Laboratory Evaluation of the Toxicity of Systemic Insecticides for Control of *Anoplophora glabripennis* and *Plectrodera scalator* (Coleoptera: Cerambycidae)

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ABSTRACT *Anoplophora glabripennis* (Motschulsky) (Coleoptera: Cerambycidae) is one of the most serious nonnative invasive forest insects discovered in North America in recent years. *A. glabripennis* is regulated by federal quarantines in the United States and Canada and is the subject of eradication programs that involve locating, cutting, and chipping all infested trees. Other control methods are needed to aid in eradication and to form an integrated management program in the event eradication fails. We conducted laboratory bioassays to determine the toxicity of two systemic insecticides, azadirachtin and imidacloprid, for potential control of *A. glabripennis* and the cottonwood borer, *Plectrodera scalator* (F.) (Coleoptera: Cerambycidae), a closely related native cerambycid. Larvae of both cerambycid species were fed artificial diet with dilutions of azadirachtin or imidacloprid for 14 wk. Both insecticides exhibited strong antifeedant effects and some toxicity against *A. glabripennis* and *P. scalator* larvae. For *A. glabripennis*, the highest larval mortality at the end of the bioassay was 60% for larvae fed artificial diet treated with azadirachtin (50 ppm) or imidacloprid (1.6 ppm). For *P. scalator*, the highest larval mortality at the end of the bioassay was 100% for larvae fed artificial diet treated with azadirachtin (50 ppm) or imidacloprid (160 ppm). At 14 wk, the LC_{50} values for *P. scalator* were 1.58 and 1.78 ppm for azadirachtin and imidacloprid, respectively. Larvae of both species gained weight when fed diet treated with formulation blanks (inert ingredients) or the water control but lost weight when fed diet treated with increasing concentrations of either azadirachtin or imidacloprid. In a separate experiment, *A. glabripennis* adults were fed maple twigs treated with high and low concentrations of imidacloprid. *A. glabripennis* adult mortality reached 100% after 13 d on twigs treated with 150 ppm imidacloprid and after 20 d on twigs treated with 15 ppm imidacloprid. There was no visible feeding by *A. glabripennis* adults on twigs treated at the higher imidacloprid rate, and feeding was significantly reduced for adults placed on twigs treated at the low imidacloprid rate compared with adults on untreated twigs. In summary, imidacloprid and azadirachtin had both antifeedant and toxic effects against *A. glabripennis* and *P. scalator* and have potential for use in management programs. Based on our results, the delivery of high and sustained insecticide concentrations will be needed to overcome the antifeedant effects and lengthy lethal time for both larvae and adults exposed to these insecticides.

KEY WORDS *Anoplophora glabripennis*, *Plectrodera scalator*, Cerambycidae, insecticides, imidacloprid, azadirachtin

Anoplophora glabripennis (Motschulsky) (Coleoptera: Cerambycidae) is one of the most serious nonnative invasive forest insects to enter North America in recent years (Haack 2005). It is native to eastern China and Korea (Lingafelter and Hoebeke 2002). In North America, *A. glabripennis* was discovered infesting urban shade trees in New York in 1996 (Haack et al. 1997), Illinois in 1998 (Poland et al. 1998), New Jersey in 2002 (Haack 2003), and Ontario, Canada, in 2003 (CFIA 2004). In Europe, infestations were recently discovered in Austria in 2001 (Tomiczek et al. 2002), France in 2003, and Germany in 2004 (Haack 2005). Larvae feed under the bark and in the wood of a variety of hardwood trees, including members of the genera *Acer*, *Aesculus*, *Betula*, *Fraxinus*, *Populus*, *Salix*, and *Ulmus* (Haack et al. 1997). Because of its wide host range and its ability to attack and eventually kill apparently healthy trees, *A. glabripennis* has the potential to cause serious economic and ecological damage nationwide (Nowak et al. 2001).

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In North America, *A. glabripennis* generally has a 1-yr life cycle. Adult females lay eggs individually in oviposition pits they chew in the bark of branches and the main stem of host trees. Eggs hatch in 1–2 wk, and the young larvae feed on phloem just under the bark. As the larvae develop, they feed deeper in the sapwood. Larvae overwinter in their galleries in the wood and then pupate just under the bark in spring and early summer. Newly developed adult beetles chew out through the bark and emerge from round exit holes, ≈1.5 cm in diameter, on trunks and large branches. Adults are generally active from May to October and feed on bark and cambium of twigs and shoots in tree canopies. In the wild, adults may live >40 d and females lay 25–40 eggs in their lifetime (Haack et al. 1997, Becker 2000, Smith 2000). Longevity and fecundity may be higher in the laboratory. Keena (2002) found females lived an average of 73–88 d and laid an average of 50–75 eggs depending on larval food and source population. Smith et al. (2001) found females lived an average of 97–103 d and laid an average of 98–193 eggs depending on host species.

The USDA–Animal and Plant Health Inspection Service (APHIS) and the Canadian Food Inspection Agency (CFIA) have implemented eradication programs in the United States and Canada. These programs involve locating, cutting, and chipping infested trees. Millions of dollars have been spent and thousands of trees have been destroyed as a result of these programs (Nowak et al. 2001, Markham 2002, CFIA 2004). Other control methods are needed to aid in eradication and to form an integrated management program in the event eradication fails. Systemic insecticides may prove useful in controlling *A. glabripennis* adults feeding on twigs and larvae feeding in the cambium and sapwood. Human exposure and nontarget impacts of insecticide application are of major concern because of the highly urban setting of the eradication programs in the United States and Canada. Therefore, insecticides with low mammalian toxicity, minimal nontarget impacts, and systemic distribution are desirable for eradication and management programs. Systemic treatments eliminate problems associated with cover sprays such as drift, wet foliage, dermal applicator exposure, and impact on foliage-inhabiting beneficial organisms.

We selected two insecticides, azadirachtin and imidacloprid, for laboratory evaluation of toxicity to *A. glabripennis.* Azadirachtin, a tetranortriterpenoid compound, is the most active insecticidal constituent of neem, which is extracted from the seeds of the Southeast Asian neem tree, *Azadirachta indica* A. Juss (Schmutterer 1990). Neem is a natural biodegradable product, which makes its use more acceptable to the public. In addition, it has low mammalian toxicity (acute oral toxicity $LD_{50} > 2{,}000$ mg/kg), contributing to its safety. It is less toxic to nonphytophagous insect species, including pest natural enemies and bees, than many conventional insecticides (Stark 1992, McCloskey et al. 1993, Naumann and Isman 1996). Several studies have demonstrated that neem seed extracts move systemically within plants (Osman and Port 1990), making it possible to apply treatments directly to the soil or vascular system where they are translocated throughout the plant. Imidacloprid, a chloronicotinyl insecticide, is a nicotine mimic active on the nicotinic acetylcholine receptor (Bai et al. 1991). It is highly effective for control of homopteran pests and many species in the orders Coleoptera, Diptera, and Hymenoptera. It is active against some species of Lepidoptera. Imidacloprid has excellent systemic and translaminar properties and high residual activity. Mammalian toxicity is low (acute oral toxicity $LD_{50} >$ 450 mg/kg), and impacts on nontargets are generally low. Bees, however, are highly susceptible to imidacloprid (Elbert et al. 1991). Both azadirachtin and imidacloprid are currently registered for indoor and outdoor use on ornamentals, trees, shrubs, horticultural crops, and plants in and around commercial nurseries, greenhouses, and interiorscapes.

The use of azadirachtin and imidacloprid for management of tree insect pests has shown promise for other insects. Azadirachtin injected into the root flares of paper birch, *Betula papyrifera* Marsh., provided control of birch leafminer, *Fenusa pusilla* (Lepeletier) (Hymenoptera: Tenthredinidae), resulting in a fivefold reduction in leaf damage compared with untreated trees and reducing damage more effectively than conventional trunk-injection treatments with Meta-Systox-R (Marion et al. 1990). In addition, trunk-injections with neem are effective against bark-mining and defoliating forest insect pests, including the mountain pine beetle, *Dendroctonus ponderosae* Hopkins (Coleoptera: Scolytidae) (Naumann et al. 1994); the pine engraver, *Ips pini* (Say) (Coleoptera: Scolytidae) (Duthie-Holt et al. 1999); pine false webworm, *Acantholyda erythrocephala* (L.) (Hymenoptera: Pamphiliidae) (Lyons et al. 1996, Helson et al. 2001); and spruce budworm, *Choristoneura fumiferana* (Clemens) (Lepidoptera: Tortricidae) (Wanner et al. 1997). Although the efficacy of azadirachtin for controlling longhorned wood borers (family Cerambycidae) has not been evaluated by trunk-injection, Zhong et al. (1998) observed 100% mortality and complete inhibition of oviposition by *Apriona germari* (Hope) (Coleoptera: Cerambycidae) when adults fed on twigs sprayed with neem extracts. Similarly, spraying neem extract on the food and body of *Xystrocera festiva* Pascoe (Coleoptera: Cerambycidae) repelled and killed the adults, acting as a contact poison (Suharti et al. 1995). Systemic use of imidacloprid significantly reduced coneworm, *Dioryctria* spp. (Lepidoptera: Pyralidae), damage in loblolly pine, *Pinus taeda* L., during the first but not the second year after tree injection (Grosman et al. 2002). In addition, it was found to be effective against *Glycaspis brimblecombei* Moore (Homoptera: Psyllidae) when microinjected into infested eucalyptus trees, *Eucalyptus camaldulensis* Dehnh. (Young 2002), and pine shoot beetle, *Tomicus piniperda* (L.), when applied as a foliar spray to Scots pine trees, *Pinus sylvestris* L. (McCullough and Smitley 1995).

Because of federal quarantine regulations, research on live *A. glabripennis* is restricted to approved quar-

antine facilities. Artificial rearing of laboratory colonies of *A. glabripennis* is both labor-intensive and costly, limiting the availability of beetles for research. To complement studies on *A. glabripennis*, we also evaluated a native cerambycid, the cottonwood borer, *Plectrodera scalator* (F.) (Coleoptera: Cerambycidae), as a surrogate for *A. glabripennis* in our laboratory. *Plectrodera scalator* is similar in size and life history to *A. glabripennis*. Both species are members of the same subfamily and tribe (Yanega 1996) and feed on *Populus* and *Salix* spp. Moreover, *P. scalator* is a native pest of cottonwood in plantations in the southern United States, is readily collected in the field, and is able to be reared in the laboratory using recently developed methods.

Our objectives were to 1) determine the toxicity of imidacloprid and azadirachtin in *A. glabripennis* and *P. scalator* larvae in artificial diet, and 2) determine the toxicity of imidacloprid in *A. glabripennis* adults feeding on twigs.

Materials and Methods

Insects for Bioassays. *A. glabripennis* were reared in the USDA–Forest Service quarantine laboratory in Ansonia, CT (Keena 2002). Pairs of adult beetles were held in glass jars with moist paper towel to maintain humidity; freshly cut twigs of sugar maple, *Acer saccharum* Marsh, for food; and small (≈6 cm in diameter by 20 cm in length) freshly cut billets from sugar maple branches for oviposition. The oviposition billets were waxed on both ends and replaced each week. Removed oviposition billets were held for 3 wk to allow for egg hatch, at which time first instars were dissected from the billets and transferred individually to artificial diet in clear plastic jars (Keena 2005). Every other week, larvae were transferred to fresh diet in successively larger jars (59, 188, and 237 ml) as they grew. Larvae were held in the dark at 25 ± 2°C and 60 ± 5% RH. *A. glabripennis* pupae were held in 50-ml containers in a water box with grating in the bottom to support containers above the water (Keena 2002). Voucher specimens of *A. glabripennis* were deposited at the Entomology Division, Yale Peabody Museum of Natural History, New Haven, CT.

P. scalator was reared in our USDA–Forest Service laboratory at Michigan State University in East Lansing, MI. Mating pairs of beetles were held at 24°C, 70% humidity, and a photoperiod of 16:8 (L:D) h in ventilated plastic boxes with moist paper towel and freshly cut twigs of willow, *Salix* spp. Fresh sticks were provided weekly. Females oviposited in the twigs and paper toweling; eggs were removed, surface sterilized, allowed to hatch in petri dishes, and placed in individual jars on artificial diet. Our artificial diet for *P. scalator* was modified from *Prionus imbricornis* (Coleoptera: Cerambycidae) diet #3 (Payne et al. 1975) as follows: 1) increase water from 750 to 950 ml/liter diet, 2) delete formaldehyde, 3) substitute Wesson's Salt Mix without iron, and 4) add 147 mg of ferric phosphate. Larvae were reared in the dark at 25 ± 2°C and 60 ± 5% RH and transferred to fresh diet every other week in increasingly larger jars (60, 120, and 240 ml). Pupae were placed on the diet surface until adult emergence.

Larval Diet Bioassays. Bioassays were conducted with both *A. glabripennis* and *P. scalator* larvae with concentrations of the systemic insecticides imidacloprid (Imicide, 10% [AI], J.J. Mauget, Co., Arcadia, CA) and azadirachtin (Ornazin, 3.3% [AI], Amvac Chemical Corporation, Los Angeles, CA) incorporated into *P. scalator* artificial diet. Serial dilutions of the insecticide formulations were prepared in distilled water. Blank formulations containing only inert ingredients were provided by the manufacturers and served as controls. Artificial diet was prepared in 0.5-liter batches to which 10 ml of diluted insecticide, blank formulation (formulation control), or distilled water (control) was added and thoroughly mixed. For each species and insecticide, five replicates of six treatments were tested. Imidacloprid treatments were 1) 160 ppm (AI) 2) 16 ppm (AI), 3) 1.6 ppm (AI), 4) 0.16 ppm (AI), 5) 0 ppm (AI) (formulation control), and 6) water control. Azadirachtin treatments were 1) 50 ppm (AI), 2) 5 ppm (AI), 3) 0.5 ppm (AI), 4) 0.05 ppm (AI), 5) 0 ppm (AI) (formulation control), and 6) water control. Fifth instars (*A. glabripennis* mean weight, 1.23 ± 0.09 g; *P. scalator* mean weight, 0.82 ± 0.02 g) were placed individually in jars containing the treated diet and held in the dark at room temperature (20 ± 2°C) for 14 wk (22 December 2000–30 March 2001 for *A. glabripennis* and 17 November 2000–28 February 2001 for *P. scalator*). Every other week during this period, larvae were removed from the diet, individually weighed, and assessed for mortality; live larvae were transferred to fresh insecticide-treated diet.

Food consumption was estimated by measuring the length of feeding tunnel in the diet, as observed through the side of the jar. Feeding was considered heavy if tunnel length was >10 cm, medium if 5–10 cm, light if <5 cm, and absent if no tunnel was visible. Feeding scores were assigned as 3 for heavy, 2 for medium, 1 for light, and 0 for none. Overall feeding scores were calculated by averaging the semiweekly feeding scores for each insect.

Adult Twig-Feeding Bioassays. A twig-feeding bioassay was conducted from 18 July to 7 August 2002 by using *A. glabripennis* adults. Two concentrations (low, 15 ppm and high, 150 ppm) of imidacloprid (Imicide 10% [AI], J.J. Mauget Co.) were prepared by serial dilution in distilled water. Sugar maple twigs (2–7 mm in diameter, 10–15 cm in length) were dipped into the low or high concentration of imidacloprid or distilled water (control) and allowed to dry for 2 h. Twigs were placed in glass jars lined with paper towels; 10 twigs of the same treatment were used for each jar. Adult *A. glabripennis* were introduced individually into the jars with the twigs and allowed to feed. Fourteen adults (seven male, seven female) were used for each imidacloprid concentration and 12 adults (four male, eight female) for the control. Because of limited availability of *A. glabripennis*, adults averaged 44 ± 13 d old (range, 8–62 d) at the beginning of the bioassay. Adult

Table 1. Median lethal concentrations (LC_{50}) of imidacloprid or azadirachtin for *A. glabripennis* or *P. scalator* fifth instars fed insecticide-treated artificial diet for 14 wk

Insecticide	*n*	Slope ± SE	LC_{50} (ppm)	95% CL	χ^2	LC_{50}/g^a (ppm/g)
A. glabripennis						
Azadirachtin	5	0.53 ± 0.30	23.55	–	2.47[b]	19.15
Imidacloprid	5	−0.55 ± 0.42	4.92	–	0.003[b]	4.00
P. scalator						
Azadirachtin	5	1.23 ± 0.46	1.58	0.22–11.51	1.21[b]	1.92
Imidacloprid	5	0.71 ± 0.30	1.78	0.0067–21.88	2.74[b]	3.34

[a] LC_{50}/g was calculated by dividing the LC_{50} by the average initial fresh weight of larvae used for the bioassay.
[b] χ^2 value not significant at the $\alpha = 0.05$ level, indicating that the observed mortality was not significantly different from the expected mortality generated by the concentration–mortality response curve of the probit model.

A. glabripennis were randomly assigned to treatments. The jars were held at room temperature (20 ± 2°C). Beetles were checked for mortality 1 d after treatment and then weekly for 3 wk. Each week, live beetles were transferred to clean jars with freshly treated twigs. The amount of feeding was estimated by measuring the total length of bark removed by adult *A. glabripennis* feeding on the twigs in each jar. Feeding was considered heavy if the length of removed bark exceeded 20 cm, medium if it was between 10 and 20 cm, light if it was <10 cm, and absent if no bark removal was visible. Feeding scores were assigned as 3 for heavy, 2 for medium, 1 for light, and 0 for none. Overall feeding scores were calculated by averaging the weekly feeding scores for each insect.

Statistical Analyses. For both larval and adult bioassays, percentage of mortality for each treatment was corrected for control mortality using Abbott's formula (Abbott 1925). Median lethal concentrations (LC_{50}) and lethal times (LT_{50}) were estimated using probit analysis (PROC PROBIT, SAS Institute 1996). These calculations were done after 14 wk of insect exposure to the treated diets. Overall feeding scores were analyzed by treatment using the Kruskall–Wallis test followed by the Tukey test for ranked sums (PROC NPAR1WAY, SAS Institute 1996).

Results

Larval Diet Bioassays. Azadirachtin and imidacloprid were toxic to *A. glabripennis* and *P. scalator* larvae. For fifth instars of *A. glabripennis*, the estimated LC_{50} for azadirachtin was 23.55 ppm after the 14 wk of exposure to the treated diets (Table 1). Insect mortality was positively correlated with azadirachtin concentration, with the highest concentration at 50 ppm achieving 60% mortality (Fig. 1A). The estimated LT_{50} values at 5.0 and 50.0 ppm azadirachtin were 16.6 and 13.9 wk, respectively (Table 2). Weight gain for *A. glabripennis* larvae on diet at the lowest azadirachtin concentration (0.05 ppm), blank formulation, or water control increased and then stabilized as lar-

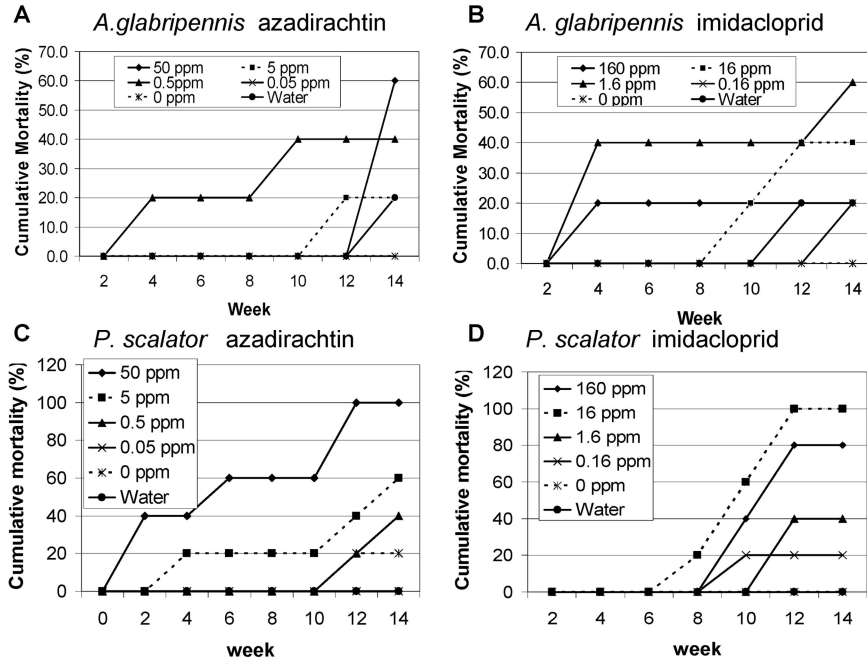

Fig. 1. Cumulative mortality (%) for *A. glabripennis* and *P. scalator* larvae fed artificial diet treated with various concentrations of azadirachtin and imidacloprid ($n = 5$).

Table 2. Median lethal times (LT_{50}) in weeks for *A. glabripennis* or *P. scalator* fed artificial diet containing azadirachtin at 5 and 50 ppm or imidacloprid at 16 and 160 ppm

Concn (ppm)	*n*	Slope ± SE	LT_{50} (wk)	95% CL	χ^2
A. glabripennis					
Azadirachtin					
5.0	5	8.73 ± 7.4	16.6	–	0.0[a]
50.0	5	108.8 ± 93	13.9	–	0.0[a]
Imidacloprid					
16.0	5	7.25 ± 3.9	14.0	–	0.72[a]
160.0	5	0.93 ± 1.1	76.0	–	0.90[a]
P. scalator					
Azadirachtin					
5.0	5	2.21 ± 1.16	15.2	–	1.62[a]
50.0	5	2.05 ± 8.6	4.0	0.41–6.74	3.98[a]
Imidacloprid					
16.0	5	16.04 ± 6.08	9.26	7.63–10.77	0.54[a]
160.0	5	11.7 ± 4.3	10.92	8.92–12.94	1.25[a]

[a] χ^2 value not significant at the $\alpha = 0.05$ level, indicating that the observed mortality was not significantly different from the expected mortality generated by the concentration–mortality response curve of the probit model.

vae approached the prepupal stage (Fig. 2A). At the higher azadirachtin concentrations (0.5, 5.0, and 50 ppm), *A. glabripennis* larvae consistently lost weight (Fig. 2A) and consumed significantly less than those exposed to the lowest concentration (0.05 ppm) and controls (Fig. 3A); this antifeedant effect was inversely correlated with concentration.

The estimated LC_{50} of imidacloprid was 4.92 ppm at 14 wk for fifth instars of *A. glabripennis* using the same bioassay method (Table 1). Unlike azadirachtin, mortality was inversely correlated with imidacloprid concentration; mortality was 20% for *A. glabripennis* larvae exposed to the 160 ppm imidacloprid versus 60% mortality for those exposed to 1.6 ppm (Fig. 1B). Estimated LT_{50} values for imidacloprid concentrations of 16.0 and 160.0 ppm were 14.0 and 76.0 wk, respectively (Table 2). *A. glabripennis* larvae fed diet treated with imidacloprid consistently lost weight, whereas those fed the formulation blank or water control gained weight (Fig. 2B). Imidacloprid exhibited a strong antifeedant effect with progressively lower feeding scores for larvae fed diet treated with higher concentrations of imidacloprid (Fig. 3B).

For *P. scalator*, the estimated LC_{50} was 1.58 ppm at 14 wk (Table 1). The highest concentration (50 ppm) of azadirachtin resulted in 100% mortality at 12 wk (Fig. 1C); as for *A. glabripennis*, mortality was positively correlated with azadirachtin concentration (Fig. 1A). The estimated LT_{50} values at 5 and 50 ppm were 15.2 and 4.0 wk, respectively (Table 2). Weight gain of *P. scalator* larvae treated at the lowest azadirachtin concentration (0.05 ppm), blank formulation, or water control initially increased then stabilized as they approached the prepupal stage (Fig. 2C). At the three higher concentrations (0.5, 5.0, and 50 ppm), *P. scalator* larvae gradually lost weight over the 14 wk (Fig. 2C). There was a strong antifeedant effect at the three highest concentrations of azadirachtin on *P. scalator* larvae, as indicated by significantly lower mean feeding scores than at the lowest rate or water and formulation controls (Fig. 3C).

The estimated LC_{50} of imidacloprid was 1.78 ppm at 14 wk (Table 1). The two highest concentrations of imidacloprid (160 and 16 ppm) resulted in 80 and 100%

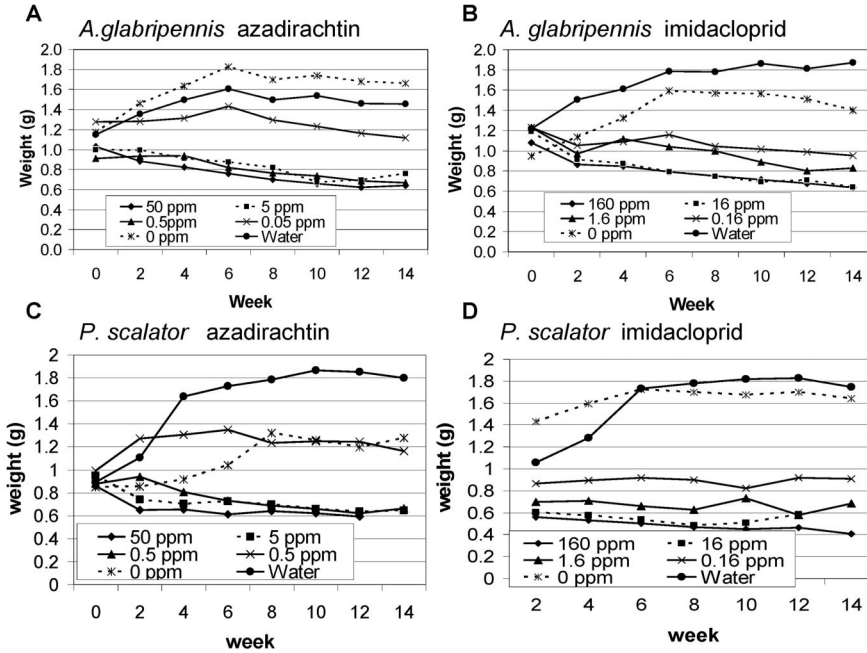

Fig. 2. Mean weekly weight of *A. glabripennis* and *P. scalator* larvae fed artificial diet treated with various concentrations of azadirachtin and imidacloprid ($n = 5$).

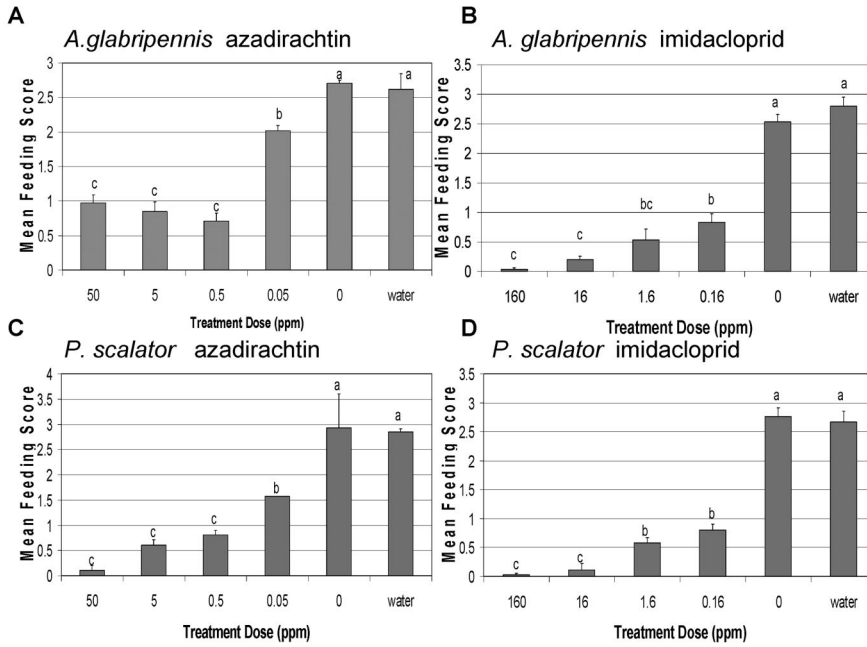

Fig. 3. Average semiweekly feeding scores (mean ± SE) for *A. glabripennis* and *P. scalator* larvae fed artificial diet with various concentrations of azadirachtin and imidacloprid. Feeding scores were assigned based on the length of feeding tunnels in the diet: 0, none; 1, light (<5 cm); 2, medium (5–10 cm); and 3, heavy (>10 cm). Bars topped with the same letter are not significantly different, Tukey test for ranked sums, $P < 0.05$ ($n = 5$).

mortality, respectively, of *P. scalator* larvae at 14 wk (Fig. 1D). The LT_{50} values for 16.0 and 160 ppm imidacloprid were similar at 9.3 and 10.9 wk, respectively (Table 2). Each concentration of imidacloprid exhibited a strong antifeedant effect for *P. scalator* larvae. Larvae gained weight when fed diet treated with the formulation blank or water control but lost weight or remained stable when fed diet treated with imidacloprid (Fig. 2D). Mean feeding scores were significantly lower for *P. scalator* larvae fed diet treated with imidacloprid compared with those treated with the blank formulation or water control (Fig. 3D).

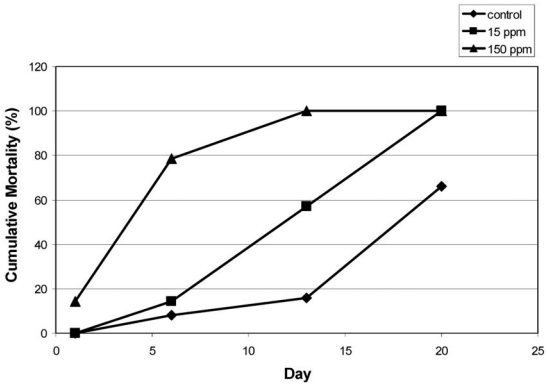

Fig. 4. Cumulative mortality (%) of *A. glabripennis* adults fed sugar maple twigs treated with 15 ppm or 150 ppm imidacloprid. ($n = 14$ for each treatment, $n = 12$ for control).

Adult Twig-Feeding Bioassay. Twigs treated at both 150 and 15 ppm imidacloprid were toxic to *A. glabripennis* adults. *A. glabripennis* adult mortality reached 100% by day 13 for twigs treated with the high concentration and by day 20 for twigs treated at the low concentration (Fig. 4). This corresponded to significantly different LT_{50} values of 2.58 and 11.5 d at the high and low concentrations, respectively (Table 3). Some natural mortality occurred during the bioassay because of the variable age of beetles used in this part of the study. Mortality of adults on untreated twigs reached 66% by day 20. Imidacloprid had a strong antifeedant effect on adults. No visible feeding was seen on twigs treated with 150 ppm, resulting in a mean feeding score of 0 ± 0. The feeding score for adults fed on twigs treated with 15 ppm was 0.32 ± 0.07 and was significantly lower than the score of 1.58 ± 0.16 for adults feeding on untreated twigs.

Table 3. Median lethal times (days) for adult *A. glabripennis* fed sugar maple twigs treated with 150 or 15 ppm imidacloprid

Imidiacloprid (ppm)	n	Slope ± SE	LT (d)	95% CL	χ^2
15	5	6.28 ± 1.52	11.5	9.12–14.00	2.45[a]
150	5	2.81 ± 0.59	2.58	1.49–3.97	0.908[a]

[a] χ^2 value not significant at the $\alpha = 0.05$ level, indicating that the observed mortality was not significantly different from the expected mortality generated by the concentration–mortality response curve of the probit model.

Discussion

Our results demonstrate that azadirachtin and imidacloprid are toxic to *A. glabripennis* and *P. scalator* larvae and *A. glabripennis* adults; however, both insecticides required lengthy exposure periods before larvae of these wood-boring beetles died. However, these insecticides exhibited an immediate and dose-dependent antifeedant effect with corresponding weight loss. Prolonged larval mortality was likely because of a combination of starvation and toxic effects accumulated during feeding. Elbert et al. (1991) demonstrated that imidacloprid is both a toxin and antifeedant. Azadirachtin from neem seed oil has insecticidal, antifeedant, growth-inhibitory, oviposition-deterrent, antihormonal, and antifertility properties against numerous insects (Jacobson 1986, 1989; Schmutterer 1988, 1990; Arnasan et al. 1989; Warthen 1989; Koul et al. 1990; Ascher 1993).

At 14 wk, LC_{50} values for *P. scalator* larvae were 1.58 ppm for azadirachtin and 1.78 ppm for imidacloprid. The highest final larval mortality levels were lower for *A. glabripennis* (60% for both azadirachtin and imidacloprid) than for *P. scalator* (100% for both imidacloprid and azadirachtin). LC_{50} values estimated for *A. glabripennis* (4.92 ppm for imidacloprid and 23.55 ppm for azadirachtin) were higher than those for *P. scalator.* The *A. glabripennis* larvae tested were somewhat larger (mean weight = 1.23 ± 0.09 g) than the *P. scalator* larvae (mean weight 0.82 ± 0.02 g) and may have required a higher concentration or longer time to experience higher levels of mortality. When adjusted for initial larval weight, the LC_{50}/g values were similar for imidacloprid (4.00 ppm/g for *A. glabripennis* and 3.34 ppm/g for *P. scalator*) (Table 1). However, LC_{50}/g for azadirachtin was higher for *A. glabripennis* (19.15 ppm/g) than for *P. scalator* (1.92 ppm/g) (Table 1). The LC_{50} values estimated for *P. scalator* larvae are within the range of levels typically achieved in leaf and twig material collected from trees injected according to label instructions (Tattar et al. 1998; Poland et al. 2006).

Imidacloprid seemed to have a somewhat stronger larval antifeedant effect than did azadirachtin for both insect species. For both *A. glabripennis* and *P. scalator* larvae, imidacloprid was a strong antifeedant at all concentrations with virtually no feeding at 160 ppm. However, the lowest azadirachtin concentration (0.05 ppm) had only a weak antifeedant effect, and some feeding occurred at the highest concentration (50 ppm). For azadirachtin, the highest concentration (50 ppm) resulted in the highest larval mortality after 14 wk for both *A. glabripennis* (60%) and *P. scalator* (100%). However, the highest concentration of imidacloprid (160 ppm) resulted in lower larval mortality after 14 wk than did some of the lower rates for both *A. glabripennis* (highest mortality = 60% for 1.6 ppm) and *P. scalator* (highest mortality = 100% for 16 ppm). It is likely that lower mortality of *A. glabripennis* and *P. scalator* larvae at higher imidacloprid concentrations was because of low feeding rates resulting in larvae ingesting less imidacloprid within the same time than larvae consuming a lower concentration. Conversely, at the lowest concentrations of imidacloprid (0.16 ppm) and azadirachtin (0.05 ppm), larvae consumed more diet, but it is likely the concentrations were too low to provide a lethal dose.

Although larvae consuming diet with high concentrations of azadirachtin and imidacloprid did not feed and lost weight, mortality was prolonged and several larvae did not die by the end of our experiment. This is typical of many wood-boring larvae that are able to remain alive with little or no feeding for prolonged periods of time (Linsley 1943, Smith 1962, Haack and Slansky 1986). However, *A. glabripennis* adults fed twigs treated with 150 ppm imidacloprid died rapidly (Fig. 4), although no visible feeding was evident. The rapid mortality of adults may have resulted, in part, from faster starvation of short-lived active adults compared with relatively sedate and long-lived larvae. Moreover, the toxicity of imidacloprid may be higher for adults than for larvae because a strong and immediate knockdown effect was observed for adults fed twigs treated with the high imidacloprid concentration (T.M.P., unpublished data).

The LT_{50} values for *A. glabripennis* adults fed twigs treated with imidacloprid were found to be 2.58 d (150 ppm) and 11.5 d (15 ppm) (Table 3). Typical residue levels of imidacloprid in leaves and twigs of injected trees are in the range of 1–2 ppm (Poland et al. (2006). Therefore, *A. glabripennis* adults may survive for several days or even a few weeks in the field after feeding on treated trees. Further studies on the effects of insecticides on mating and oviposition behavior are required to determine whether beetles that feed on treated trees are capable of ovipositing before they die.

Because of limited availability of *A. glabripennis* reared under quarantine conditions, replication of the experiments was low, resulting in limited confidence in LC_{50} and LT_{50} values and limited ability to compare results for *A. glabripennis* and *P. scalator.* Nevertheless, the overall finding of similar toxic and antifeedant effects of imidacloprid and azadirachtin against *A. glabripennis* and *P. scalator* indicates that *P. scalator* serves as a suitable surrogate for *A. glabripennis.*

Implications for Management. To successfully implement systemic insecticides for *A. glabripennis* management, our data suggest that it is critical to deliver a high and sustained dose of insecticide. At sublethal concentrations, larvae can survive for prolonged periods of time. If insecticide levels are not persistent, it is possible that larvae may eventually resume feeding and complete development. Adults also may survive for several days after feeding on twigs treated with imidacloprid. Therefore, it is possible that adults could leave treated trees because of antifeedant effects of the insecticides. Further investigations are required to determine whether all potential host trees in an area must be treated to eliminate the possibility of surviving adults switching to nearby untreated hosts. Overall, the systemic insecticides, imidacloprid and azadirachtin, demonstrated antifeedant and toxic effects

on *A. glabripennis* and *P. scalator* and have potential for use in an *A. glabripennis* management program.

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