# Insecticide Dip Treatments to Prevent Walnut Twig Beetle Colonization of Black Walnut Logs

Jackson Audley Adam Taylor William E. Klingeman
Albert E. Mayfield Scott W. Myers

**Abstract**

The health, sustainability, and commercial viability of eastern black walnut (*Juglans nigra*) are currently under threat from thousand cankers disease. The disease is caused by an invasive bark beetle species, the walnut twig beetle (*Pityophthorus juglandis*), and its associated fungal pathogen (*Geosmithia morbida*). Range expansion of the beetle and pathogen has likely been facilitated by transport of infested walnut forest products. Preventing colonization of these products is crucial to limiting further spread of thousand cankers disease. This study evaluated three insecticides for their ability to induce walnut twig beetle mortality and prevent colonization of black walnut bolts, 3 to 5 cm in diameter, after dip treatment applications. Treatments included 0.003 percent azadirachtin, 15 percent disodium octaborate tetrahydrate (DOT), 0.5 percent permethrin, and water in Trial 1, and 0.013 percent azadirachtin, 30 percent DOT, 0.5 percent permethrin, and water in Trial 2. A total of 40 beetles, 4 beetles per sample, were exposed to treated samples and observed for 120 hours in each trial. Permethrin was the only treatment to achieve 100 percent mortality and prevent all colonization activity. The 30 percent DOT treatment increased mortality compared with the control; however, it did not reduce the mean number of attacks or mean gallery length. Azadirachtin was not effective at either concentration. Results suggest that insecticide dip treatments can prevent walnut twig beetles from colonizing cut black walnut logs. Treatments could be used in conjunction with phytosanitation to help prevent further spread of thousand cankers disease while allowing for the continued transport of bark-on walnut forest products.

Eastern black walnut (*Juglans nigra*) and several other *Juglans* species in North America are currently under threat from an emergent, often fatal disease known as thousand cankers disease (Tisserat et al. 2009, Seybold et al. 2011). The disease is caused by the phloem-feeding walnut twig beetle (*Pityophthorus juglandis*) and an associated fungal pathogen (*Geosmithia morbida*; Tisserat et al. 2009, Kolařík et al. 2011). Adult walnut twig beetles introduce the pathogen upon entering the phloem of a host tree. *G. morbida* is not a systemic pathogen and numerous beetle attacks are required to induce mortality (Tisserat et al. 2009). Mortality generally occurs in the branches of the canopy first and progresses down the main stem until the host tree dies. Dieback is the result of cankers and beetle gallery formation coalescing in the phloem, girdling branches, and ultimately the main stem (Tisserat et al. 2009).

Neither the beetle nor the pathogen is native to the eastern United States. Rather, the walnut twig beetle is historically known from the American Southwest, originally associated with Arizona walnut (*Juglans major*; Cranshaw 2011, Seybold et al. 2012). Arizona walnut is hypothesized to be the primary host of the beetle and pathogen in its native range (Moltzan 2011, Utley et al. 2013), and tree mortality due to thousand cankers disease on Arizona walnut has not been reported. Over the past 2 decades, the range of the walnut twig beetle has greatly expanded. The beetle has been collected primarily from declining black walnut across the western states where it is not native, but has been extensively outplanted (Cranshaw 2011, Tisserat et al. 2011, Seybold et al. 2012). In 2010, walnut twig beetles were first discovered within the native range of black walnut on infested trees in Knoxville, Tennessee (Grant et al. 2011). Since that time, the beetle and pathogen have been recovered in Pennsylvania, Virginia (Seybold et al. 2012),

The authors are, respectively, Graduate Student and Assistant Professor, Forestry, Wildlife, and Fisheries Dept., Univ. of Tennessee, Knoxville (jaudley@vols.utk.edu [corresponding author], adamtaylor@utk.edu); Professor, Dept. of Plant Sci., Univ. of Tennessee, Knoxville (wklingem@utk.edu); Research Entomologist Project Leader, USDA Forest Serv., Southern Research Sta., Asheville, North Carolina (amayfield02@fs.fed.us); and Entomologist, USDA Animal and Plant Health Inspection Serv., Plant Protection and Quarantine, Buzzards Bay, Massachusetts (scott.w.myers@aphis.usda.gov). This paper was received for publication in July 2015. Article no. 15-00038.

Forest Prod. J. 66(3/4):233–240.
doi:10.13073/FPJ-D-15-000038

North Carolina (Hadziabdic et al. 2013, Wiggins et al. 2014), Ohio (Fisher et al. 2013), Maryland (Maryland Department of Agriculture 2013), and Indiana (Marshall 2015). Both the beetle and pathogen were also recovered from a walnut grove in Italy (Montecchio et al. 2014), constituting the first international occurrence of thousand cankers disease. Given the tremendous distance and disjoint nature of these introductions, anthropogenic influence is likely involved in the transport of the beetle. Introductions may be the result of the commercial transport of infested walnut material in which the bark was left intact (e.g., logs, unedged lumber, or firewood; Newton and Fowler 2009, Turcotte et al. 2013).

Continued spread of thousand cankers disease threatens one of North America's most economically valuable hardwood species in eastern black walnut (Shifley 2004, Newton and Fowler 2009, Moltzan 2011). Total value of standing black walnut timber for lumber and veneer production is estimated at more than $500 billion (Newton and Fowler 2009). Black walnut wood is commonly used for gun stocks, cabinetry, and several other finished wood products and is prized for its strong and durable wood, especially the distinctive, dark-brown heartwood (Williams 1990, Kirkman et al. 2007, Moltzan 2011). The highest-quality logs are sold as veneer logs and veneer-quality walnut is exceptionally valuable (Moltzan 2011).

In response to the spread of thousand cankers disease, several states have established quarantines restricting the movement of walnut logs and wood products (Newton and Fowler 2009, Moltzan 2011). In Tennessee, regulated articles include: ''the walnut twig beetle; *Geosmithia morbida*; all plants and parts of plants in the genus *Juglans* including nursery stock, budwood, scionwood, green lumber, and other material living, dead, cut, or fallen, including logs, stumps, roots, branches, mulch and composted and un-composted chips; and any hardwood firewood'' (Tennessee Department of Agriculture [TDA] 2014). Other states that have enacted quarantines have similar lists of restricted materials. To remove any of these items from counties in which thousand cankers disease has been confirmed, a phytosanitation certification or a compliance agreement from the transporter must first be obtained (Newton and Fowler 2009, TDA 2014).

Although effective phytosanitation methods, such as steam heat (Mayfield et al. 2014), have been reported for walnut twig beetle and *G. morbida*, recent evidence suggests that the beetle can colonize the bark of treated logs (Audley 2015). In experiments assessing the efficacy of several cultural and chemical treatments for eradication of the walnut twig beetle from felled black walnut logs, Peachey et al. (2011) found beetle emergence up to 21 months posttreatment from both treated and untreated logs. Beetle emergence was also reported from logs from which no pretreatment emergence was noted. This represents further evidence to support the notion that walnut twig beetles can colonize the bark of treated walnut logs. Haack and Petrice (2009) also reported colonization of heat-treated logs and boards of lumber with bark left intact by species of bark and ambrosia beetles (Coleoptera: Curculionidae: Scolytinae) and species of long horned beetles (Coleoptera: Cerambycidae). Their findings provided significant evidence to support a revision in the International Standards for Phytosanitary Measures (ISPM) 15 guidelines requiring complete debarking before phytosanitation for wood packing materials (Haack and Brockerhoff 2011). The requirement for debarking logs or lumber can be burdensome to the walnut wood industry. Producing lumber that is completely bark free reduces the volume of top-quality boards as a result of overedging (National Hardwood Lumber Association 2003), and veneer log buyers require the bark to remain intact on the log to protect the wood from drying and fungal discoloration before processing at the mill.

The potential for walnut twig beetle colonization of phytosanitized wood coupled with industry's needs to transport intact logs creates an environment in which further precautions may be required to ensure that no walnut twig beetles are transported on treated wood while not overly restricting industry. One approach could be the use of insecticides applied to the surface of logs. Insecticides are a commonly used management technique for protecting individual trees from bark beetle attack (Fettig et al. 2006) and have been shown to be effective against attack on cut logs and lumber (Strom and Roton 2009, Fettig et al. 2011).

Several active ingredients are commercially available in insecticide products labeled for treatment of logs and wood products that may reduce or prevent colonization of phytosanitized walnut logs by adult walnut twig beetles. Azadirachtin is a terpenoid extract from the neem tree (*Azadirachta indica*) that provides broad-spectrum insecticidal activity and low mammalian toxicity (Schmutterer 1990, Naumann et al. 1994, Newberry et al. 2013). The chemical structure of azadirachtin is similar to that of ecdysone, an insect hormone that regulates metamorphosis. Azadirachtin acts as an ecdysone blocker, preventing successful development of insect larval stages; thus its primary mode of action is as a growth regulator (Schmutterer 1990, Newberry et al. 2013). Studies with azadirachtin have also demonstrated population regulation through feeding deterrence, mating disruption, adult sterilization, or repellency of all life stages of several insect pests (Schmutterer 1990, 1995; Naumann et al. 1994; Xie et al. 1995; Newberry et al. 2013).

Efficacy trials using azadirachtin to manage bark- and wood-boring beetles have yielded mixed results. In a study testing systemic injections of azadirachtin on emerald ash borer (*Agrilus planipennis*) in ash (*Fraxinus* spp.) trees, 100 percent of larvae failed to complete development at doses $\geq$13.6 mg/cm diameter breast height; however, all doses tested were not effective in controlling adults (McKenzie et al. 2010). Asian longhorned beetle (*Anoplophora glabripennis*) larval mortality reached 60 percent when fed a diet with an azadirachtin concentration of 50 parts per million (Poland et al. 2006). Systemic treatments were also effective against larvae of mountain pine beetle (*Dendroctonus ponderosae*) in treated lodgepole pine (*Pinus contorta* var. *latifolia*; Naumann et al. 1994, Naumann and Rankin 1999). However, no reduction in the attack rate of adult mountain pine beetles on lodgepole pine treated with a topical azadirachtin emulsion was found (Duthie-Holt and Borden 1999).

Borates are commonly used wood preservatives (Taylor and Lloyd 2009) and demonstrate broad-spectrum insecticidal and fungicidal efficacy, with low mammalian toxicity, with labeled uses for agricultural and domestic applications (Lloyd 1998). Slahor et al. (2005) demonstrated borate to be a cost-effective preservative for treating pallet lumber, especially when combined with a heat treatment, thus lending support to the idea of practical application in an

industry setting for black walnut lumber and veneer logs as well. Previous tests of dip and spray treatments of disodium octaborate tetrahydrate (DOT) indicate only a marginal effect as a sanitizing agent (postcolonization) when tested on the emerald ash borer; however, only low doses (1.22% to 6.6%) were tested (Nzokou et al. 2006). In an assay of the effect of DOT on the structure-infesting beetle *Hemicoelus gibbicollis*, larvae were prevented from entering Douglas-fir timbers with >95 percent efficacy (Suomi and Akre 1992).

Permethrin is a synthetic pyrethroid similar to the pyrethrin extracts of pyrethrum flowers (Casida et al. 1983). Pyrethroids are characterized by an acute toxicity to a wide range of insects, with low toxicity in mammals. Permethrin has been effective in reducing attacks of several species of Curculionids including *Dendroctonus brevivomis* (Shea et al. 1984), *Dendroctonus frontalis* (Strom and Roton 2009), and *Xyleborus glabratus* (Carrillo et al. 2013). In prior work related to this study, a permethrin spray treatment applied to black walnut bolts that were subsequently suspended in tree canopies for field exposure tests also prevented walnut twig beetle attack (Mayfield and Juzwik, unpublished data, 2013).

Building upon preliminary trials and field observations, the objectives of this study were to assess the efficacy of azadirachtin, DOT, and permethrin insecticides for protecting previously phytosanitized small black walnut logs from colonization by the walnut twig beetle. Two bioassay trials were used to evaluate beetle mortality and colonization success after exposure to small bolts dipped in insecticide solution.

## Materials and Methods

### Preparation of phytosanitized walnut wood and collection of walnut twig beetles

To prepare samples, 1-m-long walnut limbs between 2 and 5 cm in diameter were cut from three black walnut trees felled at the University of Tennessee Arboretum (35°59′56″N, 84°13′5″W) in Oak Ridge, in July 2014. Limbs were sectioned into 10 branch segments and the ends of each segment were coated with Anchorseal (UC Coatings Corp., Buffalo, New York) to reduce moisture loss. Although the Arboretum is located in Anderson County, which is a thousand cankers disease-positive, quarantined county, previous walnut twig beetle trapping and limb inspections had not revealed presence of thousand cankers disease at that site (W. E. Klingeman, unpublished data, 2013). Limb pieces were returned to the University of Tennessee in Knoxville, where they were kept in refrigerated (4.5°C) storage. In August, branches were removed from storage, placed onto wooden racks in a walk-in kiln (SII Dry Kilns, Lexington, North Carolina), and then steam heated to achieve a core temperature of 60°C for 60 minutes (Mayfield et al. 2014). After steam heating, branches were cut into approximately 4.5-cm-long bolts with a radial arm saw. Bolt pieces were subdivided into eight groups of 10 bolts measured to be similar in diameter, and were then further partitioned into two sets of four visually estimated, uniform cohorts, with four sections used per trial. Cut ends were again coated with Anchorseal and samples were housed in the laboratory, maintained at approximately 20°C. All bolts were visually inspected for any bark- and wood-boring beetle activity (entrance and exit holes) before testing. Any holes were marked so as to not confound walnut twig beetle colonization activity measurements.

Beetles were obtained by hanging logs within the canopy of infested walnut trees located across Knoxville, Tennessee. Logs were baited to attract beetles using a single walnut twig beetle pheromone lure (Contech Enterprises Inc., Victoria, British Columbia, Canada; Seybold et al. 2013, Mayfield et al. 2014). After 30 days of exposure in the field, logs were returned to campus and beetles that emerged into emergence containers from wood sections were collected in dry collection cups and kept refrigerated (4.5°C) on moistened filter paper inside of petri dishes until they could be used in the experiments. Only beetles kept refrigerated for ≤3 days were used. All beetles were sexed using a dissecting stereomicroscope and sorted into male and female pairs before use in the bioassays.

### Insecticide treatments

The eight cohorts of treated branch sections were halved for use in Trials 1 and 2 ($n = 40$ each). Mean branch diameters were compared by analysis of variance (ANOVA) and the assumption of equal variance checked using Levene's test ($P > 0.05$). Mean bolt diameters were not different in Trial 1 ($F_{3,36} = 0.16$, $P = 0.93$). ANOVA comparisons of the Trial 2 bolts indicated a difference in the mean diameters ($F_{3,36} = 3.30$, $P = 0.03$); however, post hoc Tukey's honestly significant difference (HSD) test did not indicate a variance among the means.

To prepare beetle exposure trials, subdivided branch cohorts used in each trial were randomly assigned to one of the following treatments: DOT (Tim-bor active ingredient [AI] 98%; Nisus Corp., Rockford, Tennessee); azadirachtin (AzaSol AI 6%; Arborjet Inc., Woburn, Massachusetts); permethrin (Astro AI 36.8%; FMC Corp., Philadelphia, Pennsylvania); and water (control). Insecticide products were dissolved in water to create a solution bath into which bolts were submerged. Concentrations (as percent grams per milliliter of water) in Trial 1 were as follows: 0.003 percent azadirachtin, 15 percent DOT, and 0.5 percent permethrin. Concentrations were determined using the label-recommended application rates for DOT and permethrin, and using the medium recommended rate for azadirachtin. All product solutions were dissolved in 1,892.7 mL of water at ambient room temperature (approximately 20°C). For the control group, 1,892.7 mL of ambient-temperature tap water was used. Each bolt was submerged into its respective treatment solution and held submerged with tongs for 120 seconds to ensure complete coverage of the bark surface. All bolts were allowed to dry overnight (approximately 16 h) before beetle introductions.

Trial 2 was a replication of Trial 1 with azadirachtin and DOT treatments increased to 30 percent by heating the solution to approximately 50°C to increase the solubility (Nisus Corp. 2014). The solution was maintained at 50°C during treatment. Permethrin and control treatments were identical to Trial 1. All bolts were again submerged for 120 seconds and then allowed to dry overnight.

### Observations of beetles exposed to treated samples

A laboratory-scale bioassay, similar to the design used by Strom and Roton (2009), was used to evaluate the efficacy of the insecticide treatments. Bioassay arenas consisted of a

125-mL Nalgene cup (6 cm in diameter by 6 cm tall) with a 19.6-$cm^2$ mesh screen lid to allow gas circulation within the arena. Branch samples were cut, dip treated, and placed into the arena on top of a sheet of filter paper. On the morning of deployment for each trial, two pairs (two male, two female) of walnut twig beetles were placed onto the filter paper next to each of 10 bolts ($n = 40$ adult walnut twig beetles per treatment). Beetles were handled and transferred on the bristles of a fine-tipped paintbrush to avoid injury. Lids to each arena were immediately fastened and housed inside a fume hood in the laboratory and the fume hood fan was turned off. Lights to the fume hood were turned on each morning at 8 a.m. and turned off each night between 6 and 7 p.m. to simulate daily photoperiod cycles.

A total of eight observations per sample were made on beetle condition across the 120-hour trial (Fettig et al. 2011). Two observations per 24-hour period were made during the first 72 hours after exposure (HAE), followed by a single observation per 24-hour period for the remaining 48 hours. During each observation under a stereomicroscope, all live and dead beetles were counted and attack holes were noted. Beetles were confirmed dead by gently probing the body with the bristles of a fine-tipped paintbrush. Mortality was recorded as the proportion of beetles deceased per sample, and the number of HAE was used to estimate the time to mortality for each individual.

After the final observation at 120 HAE, a final visual inspection tallied all walnut twig beetle attack holes. Bark was then removed using a wood chisel and any adults found within the bark were recorded as alive or dead. Gallery lengths were measured using a Scalex MapWheel (Scalex Corp., Carlsbad, California) to provide an indication of successful colonization (Strom and Roton 2009). Trial 1 was conducted from September 3 to 8, 2014, and Trial 2 was conducted from September 16 to 21, 2014 (both ranges include the day of insecticide treatment followed by 5 days of beetle exposure).

## Statistical analysis

Walnut twig beetle survival rates were estimated for each treatment using Kaplan–Meyer product-limit survival analysis (Lee and Wang 2003), a similar method to the life-table method used by Fettig et al. (2011). Survival curves for each of the four treatments were compared using multiple pairwise comparisons with the nonparametric log-rank test and the Bonferroni adjustment (SAS Institute Inc. 2013b). The log-rank test was used instead of the Wilcoxon test because of the relatively large number of censored values (101 of 320), which indicate individuals that did not die during the testing period (Lee and Wang 2003). Survival curves were estimated using the SAS Lifetest procedure in the SAS Enterprise Guide software package (SAS Institute Inc. 2013b). All analyses were considered significant at $\alpha = 0.05$.

ANOVA was used to test the null hypothesis that mean number of attack holes and mean gallery lengths would not differ across insecticide treatments. Post hoc means comparisons were made using Tukey's HSD test ($\alpha = 0.05$). Data from Trial 1 did not require any adjustments for normality or homogeneity of variance. Mean total attack holes per treatment were $\log(y+1)$ transformed in Trial 2 so the data adhered more closely to a normal distribution to satisfy the assumptions of ANOVA. Mean gallery lengths for Trial 2 were also $\log(y+1)$ transformed to satisfy the equality of variance assumption. All reported statistics are based on the analysis of transformed data (where applicable); however, means and standard errors reported in all tables and figures reflect nontransformed data. Analysis was performed using the JMP Pro 11.1.1 statistical software package by SAS (SAS Institute Inc. 2013a).

## Results

A higher percentage of walnut twig beetles died after exposure to 0.5 percent permethrin as a wood treatment in Trial 1 than those exposed to the 15 percent DOT or the 0.003 percent azadirachtin ($\chi^2 = 46.89$, $P < 0.01$ and $\chi^2 = 54.82$, $P < 0.01$, respectively). Conversely, no differences were found among the DOT, azadirachtin, and control treatments (Table 1). Permethrin was the only treatment to achieve 100 percent beetle mortality within 120 HAE; all individuals were dead by 72 HAE (Fig. 1). Mortality after exposure to the other two insecticide treatments, as well as in the control groups, only increased to approximately 50 percent.

Beetle mortality was again consistent after exposure to bolts treated with permethrin in Trial 2, yet 100 percent mortality was achieved by 36 HAE (Fig. 1). Unlike in Trial 1, however, exposure to the higher (30%) DOT concentration increased beetle mortality above that of the control ($\chi^2 = 10.90$, $P = 0.01$) and azadirachtin ($\chi^2 = 22.889$, $P < 0.01$) treatments. Increasing the azadirachtin concentration to 0.3 percent in Trial 2 did not increase beetle mortality percentage, with results similar to death that occurred among beetles in the Trial 2 control group (Table 1). In fact, beetles exposed to azadirachtin had slightly higher survival than walnut twig beetles exposed to bolts only treated with water (Fig. 1).

Investigation of the bark surface for attack holes at 120 HAE revealed no walnut twig beetles had tunneled into the bark of bolts treated with permethrin in either Trial 1 or Trial 2 (Fig. 2). Therefore, permethrin treatments were not included in ANOVA analyses of mean attack holes or mean

*Table 1.—Multiple pairwise comparisons of walnut twig beetle survival analysis using the Bonferroni corrected nonparametric log-rank test among four topically applied insecticide treatments.*

| Treatment comparison[a] | | | $P$ values[b] | |
|---|---|---|---|---|
| Topical insecticide | Topical insecticide | Chi-square | Raw | Bonferroni |
| Trial 1 | | | | |
| Azadirachtin 0.003% | DOT 15% | 0.34 | 0.56 | 1 |
| Azadirachtin 0.003% | Permethrin 0.5% | 56.26 | <0.01 | **<0.01** |
| Azadirachtin 0.003% | Water | 0.02 | 0.90 | 1 |
| DOT 15% | Permethrin 0.5% | 46.89 | <0.01 | **<0.01** |
| DOT 15% | Water | 0.21 | 0.64 | 1 |
| Permethrin 0.5% | Water | 54.82 | <0.01 | **<0.01** |
| Trial 2 | | | | |
| Azadirachtin 0.013% | DOT 30% | 22.89 | <0.01 | **<0.01** |
| Azadirachtin 0.013% | Permethrin 0.5% | 83.13 | <0.01 | **<0.01** |
| Azadirachtin 0.013% | Water | 1.82 | 0.18 | 1 |
| DOT 30% | Permethrin 0.5% | 11.57 | <0.01 | **<0.01** |
| DOT 30% | Water | 10.90 | <0.01 | **0.01** |
| Permethrin 0.5% | Water | 52.73 | <0.01 | **<0.01** |

[a] DOT = disodium octaborate tetrahydrate.
[b] $P$ values in bold indicate significant differences at $\alpha = 0.05$.

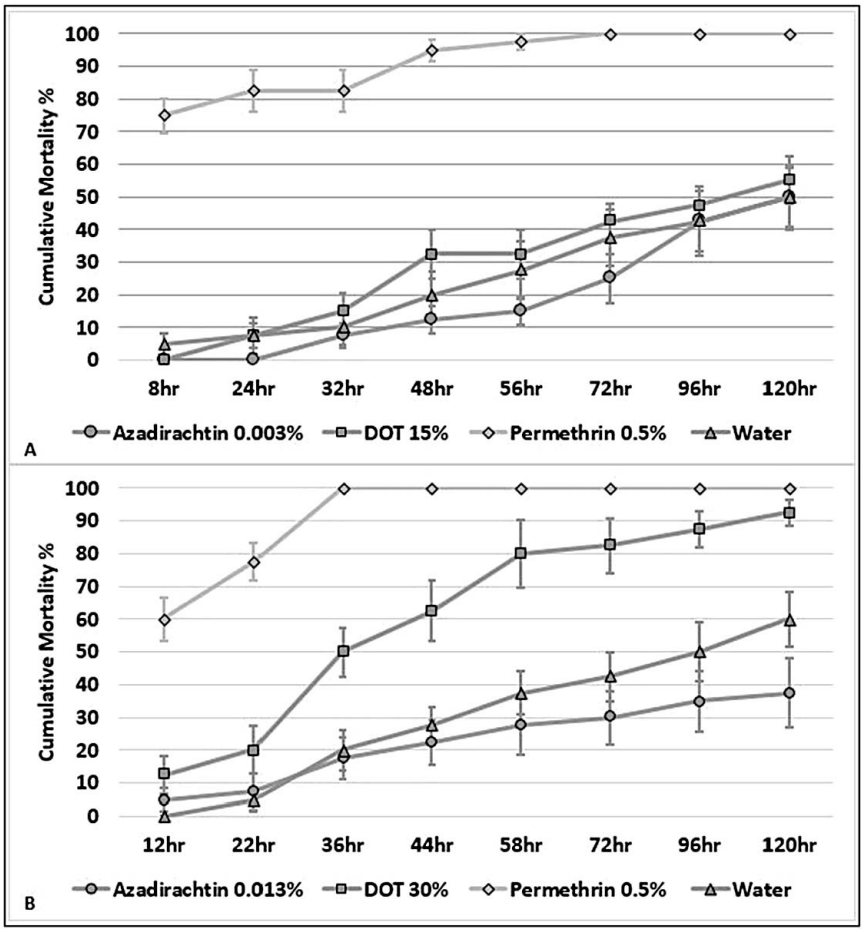


*Figure 1.—Mean cumulative walnut twig beetle mortality percent with standard error bars for each observation period per insecticide treatment for Trial 1 (A) and Trial 2 (B).*

total gallery length so as to not violate the assumption of homogeneity of variance. The mean number of walnut twig beetle attack holes did not differ after exposures to wood treated with azadirachtin, DOT, or water in Trial 1 (Fig. 2A). Similarly, no differences were found among the three treatments in mean gallery lengths (Fig. 3A). In Trial 2, the mean number of attack holes differed by treatment ($F_{2,27} = 3.96$, $P = 0.03$). The higher treatment concentration of DOT reduced the number of attacks compared with the azadirachtin treatment (Fig. 2B). Despite what appears to be an improved performance, however, Tukey's HSD test indicated that DOT did not perform significantly better than water. The same trend was also mirrored in the ANOVA tests of mean total gallery length (Fig. 3B).

## Discussion

When applied to steam-heat–phytosanitized black walnut logs, a 0.5 percent permethrin dip was the only bolt treatment in either trial that successfully prevented any walnut twig beetle colonization and caused 100 percent adult beetle mortality within 120 HAE. Among wood exposed in permethrin treatments, no attack holes were observed, and thus there were no galleries to be measured (Fig. 2). The same level of control (no colonization) was found in a field exposure study in which larger walnut bolts were exposed to walnut twig beetles or 30 days by hanging bolts in the canopy of infested black walnuts (Juzwik and Mayfield, unpublished data, 2013). In previous bioassays with other bark and ambrosia beetle species (Pajares and Lanier 1989, Fettig et al. 2006), permethrin presents acute toxic effects as a contact insecticide and may similarly affect the walnut twig beetle. Consequently, because beetles are unlikely to be transported unless they are capable of residing under the surface of the bark, permethrin shows promise as a viable treatment option to assist in preventing accidental anthropogenic, postphytosanitation spread of the walnut twig beetle.

Although permethrin has a relatively low mammalian toxicity (Schmutterer 1995, Newberry et al. 2013), the US Environmental Protection Agency (EPA) notes the potential of permethrin as a human carcinogen, and also cites risks to aquatic ecosystems if improperly applied or disposed (EPA 2015). Per the Astro label, treated surfaces should not be touched with exposed skin until the surface is completely dry (FMC Corp. 2014). This application constraint may present logistical issues that would restrict industry adoption of permethrin treatment, primarily because of constraints that could introduce bottlenecks in posttreatment processing (e.g., waiting for logs to dry posttreatment before packaging) or via increased costs incurred for investment in application and personal protective equipment. Although risk of toxic effects may be low, future studies could consider the lowest dose requirement needed to provide

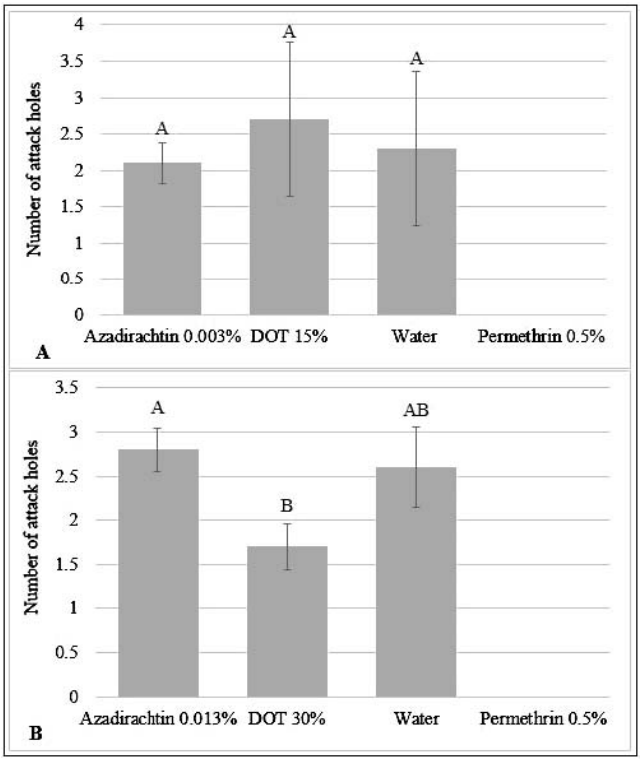


*Figure 2.—Analysis of variance comparisons of mean number of attack holes (±standard error) per treatment for Trial 1 (A) and Trial 2 (B). No difference was found across treatments in Trial 1 ($F_{2,27} = 0.930$; $P = 0.407$). A difference in the mean number of attack holes did exist in Trial 2 ($F_{2,27} = 3.960$; $P = 0.031$). Different letters indicate different means based on a post hoc Tukey's honestly significant difference test ($\alpha = 0.05$). Permethrin was excluded from analysis in both trials because there were no attack holes observed.*

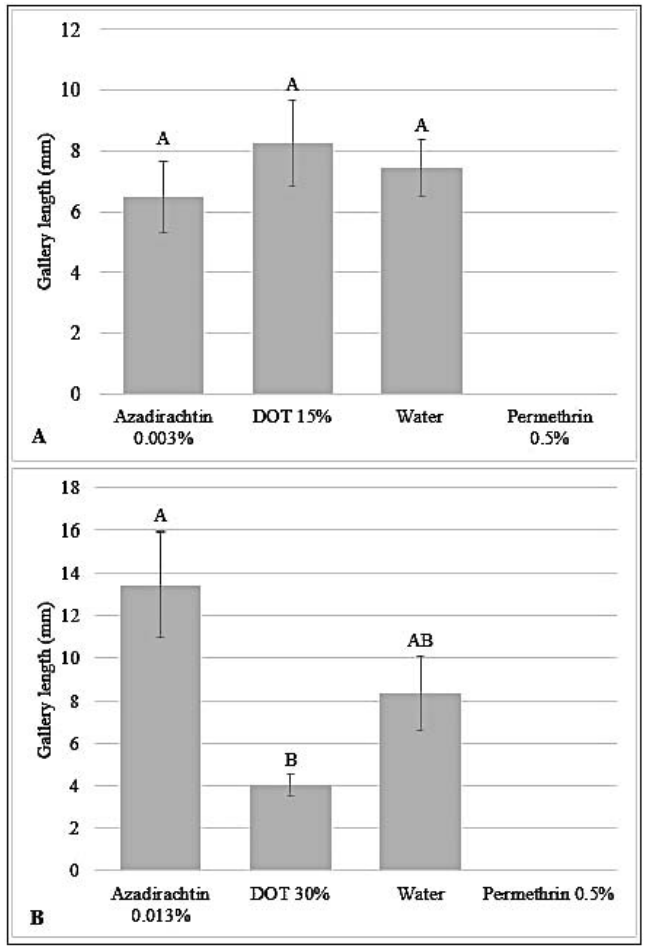


*Figure 3.—Analysis of variance comparisons of the mean total gallery lengths (±standard error) per treatment for Trial 1 (A) and Trial 2 (B). No difference was found across treatments in Trial 1 ($F_{2,27} = 0.541$; $P = 0.589$). A difference in the mean number of attack holes did exist in Trial 2 ($F_{2,27} = 8.766$; $P = 0.001$). Different letters indicate different means based on a post hoc Tukey's honestly significant difference test ($\alpha = 0.05$). Permethrin was excluded from analysis in both trials because there were no attack holes observed, and thus no gallery lengths to measure.*

effective beetle management, thus reducing exposure to industry workers.

Unlike permethrin, DOT is not a skin irritant and thus presents little health concern should solution come into contact with the skin (Nisus Corp. 2014). The 15 percent DOT treatment in Trial 1 did not perform any better than azadirachtin or the water control (Table 1; Fig. 3). Increasing the DOT concentration to 30 percent in Trial 2 improved performance and the observed beetle survival rate was reduced compared with the control and azadirachtin (Table 1). Regardless, even the 30 percent DOT dip treatment failed to significantly reduce beetle attack counts and gallery lengths. In part, these results can be explained by the insecticidal mode of action of DOT, which must be ingested to become effective (Suomi and Akre 1992, Strong et al. 1993). Therefore, DOT may not be an effective tool in preventing the spread of beetles. Although the liquid Tim-Bor solution applied to samples in this study did not prevent colonization activity, DOT can be applied as a thicker emulsion in concentrations up to 50 percent, for example via commercially available CelluTreat 50 (Nisus Corp. 2014). The resulting surface barrier may deter walnut twig beetle attacks better on treated logs; however, applying the more viscous emulsion may not be practical within an industry setting.

Azadirachtin was the least effective insecticide product tested. No reductions were observed in either walnut twig beetle survival rate, in the number of attack holes induced, or in the gallery lengths excavated when compared with the water controls in both trials. The lack of efficacy on adult walnut twig beetles in this study is consistent with the findings of McKenzie et al. (2010), who reported no effect on adult emerald ash borer behavior or survival, and with Duthie-Holt and Borden (1999), who observed no reduction in mountain pine beetle attacks. Although azadirachtin can induce mortality and reduce adult feeding in some species of herbivorous beetles (Xie et al. 1995), direct effects are less conclusive and not consistent among bark- and wood-boring species.

Another possible explanation for the limited observed efficacy of azadirachtin may be that the concentrations tested in these trials were too low. Products are available that contain a higher concentration of the active ingredient, including NeemAzal (42.3% AI; McKenzie et al. 2010). A higher concentration may be more effective in reducing adult walnut twig beetle survival rates on treated bolts.

However, even in higher concentrations, azadirachtin may not have the acute toxicity required, from a regulatory perspective, to effectively prevent short-term colonization by bark beetles. In systemic application bioassays using cerambycid larvae, Poland et al. (2006) found that a prolonged feeding period was required before azadirachtin produced significant mortality. Given the limited effectiveness at controlling adult walnut twig beetles, azadirachtin is not likely to be an effective management tool at preventing beetle transport in treated black walnut logs.

## Conclusions

Results of this study suggest that permethrin, when applied as a dip treatment to steam-heat–phytosanitized logs, can be an effective tool in preventing adult walnut twig beetle colonization on black walnut logs and wood products. Although the highest concentration of DOT tested in this study did not prevent adult beetle colonization, these results suggest that additional investigation using higher concentrations of DOT may be worthwhile. Submerging logs into insecticide solutions may provide an effective and efficient means of protecting walnut logs from subsequent exposures to adult walnut twig beetles with limited regulatory interference of commercial operations.

## Acknowledgments

We gratefully acknowledge the following individuals and agencies for their assistance on this project: Robert Camp (University of Tennessee–Center for Renewable Carbon); Phil Flanagan (University of Tennessee Plant Sciences Department). This work was funded by the US Department of Agriculture Forest Service (USDA-FS) Forest Health Protection, USDA-FS Southern Research Station, USDA Animal and Plant Health Inspection Service, and the University of Tennessee.

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