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Methods to Evaluate Host Tree Suitability to the Asian Longhorned Beetle, *Anoplophora glabripennis*¹

Scott W. Ludwig², Laura Lazarus³, Deborah G. McCullough³, Kelli Hoover⁴, Silvia Montero, and James C. Sellmer⁵ Department of Horticulture, The Pennsylvania State University, University Park, PA 16802

– Abstract –

Two procedures were evaluated for assessing tree susceptibility to *Anoplophora glabripennis*. In the first procedure, adult beetles were caged with a section of sugar maple, northern red oak, white oak, honeylocust, eastern cottonwood, sycamore or tulip poplar wood. Results showed that females laid viable eggs on sugar maple, red oak, white oak and honeylocust. Oviposition did not occur on cottonwood, sycamore, or tulip poplar. Eighty-seven percent of the first instar larvae survived in white oak, followed by sugar maple (82%), honeylocust (50%), and red oak (39%). In the second procedure, first instar larvae were manually inserted into potted sugar maple, green ash, and red oak trees and allowed to feed for 60 or 90 days. Significantly more larvae survived for 90 days within the red oak (67%) compared to green ash (17%). Larvae recovered from red oak weighed significantly more than larvae from sugar maple or green ash. Larval survival was positively related to height of insertion. These results indicate: 1) controlled laboratory and greenhouse-based procedures can be used to assess tree suitability to *A. glabripennis* and 2) *A. glabripennis* will oviposit and larvae can develop in northern red oak for up to 90 days, suggesting that this species may be a potential host.

Index words: host plant resistance, invasive pest, Populus deltoides, Fraxinus pennsylvanica, Gleditsia triacanthos, Quercus rubra, Acer saccharum, Platanus occidentalis, Liriodendron tulipifera, Quercus alba.

Species used in this study: eastern cottonwood (*Populus deltoides* Marsh.); green ash (*Fraxinus pennsylvanica* Marsh.); honeylocust (*Gleditsia triacanthos* L.); northern red oak (*Quercus rubra* L.); sugar maple (*Acer saccharum* Marsh.); striped maple (*Acer pensylvanicum* L.); sycamore (*Platanus occidentalis* L.); tulip poplar (*Liriodendron tulipifera* L.); white oak (*Quercus alba* L.).

Significance to the Nursery Industry

The Asian longhorned beetle, *Anoplophora glabripennis*, was inadvertently introduced into the United States from China, probably on infested wooden packing material on cargo ships. Populations of this wood-boring beetle were discovered in 1996 in New York and in 1998 in Chicago; more than 8000 street trees have been destroyed so far in New York and Chicago. This exotic insect pest has the potential to impact every aspect of the landscape and nursery industry, from production scheduling and product mix through landscape plant selection for new and infested sites to maintenance and pest management strategies. Currently, infested trees must be destroyed and control options are limited, costly and only somewhat effective. Little information is available

¹Received for publication February 25, 2002; in revised form April 22, 2002. Journal paper 417 of the Pennsylvania State University, Dept. of Horticulture. This research was supported in part by a grant from **The Horticulture Research Institute**, **1000 Vermont Avenue**, **NW**, **Suite 300**, **Washington**, **DC 20005**, the Pennsylvania Department of Agriculture, the International Society of Arboriculture, the National Biocontrol Institute of USDA, and WPWR-TV Channel 50 Foundation in Chicago, and cooperative agreements with the USDA-APHIS-PPQ, USDA-FS North Central Research Station and McIntire-Stennis Cooperative Forestry Research Program. We thank J. Frank Schmidt and Son Co. for the donation of trees, Nancy Ostiguy for assistance with data analysis, Melody Keena, Bailey Klinestiver, Heather Read, Laura Ludwig, Tracey Harpster and Christina Mullen for technical support, and Greg Kowalewski and staff at the W.K. Kellogg Forest. We extend special thanks to Larry Kuhns and Robert Berghage for reviewing an earlier draft of the manuscript.

²Texas A&M University Agricultural Research and Extension Center at Overton, P.O. Box 38, Overton, TX 75684.

³Department of Entomology, 243 Natural Science Building, Michigan State University, East Lansing, MI 48824.

⁴Department of Entomology, 501 Agricultural and Industries Building, The Pennsylvania State University, University Park, PA 16802.

⁵To whom all correspondence should be sent: Department of Horticulture, The Pennsylvania State University. University Park, PA 16802. on the potential host range of this beetle in North America. This paper represents a preliminary step in experimentally defining the susceptibility of commonly grown and planted urban trees to the Asian longhorned beetle.

Introduction

Anoplophora glabripennis (Motschulsky) (Coleoptera: Cerambycidae), the Asian longhorned beetle, is believed to have been introduced into North America as a stowaway in solid wood packing materials, which includes crating, pallets, dunnage, and stowage, originating from China (5). The first established population in North America was identified in 1996 on Long Island, NY, (5) and a second population was discovered two years later in the Chicago, IL, metropolitan area (8). The United States Department of Agriculture (USDA) is currently attempting to eradicate A. glabripennis from both areas. The eradication programs require time and labor intensive surveys of trees throughout neighborhoods with known infestations. All infested trees are felled, chipped and stumps are removed (11). In New York and Illinois, 8077 trees have been destroyed from 1996 to 2001 (C. Markham, USDA-APHIS, personal communication). Recent projections suggest that if A. glabripennis spreads to urban trees across North America, there would be a loss of 35% of total canopy cover (1.2 billion trees) and a compensatory value loss of \$669 billion (7). While species within the genus Acer are the most commonly infested trees in New York and Chicago, trees within the genera Aesculus, Betula, Fraxinus, Salix, and Ulmus have also been attacked at these two locations (A. Sawyer, USDA-APHIS, personal communication).

The life cycle of *A. glabripennis* in North America is generally completed in one year. An adult female chews a small depression called an egg niche into the bark and inserts a single egg at the phloem-bark interface. Eggs hatch in 1 to 2 weeks and the young larvae feed on phloem, just under the bark. Older larvae tunnel deeper and feed in the sapwood of the branches or stem, periodically pushing coarse sawdust and fecal particles out of their galleries. Larvae spend the winter in galleries in the wood, and then pupate in spring or early summer. Newly developed adult beetles leave a round hole, roughly 18 mm in diameter, where they exit from the tree. Adult beetles may be present from May to October and often feed on bark and cambium of twigs and shoots in tree canopies (1, 10).

Given the potential damage that A. glabripennis may cause in urban forests, there is an urgent need to evaluate the suitability of commonly planted landscape trees to beetle oviposition and larval development. By proactively screening landscape trees, we can identify species and varieties of trees that are not suitable hosts for A. glabripennis. Replacing infested trees with species or cultivars that are not susceptible to A. glabripennis will reduce the overall vulnerability of the infested areas in New York and Chicago. This information will also help to prioritize species for surveys and could help to limit the damage to urban forests if populations spread or new populations are discovered in the future. The objectives of this study were to (1) evaluate two techniques for assessing susceptibility of selected species to A. glabripennis under controlled laboratory and greenhouse-based conditions, and (2) assess the suitability of selected North American species for A. glabripennis oviposition and larval development.

Materials and Methods

Oviposition preference on wood sections: Suitability of sugar maple (Acer saccharum), northern red oak (Quercus rubra), white oak (Quercus alba), honeylocust (Gleditsia triacanthos), eastern cottonwood (Populus deltoides), sycamore (Platanus occidentalis) and tulip poplar (Liriodendron tulipifera) for A. glabripennis oviposition and early larval survival was assessed at the USDA Forest Service, Northeastern Research Station Quarantine Laboratory in Ansonia, CT. Branches and twigs of sugar maple, white oak, sycamore and tulip poplar were collected from a woodlot in Ansonia. The other species were collected at Michigan State University's W.K. Kellogg Forest, Kalamazoo Co., MI. Cut ends of bolts and branches were waxed and all material was shipped in coolers by overnight mail to the Ansonia laboratory. Sections of wood were cut with a band saw before each host preference test to a size of 5×20 cm (2.0×8 in) and the cut ends were waxed with paraffin to slow desiccation. Wood and twigs were stored in a cool growth chamber for no more than 2 days before tests.

Beetles used in the oviposition trials had emerged from infested maple bolts collected in New York and transported to the Ansonia facility. One male-female pair of adult beetles was placed in a 3.8 liter (1 gal) glass jar that contained a section of wood with bark attached of one of the test species. Sugar maple twigs were included in each jar to ensure that beetles would survive during the test period. Nearly all of the twigs had some evidence of feeding. Jars with beetles were held in a growth chamber at 21C (70F) and 16:8 light:dark photoperiod. The number of host species that could be tested was determined by availability of healthy beetles. Four male:female pairs of beetles were used in Test 1 to evaluate oviposition on northern red oak, honeylocust, eastern cottonwood and sugar maple. Four different pairs of beetles were used in Test 2 to evaluate white oak, sycamore, tulip poplar and sugar maple. Beetles used in Test 1 were 25 to 48 days old, and those used in Test 2 were 54 to 55 days old

when bioassays began. Beetles used in Test 1 had intact antennal segments, but one female was missing her left front tarsus, while one female in Test 2 had a malformed antennae.

In Test 1, three pairs of beetles were randomly assigned to northern red oak, honeylocust, or eastern cottonwood, while one beetle pair remained on sugar maple for the duration of the test. Beetles were allowed to mate, feed, excavate egg niches and oviposit on the wood in each jar for four days. After four days, each mating pair was placed into a new jar containing fresh sugar maple twigs and a sugar maple wood section for two days to allow beetles to recover. Each mating pair was then assigned to a new species for four days, followed by a two-day recovery period on sugar maple. This process was repeated again, so that each pair of beetles was exposed to three different host species. Test 2 included white oak, sycamore and tulip poplar along with sugar maple and followed the same methods described for Test 1. A 61-dayold female of one of the mating pairs died during the second resting period in Test 2, so another mating pair of beetles was used to complete the test. At the end of each 4-day period, wood sections were examined to determine the number of egg niches on the wood where the female beetle had clearly used her mandibles to scrape the bark. Wood sections were placed on end in a 21C (70F) growth chamber for rearing, then were carefully dissected 21 days after completion of each test. Number of eggs and first instar larvae were recorded for each section.

Data were tested for normality using the Shapiro-Wilkes residual test and residual plots. Data were not normally distributed so the nonparametric Kruskal-Wallis test (p < 0.05) was used to determine whether the number of egg niches and first instar larvae differed significantly among host species that were tested. When the Kruskal-Wallis test was significant, the Kruskal-Wallis multiple comparison procedure (p < 0.05) was used to identify differences among species (3).

Larval performance in potted trees: Two larval insertion experiments were conducted within a 111 sq m (1200 sq ft) quarantine greenhouse at The Pennsylvania State University. This facility was equipped with screened cages measuring 3 \times 2.7 \times 2.1 m (10 \times 9 \times 7 ft) in which the trees and larvae were confined. Sugar maple, green ash (Fraxinus pennsylvanica), and northern red oak trees averaging 2 cm (5.1 in) in caliper were cultured in #20 containers containing Fafard 52 (FAFARD Inc, Agawam, MA) pine bark medium within an adjacent greenhouse for 6 to 12 months prior to the initiation of the experiments. First instar A. glabripennis larvae were implanted into the trees by making a downward tangential incision to a depth of 5 mm along the trunk of each tree at a predetermined height with a scalpel through the bark, near the bark-cambium-phloem interface, creating a bark flap. One larva was inserted under each bark flap. The flap was carefully closed and sealed with a 5×15 cm (2×6 in) piece of plastic wrap and anchored with tape at both ends to confine the larva to the insertion site. The plastic wrap was removed after 14 days and larval status was observed and recorded when possible without harming the larvae. In the second experiment, the insertion area was covered with gauze after the plastic wrap was removed. The gauze remained in place for 28 days. Both experiments were terminated by destructively harvesting the implanted sections of trunk, carefully splitting open the section with a chisel and hammer,

and removing each implanted larva. Weight, length, gallery size measurements, and observations on gallery characteristics were recorded.

The first insertion trial was initiated in September 2000 using larvae provided by the USDA Otis Plant Protection Center (Otis Air National Guard Base, MA). These larvae were reared in the laboratory from oviposition logs of striped maple (Acer pensylvanicum) and placed on northern red oak borer diet (4) prior to transport to the quarantine testing facilities in Pennsylvania. The trial was set up as a randomized complete block design with two cages each containing two sugar maple, northern red oak and green ash trees. Larval insertions were made in each tree at heights of 1, 1.5, 1.75, and 2 m (3.3, 4.9, 5.7, and 6.6 ft) above the soil line following the implantation protocol described above. The mean caliper and standard error of the mean (\pm SEM) in mm for each tree species measured at 0.15 m (6 in) above the soil line were: green ash 33.7 ± 0.9 , sugar maple 25.3 ± 0.7 , and northern red oak 20.8 \pm 0.6. Sixty days after implantation the trees were destructively harvested.

The second trial was initiated in April 2001. Sugar maple logs containing eggs were obtained from the Cornell University Department of Entomology and transported to the quarantine greenhouse. Once the larvae began feeding on the host log, they were removed and immediately placed into the insertion site. Larvae were inserted into each of the three tree species at heights of 1.0, 1.25, 1.5, 1.75 and 2.0 m (3.3, 4.1, 4.9, 5.7, and 6.6 ft) above the soil line. The insertions were replicated in three cages containing two trees of each species. The mean caliper (\pm SEM) in mm for each tree species measured at 0.15 m (6 in) above the soil line were: green ash 33.0 \pm 1.0, sugar maple 23.2 \pm 1.1, and northern red oak 22.6 \pm 0.5. Ninety days after implantation the trees were destructively harvested and data collected as described above.

Differences among species in larval length and weight were subjected to analysis of variance by general linear model procedures followed by the protected least significant difference test (PLSD) at the P < 0.05 level (9). Two methods were used to analyze survival of implanted larvae at 60 and 90 days post-implantation. First, logistic regression with entry of

variables specified (height, tree species, block, followed by interaction terms) was performed to determine which variables influenced survival (2). Differences in survival among tree species were then compared using Pearson's Chi-square Test of Independence (13). Data were analyzed using SPSS (Mac version 10) (SPSS Inc, Chicago, IL).

Results and Discussion

Oviposition preference on wood sections: Excavation of egg niches, number of eggs laid, and survival of first instar larvae varied widely among the host species tested (Table 1). The number of eggs laid on the wood sections of the species tested ranged from 0 to 23 per section. Overall, 68% of eggs hatched and those that did not hatch often appeared to be desiccated. Relatively little phloem feeding had occurred at the time of dissection; by the end of the 21-day rearing period, most first instar larvae had consumed an area slightly larger than their body size.

In Test 1, more eggs and larvae were recovered from sugar maple than from any of the alternate species (Table 1) and the numbers were comparable to numbers of eggs and larvae reared in other A. glabripennis projects at the Ansonia facility (M. Keena, USDA Forest Service, unpublished data). In Test 2, however, relatively few eggs and larvae were recovered from the sugar maple sections, probably because the beetles assigned to sugar maple in Test 2 were relatively old. At the end of Test 2, the female beetles were up to 73 days old, while at the end of Test 1, beetles were no more than 66 days old. Recent research has shown that egg production decreases as A. glabripennis females age, although the proportion of viable eggs may increase (6, 12). Female A. glabripennis, however, are fairly long-lived beetles and average adult female survival may exceed 100 days on some hosts (12). Northern red oak (Test 1) and white oak (Test 2) appeared to be more suitable hosts for ovipositing A. glabripennis beetles than we originally expected. Female beetles assigned to northern red oak sections in Test 1 usually excavated egg niches and oviposited around branch nodes where the bark was relatively rough and thick. The total num-

0

0.0c

Table 1.	Mean and standard error of the mean (\pm SEM) are listed for number of <i>A. glabripennis</i> eggs, egg niches and first instar larvae on 5 × 20 cm				
	wood sections of six North American trees; n = 3 logs per species. Sugar maple, a favored host, was used for comparison ^z .				

Test 1				
	Northern red oak	Honeylocust	Eastern cottonwood	Sugar maple
Total number of egg niches	28	16	5	40
Mean number of egg niches per wood section	$9.3 \pm 2.40a$	$5.3 \pm 1.33b$	$1.7 \pm 1.20c$	$13.3 \pm 0.88d$
Total number of eggs	23	10	0	22
Mean number of eggs per wood section	$7.7 \pm 1.45a$	$5.3 \pm 1.20a$	0.0a	$7.3 \pm 3.28a$
Total number of larvae	9	5	0	21
Mean number of larvae per wood section	$3.0 \pm 1.53a$	$1.7\pm0.67a$	0.0a	$7.0 \pm 3.46a$
Test 2				
	Whiteoak	Sycamore	Tulip poplar	Sugar maple
Total number of egg niches	122	8	0	54
Mean number of egg niches per wood section	$40.7 \pm 1.20a$	$2.7 \pm 1.45c$	0.0c	$18.0 \pm 3.46b$
Total number of eggs	16	0	0	7
Mean number of eggs per wood section	$5.3 \pm 0.88a$	0.0a	0.0a	$2.3 \pm 1.45a$

0

0.0c

^zWithin rows, means followed by the same letter are not significantly different (Kruskal-Wallis test and multiple comparison procedure; P < 0.05).

14

 $4.7 \pm 1.45a$

Total number of larvae

Mean number of larvae per wood section

4

 $1.3 \pm 0.88b$

Table 2. Mean (\pm SEM) caliper (mm) and percent larval survival at four heights for each tree species and for each trees species measured at 0.15 m above the soil line in potted tree trial 1, n = 4 trees per species.

		Height above soil line (m)				
	1.00	1.50	1.75	2.00	Tree caliper (at 0.15 m)	
Green ash						
Caliper (mm)	36.9 ± 1.6	35.4 ± 0.9	33.4 ± 0.3	29.3 ± 3.8	33.7 ± 3.5	
Larvae survival	0%	0%	75%	25%	25%	
Sugar maple						
Caliper	26.9 ± 2.2	26.5 ± 2.0	24.3 ± 2.0	23.6 ± 3.3	25.3 ± 2.6	
Larvae survival	50%	50%	75%	50%	56%	
Northern red oak						
Caliper	22.4 ± 0.5	21.2 ± 1.2	19.8 ± 2.3	19.6 ± 3.1	20.8 ± 2.2	
Larvae survival	25%	50%	75%	100%	62%	

ber of eggs laid on northern red oak (23) was comparable to the number laid on sugar maple in Test 1 (22), although only 39% of the eggs on northern red oak hatched compared with 82% of the eggs on sugar maple. At least 8 of the unhatched eggs on northern red oak appeared desiccated at the time of dissection and the northern red oak appeared to dry out more rapidly than sections of other species. White oak sections had significantly more egg niches than any other species in Test 2 (T = 9.43; p < 0.05) (Table 1), but the shape of the niches was unusual. We observed females repeatedly scraping the thick, corky bark on the white oak sections, presumably to find suitable sites for oviposition, but they actually laid an egg in less than half of the egg niches. Females laid significantly more eggs on white oak than on other species in Test 2 (T = 8.50; p < 0.05) and a total of 87% of the eggs on white oak sections hatched successfully. Significantly more larvae were recovered from white oak sections than from the other Test 2 species (T = 8.77; p < 0.05). The other tree species appeared less suitable for egg niche excavation and oviposition. The number of egg niches differed significantly among honeylocust, northern red oak and sugar maple (T = 8.59, p < 0.05), but the number of eggs and larvae did not differ significantly between honeylocust and northern red oak or honeylocust and sugar maple (Table 1). However, less than half as many eggs were laid on honeylocust as on the sugar maple in Test 1, and only 50% of the eggs on honeylocust hatched. Beetles did excavate a few egg niches on rough-barked areas of cottonwood and sycamore sections, but no eggs were laid on any of these sections. Tulip poplar appeared to be highly unsuitable as a host; there was no evidence of any attempts to excavate egg niches or oviposit on these sections.

Based on these results, it appears that northern red oak and white oak may be acceptable hosts for ovipositing *A. glabripennis* beetles, especially in situations where more preferred hosts are not available. This may also be true for honeylocust. In contrast, females laid no eggs on eastern cottonwood, sycamore and tulip poplar. The lack of oviposition on eastern cottonwood is interesting given that hybrid poplars in China are readily attacked by *A. glabripennis* and are suspected of being the dissemination source for *A. glabripennis* throughout the world in the form of solid wood packing materials (11). These results should, however, be considered preliminary, given that relatively few beetles were exposed to each of the species we tested. Additional research is needed to further assess how *A. glabripennis* responds to *Populus* species, as well as hybrid poplar varieties and to evaluate whether the response of female beetles to live trees is similar to their response to cut sections of wood that we used in this study.

Larval performance in potted trees: It was not possible to accurately determine survival at 14 days without injuring the larvae or trees. It appears, however, that if the larvae survived the insertion process and started to tunnel into the tree, then they survived until they were harvested from the tree. In the first insertion trial, tree species was not predictive of survival (Logistic regression: $X^2 = 3.18$, df = 1, p = 0.0744); thus, larval survival did not differ significantly among the three tree species 60 days after insertion ($X^2 = 5.18$, p = 0.0752). Overall survival was 62%, 56%, and 25% on northern red oak, sugar maple, and green ash, respectively (Table 2). Sixty days after insertion, larvae in green ash weighed significantly more (F = 8.11; df = 6; P = 0.0003) (Fig. 1) and were significantly longer (F = 6.22; df = 6; P = 0.0013) (Fig. 2) than larvae inserted into sugar maple or northern red oak.

In the second trial, tree species was predictive of survival (Logistic regression equation: probability of survival = 1.16 – 0.70, tree species; oak = 0, ash = 1, maple = 2; $X^2 = 6.89$, df = 1, p = 0.0087). In addition, significantly more larvae survived in northern red oak (67%) than in green ash (17%),

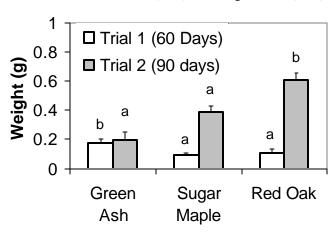


Fig. 1. Mean larval weight (± SEM) in grams of A. glabripennis larvae reared in green ash, northern red oak, and sugar maple, n = 4 trees per species. Different letters indicate a significant difference between treatments within each trial (PLSD, P < 0.05).

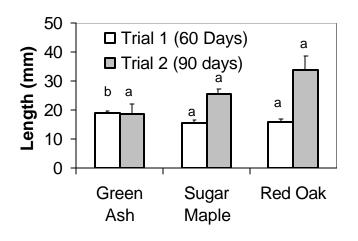


Fig. 2. Mean larval length $(\pm SEM)$ of *A. glabripennis* larvae reared in green ash, northern red oak, and sugar maple, n = 6 trees per species. Different letters indicate a significant difference between treatments within each trial (PLSD, P < 0.05).

while survival in sugar maple (50%) was not significantly different from larval survival in northern red oak or green ash ($X^2 = 15.75$, p = 0.0004) (Table 3). Larvae harvested from northern red oak weighed significantly more than larvae from sugar maple or green ash (F = 3.53; df = 7; P = 0.0064) (Fig. 1), but no significant difference was found in larval length among the three tree species (F = 0.90; df = 7; P = 0.5167) (Fig. 2).

In both studies, the higher in the tree the beetles were inserted, the greater chance of survival (Logistic regressions: Trial 1: probability of survival = -5.28 + 3.81(height); X² = 54.48, df = 4, p = 0.018. Trial 2: probability of survival = -2.4 + 1.84(height); X² = 100.84, df = 7, p = 0.002) (Tables 2 and 3). In addition the caliper of the tree at the insertion site was negatively correlated with survival (Logistic regression: probability of survival = 1.85 - 0.08(caliper); $X^2 = 11.4$, df = 1, p = 0.0007). In other words, as caliper decreased, the number of larvae that survived increased. From these results it is not possible to determine if the increased survival is a result of caliper or the height of the insertion site because these two variables were highly correlated (-0.42, p < 0.001). Further testing needs to be completed to conclude whether tree caliper or height in the tree is responsible for increased survival. Field observations indicate that when a tree is first colonized by A. glabripennis, the adults lay their eggs in the

upper part of the tree canopy. This may be a result of higher nitrogen levels, thinner bark or thicker phloem in upper canopy, which could facilitate egg or early larval survival (M. Smith, USDA-ARS, personal communication). This pattern may also occur because adult beetles often feed on small twigs and shoots in the canopy and females may simply oviposit near their twig-feeding sites.

Green ash exhibited dramatic sap flow after incisions were made in both insertion trials, while northern red oak exhibited a delayed but prolonged sap flow during both insertion trials and sugar maple exhibited the least sap flow of the trees. Sap flow in green ash was more pronounced at the insertion sites closer to the soil surface than higher in the stem. Heavy sap flow may have contributed to the relatively high rate of larval mortality in the green ash. Further research is needed to determine if sap flow is similarly pronounced when adult beetles are twig-feeding or ovipositing.

These preliminary experiments demonstrate that host suitability to A. glabripennis can be evaluated under laboratory and greenhouse conditions. Evaluating oviposition and early larval survival on wood sections in the laboratory and the insertion of larvae into potted trees both provide a means for evaluating host suitability. In these tests, we were able to quantify egg niche excavation, oviposition, and larval development including growth, gallery size, and survival. We also found that larval insertion can be accomplished without a significant reduction in larval survival. Results from the oviposition tests together with the potted tree trials indicate that northern red oak may be an acceptable host for oviposition by A. glabripennis females, at least under limited choice conditions, and that larvae are able to develop on northern red oak phloem and wood. Likewise, white oak and honeylocust appeared to be acceptable to ovipositing females, but the ability of larvae to develop in these species has not yet been determined. Results of the oviposition study indicate that eastern cottonwood, sycamore and tulip poplar are unlikely to be used as hosts for A. glabripennis. Further research on A. glabripennis host preference and larval development with living trees is underway.

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Table 3. Mean (± SEM) caliper (mm) and percent larval survival at five heights for each tree species, and for each trees species measured at 0.15 m above the soil line in potted tree trial 2, n = 6 trees per species.

	Height above soil line (m)					
	1.00	1.25	1.50	1.75	2.00	Tree caliper (at 0.15 m)
Green ash						
Caliper (mm)	35.9 ± 3.0	37.8 ± 3.3	35.6 ± 1.7	29.4 ± 4.2	20.8 ± 2.6	33.0 ± 5.6
Larvae survival	0%	50%	17%	0%	17%	17%
Sugar maple						
Caliper	23.2 ± 5.9	26.4 ± 2.8	27.6 ± 2.1	24.8 ± 2.2	23.0 ± 5.9	14.6 ± 4.4
Larvae survival	33%	50%	67%	83%	33%	50%
Northern red oak						
Caliper	22.6 ± 3.0	25.7 ± 2.3	24.4 ± 2.1	23.0 ± 1.7	20.9 ± 1.9	18.9 ± 1.3
Larvae survival	67%	67%	50%	100%	50%	67%

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