Ishii 2001; Kearney and Porter 2009) and likely their biological relevance as well.

Our objectives were to (1) determine the physiological limits, mortality rates, and cold tolerance of *X. glabratus* in response to exposure to cold temperatures, (2) model the establishment potential of *X. glabratus* in the eastern United States based on historical North American climatological data, host distribution and density data, and the findings from objective 1, and (3) model the effects of climate change on the establishment potential of *X. glabratus* in the United States.

Methods

Summer acclimatized *Xyleborus glabratus*

Beetles were reared from 33 cm long bolts of redbay cut from laurel wilt-symptomatic trees on the Grandbay National Estuarine Research Reserve (NERR) in

Jackson County, Mississippi (N 30.4297° W − 88.4279°; 2 m a.s.l.). Trees were felled during the spring of 2012 and were promptly placed in 50 gallon rearing containers after being cut. The rearing containers were kept outside in a shaded area and checked daily. In August 2012, 340 fully sclerotized and apparently healthy adult female *X. glabratus* (> 30 days old; Bates et al. 2013) were collected from jars attached to the rearing containers and tested for lower lethal temperature.

Winter acclimatized *Xyleborus glabratus*

Adult female *X. glabratus* were extracted directly from laurel wilt-symptomatic redbay from 10 December to 14 December 2014. Ambient air temperatures over the course of the collection dates averaged 7.8 °C with a maximum and minimum of 15.5 and 0.6 °C, respectively. Laurel-wilt symptomatic redbay were individually felled, cut into 33 cm long bolts, and transported ~ 3 km to the field station at the Grand Bay NERR for beetle extraction. At the field station, each 33 cm long bolt was placed on a sheet of white cardboard (to help with the location/identification of the 2 mm long beetle) and split (~ halved) repeatedly with a hatchet along its length until it was reduced to small kindling. After each swing of the hatchet both the bolt and cardboard were examined for female *X. glabratus*. Beetles within galleries near the surface of the wood were removed without any apparent damage, usually after several hard taps on the end of the split bolt with a hammer. Beetles found during extraction were carefully examined under a 10× hand lens for damage caused by the extraction process. Only beetles that appeared undamaged were retained for laboratory testing. The extraction process was repeated until all the bolts from a single tree were reduced to kindling and examined for the presence of *X. glabratus*.

Once extracted, the beetles were taken immediately into the laboratory and allowed to acclimate to room temperature (~ 21 °C) for 10 min. The beetles were then re-examined under stereomicroscope to assess physical condition and mobility. Only beetles that appeared healthy and were able to crawl normally were retained for experimentation. In total, 82 fully sclerotized and apparently mature *X. glabratus* (> 30 days old; Bates et al. 2013) were collected in December 2014 and immediately used for lower lethal temperature studies. The entire process from tree felling to beetle testing took ~ 2 h and the beetles spent no more than 30 min exposed to ambient indoor temperatures.

Artificially acclimatized *Xyleborus glabratus*

On 14 July 2014, approximately 300 apparently healthy, adult female *X. glabratus* (> 30 days old; Bates et al. 2013) were collected from rearing containers (following the methods used to rear summer acclimatized beetles). The beetles were placed in a low temperature incubator (VWR International, Model 2015, Radnor, PA, USA) for 31 days with a thermo-photoperiod of 7 °C:2 °C (10:14 h L:D) to acclimate beetles to conditions that simulate late autumn near the northerly, inland limits of sassafras.

Initially, female *X. glabratus* were introduced into a 20 °C incubator. Immediately following introduction, the temperature was lowered to 7 °C at a rate of − 1.0 °C/day. When 7 °C was reached (day 14) the acclimatization thermoperiod was initiated. At 31 days (14 August 2012), 40 beetles were removed from the incubator and warmed to ambient temperatures (~ 21 °C) for 2 h. At the end of the 2 h each beetle was given a survival rating (dead, limited response, or highly responsive) based on a response to stimulus (i.e. a small, fine-tipped paintbrush). In total, 20 highly responsive and apparently healthy *X. glabratus* were artificially acclimatized and used for lower lethal temperature determination.

Determination of lower lethal temperature

The lower lethal temperature of *X. glabratus* was determined by subjecting the different treatments (summer, winter, and artificially acclimatized beetles) to a variety of temperatures (5, 0, − 5, and − 10 °C) and exposure times (6, 8, 10, and 24 h). However, not all treatment types were exposed to every combination of time and temperature regimes. Summer acclimatized beetles were tested at all exposure times and temperatures, naturally acclimatized beetles were exposed to 24 h of 0, − 5, and − 10 °C, and artificially acclimated beetles were subjected to 6 h of − 10 °C only (because − 10 °C was the temperature at which all the summer acclimatized beetles died).

Lower lethal temperatures of *X. glabratus* were tested in a low-temperature incubator. Beetles were introduced to the incubator set at an initial temperature matching the conditions of the cohort prior to testing. For example, beetles collected on 12 December 2012 were introduced to an incubator set at 8 °C (this was the ambient air temperature at the time of collection); whereas, artificially acclimated beetles were placed into a 7 °C incubator (the incubator temperature at time of collection). After the exposure time was reached, the beetles were removed from the incubator, warmed to room temperature (~ 21 °C) for 10 min, checked for survival, and given a survival rating (live, dead, or injured). Each beetle was then placed into a 1.5 ml microcentrifuge tube containing damp filter paper (to minimize desiccation) and held at room temperature for 24 h. Beetles were reexamined after 24 h and given another survival rating.

Microclimate correction

To examine the effect of thermal buffering on *X. glabratus,* a mature, living sassafras (25.0 cm DBH; *S. albidum* (Nutt.) Nees) tree in northwest Mississippi (Desoto County, Mississippi) was equipped with HOBO U23-001 Pro v2 data loggers (Onset Corporation) in late July 2013. The research site in northwest Mississippi averages extreme minimum temperatures between − 15.0 to − 12.2 °C (USDA Zone 7a) annually.

This particular sassafras was chosen because it was entirely sheltered within a forest stand. There were other mature sassafras trees in the area; however, most of them were more exposed, often growing along fence lines or in open fields. The topography of the site was mostly flat and the tree was sheltered on all sides from direct wind by codominant, intermediate, and overtopped/understory trees. The tree was surrounded by mature oaks, other mature broadleaf trees, and some mature and juvenile eastern red cedars (*Juniperus virginiana* L.); thus, the canopy around the tree was mostly closed to direct sunlight, even during winter months. This tree was specifically chosen with these characteristics to minimize the effects of wind and insolation on our measurements.

The sample size of one sassafras tree, or any experimental unit, is generally considered inadequate for scientific inquiry, because the lack of repeated observations makes it impossible to estimate values with a definable level of accuracy. However, there are several confounding issues of scale that make a larger sample size irrelevant in this case, as they undercut the practicality of an accurate, yet generalizable, model of the thermal buffering capacity of sassafras trees. The first of these issues relates to the regional scale of the problem. Our goal is to characterize the thermal buffering capacity of sassafras across the entire range of the species in North America (i.e. for billions of sassafras trees). It would matter little if we sampled one tree, three trees, or 1000 trees, the variability in any subsequent estimate would still be extremely large, and the costs of additional sampling would far outweigh the gains in terms of a more certain estimate.

Even if enough individual trees could be sampled to build a regional-scale model, there are various other problematic details. For instance, aspect, insolation, and shade are important, albeit transient, factors that can dramatically change the temperature inside trees relative to ambient air temperatures, but because of their transience, they are essentially impossible to model or predict. Tree diameter and water content, two of the primary factors controlling thermal conductivity in living trees, are similarly dynamic, but more to the point, no data exist that spatially reference either factor at the regional scale. Therefore, sampling hundreds or thousands of trees to build a regional-scale model of thermal buffering would be futile since there are really no data to which it could applied. Lastly, there is no feasible way to account for how far the ambrosia beetles will bore into any given tree.

We acknowledge that our estimate of the thermal buffering capacity is coarse, but maintain that including this estimate in the hazard model is more logical than leaving it out. Clearly, thermal buffering affects beetle survival and mortality in some fashion. To limit the impact of the uncertainties of scale, we defined the hazard model with three broad beetle mortality classes (no mortality, some mortality, high mortality), thereby downplaying any differences between our estimate and the actual buffering capacities of individual sassafras trees. Still, it is worth noting that a − 1.2 °C correction is consistent with the limited literature available from other tree species (e.g. Bolstad et al. 1997; Tran et al. 2007; Vermunt et al. 2012).

Internal tree temperatures were recorded at depths of 5.1 and 12.7 cm (12.7 cm was approximately in the center of the tree). Ambient temperature data were recorded by a data logger mounted to a wooden stake 920 cm from the tree and 1 m from the ground (to minimize any radiant heat effects). Each data logger was connected to a thermocouple via a 1.8 m data cable. All three data loggers were attached to a mounting board (5.1 cm × 10.2 cm × 1.4 m) that was driven into the ground ~ 1.0 m away from the tree. Holes were drilled into the south side of the tree at 1.5 m above the base. Thermocouples were inserted into these openings and silicone caulk was placed around the data cable at the entrance of the hole to minimize temperature loss/gain and protect the thermocouples.

Data collection began August 2013 and continued for 1 year; however, only winter (December–February) data were used for analyses. Temperatures were recorded at 1 h intervals for the entire sampling period. The data on each logger were transferred to a computer via a HOBO® Waterproof Shuttle (Onset Corp., U-DTW-1) at the end of the sampling period.

Statistical analyses

All data were analyzed using JMP 12 statistical software. A simple linear regression was used to determine the effect of temperature on mortality and logit regressions determined the effect of time on mortality at each predetermined temperature. The temperature that caused mortality in all specimens tested was recorded as the lower lethal temperature (i.e. LLT_{100}). The mean lower lethal temperature data of each treatment were compared using the nonparametric Mann–Whitney *U* test. The microclimate correction data were analyzed with simple linear regressions to determine the relationship, if any, between the within-wood temperatures and winter air temperatures. The winter air, outer wood, and inner wood temperature data also were compared using the nonparametric Mann–Whitney *U* test.

Species distribution modeling

The establishment potential models for *X. glabratus* were constructed using climate (historical and future) and host distribution data. For historical climate data, 30-year (interpolations of observed data, representative of 1961–1990) mean annual minimum temperature data (Bioclimatic Variable 6, WorldClim Version 1) were obtained in raster form (1 km/30 arc-second resolution) and transformed to USA Contiguous Albers Equal Area Conic projected coordinate system (941.7 m spatial resolution). Next, raster climate data were reclassified in GIS into three new classes (representing three distinct ecophysiological regions) based on the lower lethal temperature results from this study, and further corrected by subtracting 1.2 °C from reclassification thresholds to account for thermal buffering afforded by within-tree microclimate, also reported herein. The microclimate-corrected reclassification thresholds representing *X. glabratus* invasion potential under current (historic) climatic conditions were: ≥ -6.2 °C (no winter mortality); -6.19 to -11.2 °C (some winter mortality); and < -11.2 °C (high winter mortality).

The future climate change scenario assumed a Representative Concentration Pathway of + 4.5 W/m^2 (RCP4.5, downscaled global climate model data from CMIP5, IPPC Fifth Assessment; Hijmans et al. 2005). RCP4.5 predicts mean global warming of 1.4 °C (likely range 0.9–2.0 °C). Predicted bioclimatic variable 6 (mean winter minimum temperature) for the year 2050 (predicted average for 2041–2060) was obtained from www.worldclim.org, imported into the GIS, and classified using the same methodology as described above.

Host distribution data (i.e. sassafras basal area) were downloaded in raster format (1 km resolution) from the USDA Forest Service Forest Health Technology and Enterprise Team ArcGIS server. Redbay, California bay laurel (*Umbellularia californica* (Hook. and Arn.) Nutt.), pondspice (*Litsea aestivalis* (L.) Fernald), silkbay (*Persea humilis* Nash), and avocado were excluded from model analyses because they grow in regions where mean minimum winter temperatures are too warm to limit establishment of *X. glabratus* (based on the results from this study). Northern spicebush (*Lindera benzoin* (L.) Blume) was excluded because there were no reliable raster datasets of its distribution. Land area (hectares) of sassafras forest occurring in each ecophysiological region was calculated via pixel counts in the raster attribute tables.

All sassafras trees, even populations of sassafras at low densities, are under high hazard because of their high susceptibility to *R. lauricola* and the ability of *X. glabratus* to locate them (Fraedrich et al. 2015). However, sassafras size and stand basal area do seem to influence the speed at which the invasion progresses (Cameron et al. 2015); therefore, some stands (stands with lower sassafras basal area or smaller trees) may take longer to die, and thus, the invasion may proceed more slowly in those forests than in others with high sassafras basal area and/or larger trees. As a result, we do not think a more complex model of invasion potential (e.g. a hazard model) that includes both sassafras population density (i.e. trees per hectare) and ecophysiological data would be very accurate or informative, primarily because a very high percentage of sassafras will likely eventually experience mortality, regardless of the population density of sassafras.

Results

Lower lethal temperatures

The physiological status (dead, injured, or uninjured) of all beetles from each treatment was recorded 10 min and 24 h after testing. The percentages of mortality and injury recorded in each treatment type (summer, winter, and artificially acclimatized) are summarized in Table 1. In summer acclimatized beetles, there was significantly more injury at − 5 °C than any other temperature ($\chi^2 = 15.66$, $P = 0.013$), including − 10 °C (which had more mortality than injury). In winter acclimatized beetles, the number of beetles injured was not significantly different among the temperatures ($\chi^2 = 1.06$, $P = 0.59$), and in artificially acclimatized *X. glabratus*, 85% of the beetles tested were injured 10 min after the experiment (the other 15% were dead). All beetles categorized as injured at 10 min were dead 24 h after the experiment, regardless of the time or temperature exposure.

In summer acclimatized *X. glabratus*, mortality decreased significantly as temperatures increased ($R^2 = 0.53$, $df = 339$; $P < 0.0001$). Likewise, in winter acclimatized beetles, mortality decreased significantly as temperatures increased ($R^2 = 0.63$, $df = 81$; $P < 0.0001$). Control treatments had significantly lower mortality than the other treatment types ($R^2 = 0.17$, $df = 3$, $P = 0.05$) and − 10.0 °C was the only temperature that had significantly higher mortality across all exposure times compared to the controls ($R^2 = 0.12$, $df = 3$, $P = 0.007$). Across all treatment types, there was a significant effect from an exposure time × temperature interaction on survival ($R^2 = 0.58$, $df = 441$, $P < 0.0001$).

Microclimate correction

Mean temperatures in both the outer wood ($R^2 = 0.80$, $df = 1775$, $P = 0.0003$) and inner wood ($R^2 = 0.89$, $df = 1775$, $P < 0.0001$) were significantly different than ambient air temperatures (Fig. 2). Additionally, inner wood temperatures lagged behind ambient air temperatures about 12 h on average. The average thermal buffering between winter air temperatures and temperatures in the outer and inner wood during the winter was 0.3 °C ± 0.12 and 1.2 °C ± 0.13 (mean ± SE), respectively. The average thermal buffering effect of the inner wood microclimate (1.2 °C) was used to correct climate datasets used in the invasion potential model.

Table 1 Percent of *Xyleborus glabratus* in each treatment killed or injured when exposed to cold temperatures (results recorded 10 min and 24 h after testing)

	Summer acclimatized			Winter acclimatized			Artificially acclimatized		
	Temp (°C)	Mortality (%)	Injured (%)	Temp (°C)	Mortality (%)	Injured (%)	Temp (°C)	Mortality (%)	Injured (%)
10 min	5	3	0	0	0	6	− 10	15	85
	0	2	1	− 5	77	10			
	− 5	6	10	− 10	85	15			
	− 10	98	2						
24 h	5	3	0	0	6	0	− 10	100	0
	0	4	0	− 5	87	0			
	− 5	17	0	− 10	100	0			
	− 10	100	0						

Xyleborus glabratus species distribution models

Figure 3 illustrates the current distribution and basal area (i.e. the cross-sectional square footage of stems per hectare 1.37 m from the ground) of sassafras in the eastern United States, and the establishment potential of *X. glabratus* in North American forest types containing sassafras, under current climate (Fig. 3a) and a projected climate change scenario (Fig. 3b). In this model, we assume that most sassafras is highly susceptible to laurel wilt and will eventually succumb unless cold winter temperatures limit the range expansion of *X. glabratus*. In current climatic conditions, our model predicted that beetle mortality from cold winter temperatures (< − 11.2 °C, reported herein) at the northernmost extent of sassafras in the United States will protect less than 1% of sassafras trees from invasion by *X. glabratus*.

However, approximately 52% of sassafras forest area and 56% of sassafras basal area will be protected by some *X. glabratus* winter mortality (Fig. 4). This ecophysiological region (some winter mortality) is based on results from laboratory cold tolerance tests (reported herein), which indicated that *X. glabratus* begins to experience cold injury at microclimate-corrected temperatures in the range of − 6.19 to − 11.2 °C. We cannot quantify how much mortality *X. glabratus* will incur in this ecophysiological region, but our lab experiments showed as much as 87% *X. glabratus* mortality at − 5 °C; even with thermal buffering provided by host trees, substantial mortality could occur after prolonged exposure to moderately cold temperatures. This may help slow the spread of *X. glabratus*, but because female beetles are capable of reproducing asexually and killing a healthy host tree on their own by inoculating it with *R. lauricola*, it is unlikely that this will offer lasting protection for sassafras trees in this ecophysiological region.

Fig. 3 Invasion potential of *Xyleborus glabratus*, the redbay ▶ ambrosia beetle, in North American forests containing sassafras (*Sassafras albidum* L.) under current (**a** mean annual minimum temperature data 1961–1990) and predicted (**b**; + 1.4 °C by 2050) climate scenarios. This model assumes that most sassafras are highly susceptible to laurel wilt regardless of population density and will eventually succumb to laurel wilt unless cold winter temperatures limit the eventual distribution of *X. glabratus*

Approximately 48% of sassafras land area and 44% of sassafras basal area occur in the most southern ecophysiological region (no winter mortality) of our model. Based on our laboratory experiments, the microclimate-corrected mean winter minimum temperatures in this region (≥ − 6.2 °C) are likely too warm to cause any appreciable winter *X. glabratus* mortality, and should not hamper laurel wilt spread and disease progression in this region. Redbay and

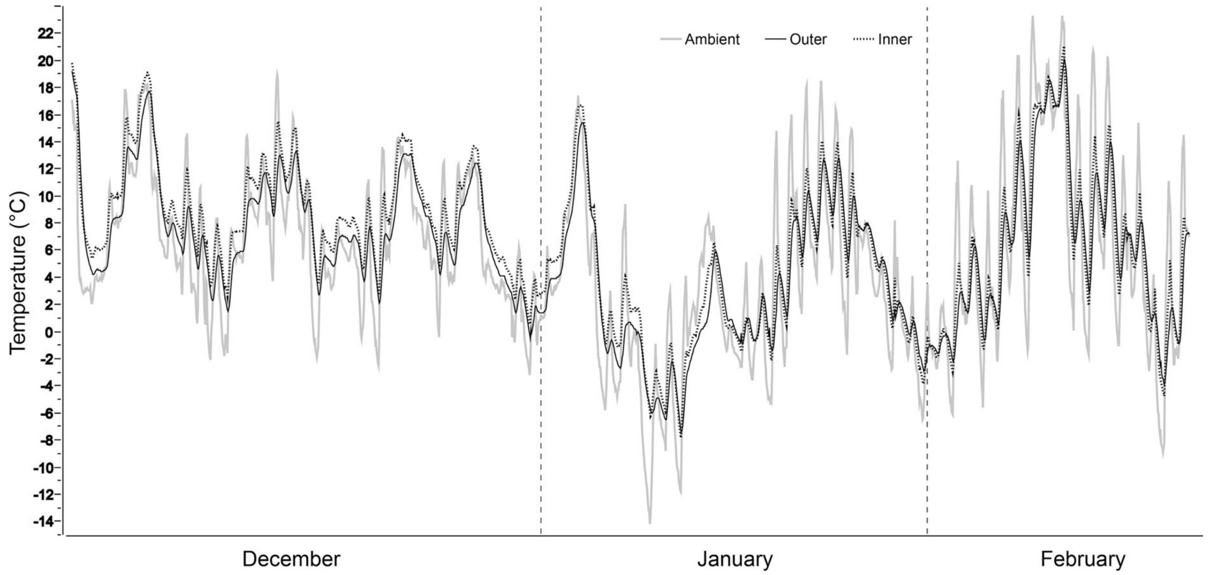
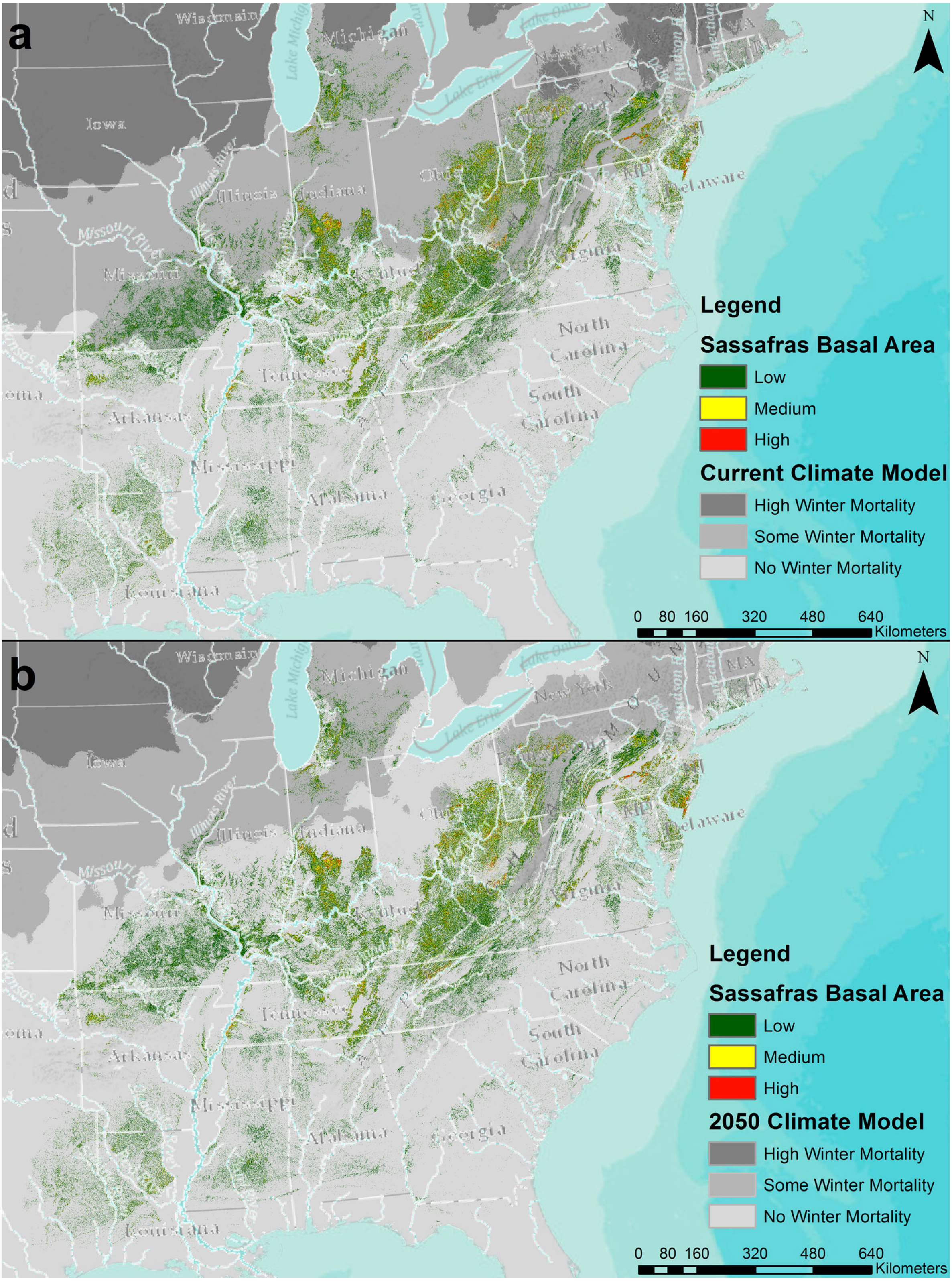


Fig. 2 Ambient air, inner (depth of 13 cm), and outer wood (depth of 5 cm) temperatures (°C) of a 25.0 cm DBH sassafras located in Desoto County, Mississippi from 1 December 2014 to 13 February 2015


a
N
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Michigan
Lake Michigan
Lake Erie
Lake Ontario
New York
Iowa
Illinois River
Illinois
Indiana
Ohio
Missouri River
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Virginia
Delaware
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South Carolina
Arkansas
Mississippi
Alabama
Georgia
Louisiana
Legend
Sassafras Basal Area
Low
Medium
High
Current Climate Model
High Winter Mortality
Some Winter Mortality
No Winter Mortality
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Kilometers
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Wisconsin
Michigan
Lake Michigan
Lake Erie
Lake Ontario
New York
MA
Iowa
Illinois River
Illinois
Indiana
Ohio
Missouri River
Missouri
Kentucky
Virginia
Delaware
MD
North Carolina
Tennessee
South Carolina
Arkansas
Mississippi
Alabama
Georgia
Louisiana
Legend
Sassafras Basal Area
Low
Medium
High
2050 Climate Model
High Winter Mortality
Some Winter Mortality
No Winter Mortality
0 80 160 320 480 640
Kilometers

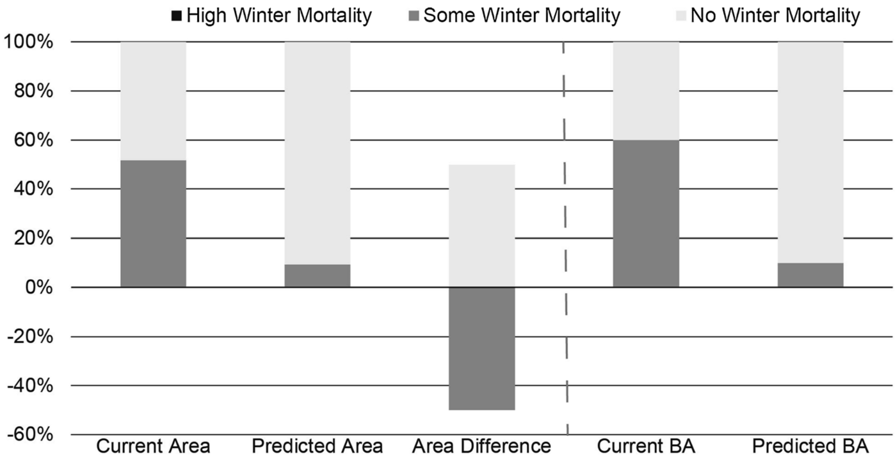

Fig. 4 Percent of US land area (hectares, rounded to nearest 1%) and *Sassafras albidum* basal area (BA; rounded to nearest 10%) reporting mortality in three ecophysiological regions (high, some, and no winter mortality) derived from *Xyleborus glabratus* cold tolerance data. Areas of high *X. glabratus* mortality were less than 1% of current total land and basal area; therefore, these percentages were so small they do not appear in the graph. Area difference represents the difference between the current and projected land area reporting *X. glabratus* mortality (i.e. approximately 40% of the land area currently protected somewhat by cold temperatures will not be protected at all after a 1.4 °C increase in mean minimum winter temperatures)

swampbay occur only along the southern Gulf and Atlantic Coasts and by extension should not benefit from any winter mortality of *X. glabratus*.

However, if global climate warms by as little as 1.4 °C on average by 2050, none of the current spatial extent of sassafras would occur in the coldest of the three ecophysiological regions in our model (Figs. 3b, 4). Even though the downscaled RCP4.5 scenario data have a 1 °C level of uncertainty per pixel, the invasion potential suggested by the model still indicates that the vast majority of sassafras throughout the United States will be threatened by *X. glabratus*. If the climate change projection holds true, by 2050, ~ 91% of the current sassafras spatial extent and ~ 90% of current sassafras basal area would fall into the southernmost and warmest ecophysiological region in our model, where no appreciable *X. glabratus* mortality should occur from projected minimum winter temperatures.

Discussion

Comparing the lower lethal temperature results reported herein with the supercooling point results reported by Formby et al. (2013) it can be concluded that *X. glabratus* is chill susceptible, because the supercooling point (− 22.0 °C) of *X. glabratus* is below its lower lethal temperature (− 10 °C). Chill susceptibility (Bale 1993) is one of four subclasses within the freeze-intolerant category insects; the other subclasses being opportunistic, mild chill tolerance, and high chill tolerance; Sinclair 1999). Knowing this is important because winter mortality in chill susceptible insects is unrelated to the supercooling point and death occurs during brief exposures (minutes to hours) from − 5 to − 15 °C (Bale 1996).

In summer acclimatized *X. glabratus*, − 5 °C caused substantial chill injury in as little as 10 h after exposure (Table 1). Chill injury results from brief exposures to cold, which occurs in the absence of extracellular ice formation (Chen et al. 1987; Chown and Nicolson 2004). Both winter acclimatized and summer acclimatized beetles experienced similar chill injury at − 5 °C. Additionally, the microclimate inside sassafras required ~ 12 h to match air temperatures, and was on average 1.2 °C warmer than air temperatures. This indicates locales where minimum winter temperatures are − 6.2 °C or colder for 12 h will likely begin limiting (via chill injury) *X. glabratus* spread. Chill injury can lead to death by inducing fluid-to-gel phase transitions in cell membranes (which can separate protein and lipid membranes), changing membrane permeability, and deactivating membrane bound enzymes (Chown and Nicolson 2004). Furthermore, chill injury can affect neurons and negatively impact neurological transmissions, inhibit enzyme activity, and change protein structure and denaturation (Hosler et al. 2000; Ramlov and Lee 2000; Košťál et al. 2004, 2006, 2007), all of which can cause irreparable harm to the insect.

It is important to consider the thermal buffering effects of living inside trees when modeling the invasion potential of woodboring insects. Wood provides protection from ambient air temperatures and extreme weather events (Wood 1982; Bolstad et al. 1997; Poland and McCullough 2006; Tran et al. 2007), enabling woodborers to persist in more extreme environmental conditions than their cold-temperature physiology would otherwise allow. Derby and Gates (1966) reported that temperatures within the heartwood of large trees can take several hours to days to respond to ambient air temperature changes. However, buffering capacity of the wood is also dependent on several other factors (e.g. amount of sun exposure, tree species and diameter, wood and tissue moisture, depth of gallery/brood chamber, location of the tree; Derby and Gates 1966; Bolstad et al. 1997; Vermunt et al. 2012) and, in higher latitudes or elevations, there may be an additional buffering effect from snowpack (e.g. Pruitt 1957).

The reproductive biology of *X. glabratus* is also an important consideration. The lethal temperature that kills half of a population (LT_{50}) is enough to limit the spread of most insect species, and is commonly used to model distribution potential (Ungerer et al. 1999; Bale 2002; Andersen et al. 2015). However, *X. glabratus* females are capable of producing and mating with male offspring from unfertilized eggs. Therefore, many of the Allee effects associated with invading organisms are reduced or removed, making it all the more likely that a single *X. glabratus* that survived a cold snap could restart reproduction, population growth, and range expansion the following spring. Because of this reproductive strategy, the limiting effects of chill injury at intermediate temperatures (warmer than the SC and LLT) such as the LT_{50} are uncertain.

Our results suggest that a previous climate match (based on data from the insect's native range) *X. glabratus* spread models could have underestimated its potential distribution in the USA (Koch and Smith 2008), while supercooling point alone as an ecophysiological basis for establishment potential (as in Formby et al. 2013) likely overestimated eventual distributions. This is likely because many insects, especially chill susceptible insects, begin to experience chill injury and/or mortality at temperatures warmer than their supercooling point (Bale 1996).

Our methods combine (1) experimentally quantified invader ecophysiological constraints, (2) host distribution, and (3) microhabitat-corrected climate datasets to produce mechanistic species distribution models. A mechanistic approach may be capable of predicting invasive species ranges and impacts with greater biological relevance than correlative models (Kearney et al. 2010). This is because mechanistic species distribution models inherently account for physiological constraints that limit invader distributions (Kearney and Porter 2009). However, the success of physiologically based species distribution models is dependent on correctly recognizing key limiting factors (Kearney et al. 2010). The vector-pathogen complex responsible for laurel wilt continues to rapidly invade the southeastern United States, and we assume few other limiting factors like natural enemies or host resistance will limit eventual laurel wilt distribution. However, the recent discovery of lateral transfer of *R. lauricola* to other native and non-native ambrosia beetle vectors (Carrillo et al. 2014), each with their own unknown physiology, adds further biological complexity that our invasion model does not attempt to capture.

The invasion of North America by *X. glabratus* and *R. lauricola* has proven to be another in a series of forest mortality events caused by human-aided transport of non-native insects and pathogens. In this case, the entire laurel family (Lauraceae) in North America is at risk. Because the invading organisms were not pestiferous in their native range, an early correlative invasion model lacked sufficient information regarding key ecophysiological variables, such as *X. glabratus* North American host preference and cold weather survival. At current global temperatures, our model predicts that less than 1% of sassafras trees occur in locales where mean winter minimum temperatures reach the threshold necessary to cause 100% mortality of *X. glabratus* within the buffered microclimate of host trees. Furthermore, with as little as 1.4 °C rise in global temperatures, the number of sassafras trees potentially protected by cold temperatures falls to zero. Our ongoing research is focused on documenting and describing the myriad ecological effects resulting from this devastating invasion.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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