Field Evaluations of Systemic Insecticides for Control of *Anoplophora glabripennis* (Coleoptera: Cerambycidae) in China

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ABSTRACT *Anoplophora glabripennis* (Motschulsky) (Coleoptera: Cerambycidae), a pest native to China and Korea, was discovered in North America in 1996. Currently, the only reliable strategy available for eradication and control is to cut and chip all infested trees. We evaluated various doses of the systemic insecticides azadirachtin, emamectin benzoate, imidacloprid, and thiacloprid for control of *A. glabripennis* in naturally infested elms (*Ulmus* spp.), poplars (*Populus* spp.), and willows (*Salix* spp.) in China between 2000 and 2002. Significantly more dead *A. glabripennis* adults were found beneath elm and poplar trees treated with imidacloprid (in 2000 and 2001) or thiacloprid (in 2001) and beneath willow trees injected with imidacloprid or thiacloprid (in 2002) compared with control trees. In 2000, 4 mo after injection, the density of live *A. glabripennis* was significantly reduced in poplar trees treated with imidacloprid (90%) and in willow trees treated with imidacloprid (83%) or emamectin benzoate (71%) compared with controls. In 2001, 9 mo after injection, the density of live *A. glabripennis* was significantly reduced in poplar (76%) and willow (45%) trees treated with imidacloprid compared with control trees. Similarly, percentage mortality of all life stages of *A. glabripennis* feeding within trees was significantly higher on poplar trees 4 mo after injection with imidacloprid (64%) in 2000 and on elms (55%) and poplars (63%) 9 mo after injection with imidacloprid in 2001 compared with control trees. Imidacloprid residue levels in leaves and twigs collected at various times from 1 d to 9 mo after injection ranged from 0.27 to 0.46 ppm. Injecting *A. glabripennis*-infested trees with imidacloprid can result in significant mortality of adults during maturation feeding on leaves and twigs and of all life stages feeding within infested trees. Imidacloprid is translocated rapidly in infested trees and is persistent at lethal levels for several months. Although, injection with imidacloprid does not provide complete control of *A. glabripennis*, systemic insecticides may prove useful as part of an integrated eradication or management program.

KEY WORDS *Anoplophora glabripennis*, imidacloprid, thiacloprid, azadirachtin, emamectin benzoate

Anoplophora glabripennis (Motschulsky) (Coleoptera: Cerambycidae) was discovered in North America in New York in 1996 (Haack et al. 1997), and since then it has been found in Illinois (Poland et al. 1998), New Jersey (Haack 2003), and Ontario, Canada (CFIA 2005, Haack 2006). It is native to eastern China and Korea (Lingafelter and Hoebeke 2002) and attacks several species of hardwood trees, including members of the genera *Acer*, *Aesculus*, *Betula*, *Populus*, *Salix*, and *Ulmus* (Haack et al. 1997). *A. glabripennis* is thought to have entered North America in solid wood packing materials associated with imports from Asia.

In North America, *A. glabripennis* adults are generally active from May to October and feed on foliage and small twigs of their host trees. Females make oviposition pits in the bark of branches and tree trunks to lay their eggs. After hatching, the larvae initially feed under the bark and later in the xylem. Larvae overwinter in their feeding tunnels and then pupate just under the bark in spring or early summer. Newly developed adult beetles leave round exit holes in the bark as they chew their way out of the tree (Haack et al. 1997, Becker 2000, Smith 2000). Larval tunneling results in branch dieback and eventually tree mortality if population densities are high or infestations persist for several years (Haack et al. 1997). Larval feeding also structurally weakens tree stems and branches.

Eradication efforts under the direction of the USDA–Animal and Plant Health Inspection Service (APHIS) and the Canadian Food Inspection Agency (CFIA) have been underway at all North American sites since the discovery of *A. glabripennis.* As part of the eradication programs, intensive surveys are con-

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ducted to locate all infested trees, which are then cut down and chipped. In New Jersey, susceptible host trees in the surrounding area are cut and destroyed. Similarly, in Canada, all host trees within 400 m of an infested tree are removed. The eradication program has cost millions of dollars and resulted in the removal of >8,000 infested trees in the United States (Nowak et al. 2001, Haack 2006). In Canada, >12,000 host trees, of which hundreds were infested, have been cut (CFIA 2005). USDA–APHIS has incorporated the use of the systemic insecticide imidacloprid in the eradication program (USDA–APHIS 2005). Uninfested host trees within the quarantine areas are treated by soil or trunk injection in the hope they will be protected from attack by *A. glabripennis.* It is thought that systemic insecticides could kill *A. glabripennis* adults during maturation twig feeding and larvae feeding in the cambial region and sapwood.

Insecticides that can be injected directly into trees and have low mammalian toxicity and minimal nontarget impacts are preferred for use in the eradication program because of concerns over human and nontarget exposure in urban areas. Based on the abovementioned criteria, we selected azadirachtin, emamectin benzoate, imidacloprid, and thiacloprid to evaluate for control of *A. glabripennis.*

Azadirachtin, a tetranortriterpenoid, is the most active insecticidal constituent of neem that is extracted from the seeds of the southeast Asian neem tree, *Azadirachta indica* A. Juss (Schmutterrer 1990). It has low mammalian and nontarget toxicity (Stark 1992, McCloskey et al. 1993, Naumann and Isman 1996) and moves systemically within plants (Marion et al. 1990). Emamectin benzoate is a semisynthetic insecticide derived from avermectin, a class of insecticidal compounds made by fermentation of the soil bacterium *Streptomyces avermitilis.* It has low nontarget and mammalian toxicity (Chukwudebe et al. 1998, Roy et al. 2000), binds tightly to soil and thus tends not to leach or accumulate in the environment (Mushtaq et al. 1996, Chukwudebe et al. 1997), and is available as a water-soluble formulation that can be injected into trees (Takai et al. 2000a, b). Imidacloprid is a chloronicotinyl insecticide that mimics nicotine and acts on the nicotinic acetylcholine receptor (Bai et al. 1991). Imidacloprid has good systemic properties, low mammalian toxicity, and generally low nontarget impacts (Elbert et al. 1991). Thiacloprid is a recently developed nicotinoid compound. It is similar to imidacloprid and has a favorable environmental profile (Tomizawa et al. 2000). Imidacloprid was found to be toxic against *A. glabripennis* adults and larvae in laboratory evaluations (Poland et al. 2006). Azadirachtin was not tested against adults but was toxic against *A. glabripennis* larvae in the laboratory.

Our overall objective was to evaluate the efficacy of azadirachtin, emamectin benzoate, imidacloprid, and thiacloprid for controlling *A. glabripennis* in naturally infested trees. Our specific objectives were to 1) determine insecticide residue levels in the leaves and twigs of trees injected with systemic insecticides; 2) determine mortality of *A. glabripennis* adults feeding on twigs of treated trees in the field; and 3) evaluate density of live *A. glabripennis* larvae, pupae, adults, and fresh exit holes and mortality of all life stages feeding within trees injected with systemic insecticides. This work was conducted in portions of China that had high *A. glabripennis* populations. Similar work could not have been conducted in the United States because infested trees are removed soon after discovery.

Materials and Methods

Three field experiments were conducted in China between 2000 and 2002. For each experiment, trees were injected with systemic insecticides; insecticide residue levels were determined in leaves and twigs; the numbers of dead *A. glabripennis* adults found beneath treated and untreated trees were compared; and density of live *A. glabripennis* larvae, pupae, adults, and fresh exit holes and mortality of all internally feeding life stages were determined by cutting down and dissecting trees.

2000 Field Experiment. The 2000 field experiment was conducted in Gansu province near Bayin City (35° N, 105° E). The field sites consisted of windrow plantings of trees surrounding agricultural fields. The agricultural fields were irrigated approximately once every 2 wk. We tested three systemic insecticides by using three common *A. glabripennis* host tree species. The test trees included a species of elm, *Ulmus pumila* L.; poplar, *Populus nigra* variety *thevestina* (Dode) Bean; and willow *Salix matsudana* Koidz. The trees were ≈8–10 yr old with mean ± SE diameters at breast height (dbh at ≈1.5 m) and heights of 8.9 ± 0.5 cm and 5.9 ± 0.3 m (elm), 6.7 ± 0.2 cm and 5.7 ± 0.2 m (poplar), and 9.6 ± 0.2 cm and 4.6 ± 0.3 m (willow). For each species, 24 heavily attacked and 24 lightly attacked trees were selected (six trees of each attack level per treatment). In addition, 12 unattacked elm and willow trees were included (three unattacked trees per treatment). No unattacked poplars were available for inclusion in the experiment. Heavily attacked trees had numerous oviposition pits and exit holes on the branches, upper trunk, and main bole within 2 m of the ground. Lightly attacked trees had some oviposition pits or exit holes visible on the branches but none on the main bole within 2 m of the ground. Unattacked trees displayed no signs of attack and had no visible oviposition pits or exit holes. Trees were injected with systemic insecticides on 11–14 June 2000 when adults were beginning to emerge, feed, and oviposit. The insecticide treatments included 1) imidacloprid (Imicide, 10% [AI], J. J. Mauget Co., Arcadia, CA), 2) emamectin benzoate (Shot Wan Liquid Formulation, 10% [AI], Syngenta, formerly Novartis, Greensboro, NC), 3) azadirachtin (Ornazin, 3.3% [AI], Amvac Chemical Corp., Los Angeles, CA), and 4) untreated control. Insecticides were injected at the label rates by using commercial injectors if available. Imidacloprid was injected using Mauget microinjection capsules, which were inserted into predrilled holes around the root flare. It was

injected at the label rate of 0.06 g/cm dbh. Emamectin benzoate was injected using Shot Wan 30-ml injectors that were inserted into predrilled holes around the root flare. It was injected at the label rate of 0.2 g/cm up to 15 cm dbh with an additional 0.6 g/cm for each 5-cm increment in dbh. Azadirachtin was injected using systemic tree injection tubes (STITs; Helson et al. 2001). The STITs were inserted into predrilled holes 50 cm above ground at the base of the tree. Each STIT contained 25 ml of Ornazin, which was injected at a rate of 0.165 g/cm dbh.

To increase the probability that oviposition would occur on each test tree, on 26–27 July 2000, 6 wk after injection, two pairs of field-collected *A. glabripennis* adults were caged on each lightly infested or uninfested tree. Wire-screen cages 1 m in height were constructed around the bole of each tree at 50 cm above ground level. Fresh twigs with foliage collected from healthy trees were placed in small cups of water and enclosed in each cage. Twigs, foliage, and any dead adults were replaced every other day for 1 mo. On 26–28 July 2000, samples (≈2 liters of loosely packed tissue) of current-year leaves and twigs were collected from two or three branches in the midcanopy of three heavily attacked trees of each species and treatment and were held frozen in plastic bags until analyzed to determine insecticide residue levels. Insecticide residue levels in leaves and twigs are indicative of insecticide translocation throughout treated trees and of dosages received by adults during maturation feeding. Insecticide residue levels in phloem and sapwood, where larvae feed, were not analyzed because of the complexity of collecting and processing wood samples and extracting residues from wood.

Insecticide residue analyses were performed at the Chinese Academy of Forestry. Leaf and twig samples were dried separately at 60°C for 48 h and then ground and passed through a 60-mesh sieve. A 5-g sample of the homogenized tissue was analyzed. Insecticide residues were extracted from the homogenate by adding 50 ml of solvent and shaking in an ultrasonic bath for 2 h. The solution was held overnight and then filtered. The filtrate was reextracted in 30 ml of solvent, shaken in an ultrasonic bath for 2 h, held overnight and refiltered. The solvent used for imidacloprid extraction was methylene chloride (CH_2Cl_2); methyl alcohol (CH_3OH) was used for azadirachtin and thiacloprid (tested in 2001 and 2002) extraction. The extracts were evaporated in a ventilated hood to dryness and then dissolved in methyl alcohol (for imidacloprid) or acetonitrile (CH_3CN, for azadirachtin and thiacloprid) and filtered through a 0.45-μm membrane. The filtrate was analyzed for insecticide residues by high performance liquid chromatography (HPLC) using a Waters model 244 HPLC fitted with a Diamaosil C18 (0.4 by 25 cm) analytic column. The mobile phase consisted of acetonitrile (35% in water for imadacloprid, 60% for azadirachtin, and 40% for thiacloprid). The flow rate was 0.7 ml/min, and a UV detector was used. Samples were quantified using external standards, and the recovery rate was >95%. Residue levels were not determined for emamectin benzoate because analytical procedures were not available at the time.

On 2 September 2000, the ground at the base of each poplar and elm tree was inspected for dead *A. glabripennis* adults. Dead adults beneath each tree were collected and tallied. Only dead adults within 1 m of the base of each tree were collected to ensure that they had fallen from the same tree. Dead adults were not tallied at the willow site because of potential disturbance at the site.

On 25–30 October 2000 (4 mo after injection), three heavily attacked and three lightly attacked trees of each species and treatment as well as two previously unattacked elms and willows of each treatment were cut down and dissected to determine density of live *A. glabripennis* larvae, pupae, adults, and fresh exit holes and mortality of all life stages within each tree. Trees were cut at ground level and bucked into 1-m sections. Bolt length and diameter were measured, and oviposition pits and exit holes in the outer bark were tallied before debarking and splitting. The number of dead and live *A. glabripennis* individuals of each life stage (eggs, small larvae, large larvae, pupae, and adults) was recorded. Small larvae included larvae that were <2 cm in length and were typically found in the cambial region or outer sapwood. Large larvae were >2 cm in length and were usually found deeper in the sapwood. On 25–30 June 2001, the remaining trees (three heavily infested and three lightly infested trees of each species and treatment and one previously unattacked elm of each treatment) were cut down and dissected according to the same procedures as in October 2000.

2001 Field Experiment. The 2001 field experiment included trees of the same three species as used in 2000. Poplar and elm trees were again located in Gansu Province at the same field site used in 2000. The trees were ≈8–10 yr old with mean diameters and heights of 7.6 ± 0.2 cm and 6.1 ± 0.2 m (poplar) and 10.9 ± 0.4 cm and 6.8 ± 0.3 m (elm). The willow trees (20.1 ± 0.4 cm in diameter) were located in eastern China in Hebei Province, near Wuji (40° N, 117° E). On 2–5 July 2001, 50 lightly infested trees of each species were injected (10 trees per treatment). The systemic insecticides tested included 1) imidacloprid (Imicide, 10% [AI]), 2) low-dose thiacloprid (Thiacide, 5% [AI], J. J. Mauget Co.), 3) high-dose thiacloprid (Thiacide, 10% [AI], J. J. Mauget Co.), 4) azadirachtin (Ornazin, 3.3% [AI]), and 5) untreated control. Azadirachtin was injected using STITS, and imidacloprid and thiacloprid were injected using Mauget microinjection capsules in the same manner as described above for the 2000 field experiment. Application rates for the insecticides were 0.9 g/cm dbh for imidacloprid, 0.45 g/cm dbh for low-dose thiacloprid, 0.9 g/cm dbh for high-dose thiacloprid, and 0.25 g/cm dbh for azadirachtin.

To increase the chances for oviposition, two pairs of field-collected *A. glabripennis* adults were released on each willow tree on 7 July 2001 (within 2 d after injection). Similarly, an additional pair of *A. glabrip-*

ennis adults was released on each willow tree on both 9 and 11 July 2001.

Dead *A. glabripennis* adults found on the ground within 1 m of the base of each test tree were collected and tallied daily from the day of injection until 15 July (willows) or every other day until 16 August 2001 (poplars and elms). Because of international travel restrictions in fall 2001, trees were not dissected, and insecticide residue analysis samples were not collected until 2002. On 12–15 April 2002, current-year leaves and twigs were collected from willow trees that had been injected with imidacloprid or azadirachtin to determine insecticide residue levels. Only twigs were collected from injected elm and poplar trees because the leaves had not flushed at the time samples were collected. Leaf and twig samples were collected in the same manner as described for the 2000 field experiment. Insecticide residue analyses were again conducted at the Chinese Academy of Forestry by using the same procedures as in 2000. All elm and poplar trees were cut down and dissected according to the same procedures as used for the 2000 field experiment. Because of local restrictions, the willow trees could not be cut down; therefore, two to three major branches (7.5 ± 0.3 cm in diameter at the base of the branch, 2.5 ± 0.07 m in length) were cut from each tree at an average height of 2.4 ± 0.03 m and were dissected in the same manner as the elm and poplar tree boles.

2002 Field Experiment. On 28 June 2002, 60 willow trees (22.6 ± 0.4 cm in diameter) were injected in Tianjin Province, in eastern China near Wuqing (39° N, 117° E). Fifteen trees were injected per treatment. The insecticide treatments included 1) low-dose imidacloprid (Imicide, 15% [AI]), 2) high-dose imidacloprid (Imicide, 25% [AI]), 3) thiacloprid (Thiacide, 15% [AI]), and untreated control. All insecticides were injected using Mauget microinjection capsules as described above for the 2001 field experiment. Application rates for the insecticides were 0.135 g/cm dbh for low-dose imidacloprid, 0.225 g/cm dbh for high-dose imidacloprid, and 0.135 g/cm dbh for thiacloprid. Dead *A. glabripennis* adults found on the ground within 1 m of the base of each tree were collected and tallied daily until 28 July 2002. On 29 June and on 27 July 2002, current-year leaves and twigs were collected from 10 trees per treatment to determine insecticide residue levels. Samples were collected in the same manner as described for the 2000 field experiment. Residue analyses were conducted at the Chinese Academy of Forestry by using the same procedures as in 2000.

Data Analyses. Density of live *A. glabripennis* was calculated as the total number of live larvae, pupae, adults, and fresh exit holes divided by the surface area of tree dissected. The surface area was calculated as the average circumference of each bolt times its height; the areas of all bolts from each tree were summed. Percentage mortality of *A. glabripennis* life stages within trees or branches was calculated as the proportion of dead individuals divided by the total number of live and dead individuals found in each tree (i.e., total number of dead eggs, larvae, pupae, and adults divided by total number of live plus dead eggs, larvae, pupae, adults, and exit holes). Trees in which no live or dead *A. glabripennis* life stages were found were dropped from the mortality analyses. For the 2000 field experiment, for each tree species and dissection date, density of live *A. glabripennis* and percentage mortality were analyzed using two-way analysis of variance (ANOVA) with main effects for infestation level and treatment. In no case was infestation level found to be significant. Therefore, to improve the power of the analysis, attack density and percentage mortality were analyzed by treatment with one-way ANOVA followed by the Ryan–Einot–Gabriel–Welsch (REGW) multiple comparison test (PROC GLM, SAS Institute 1996). For the 2001 field experiment, density of live *A. glabripennis* and percentage mortality for each tree species were analyzed by treatment with one-way ANOVA followed by the REGW test. Percentage mortality of *A. glabripennis* within the willow branches was subjected to an arcsine square-root transformation before analysis to satisfy assumptions of normality and homoscedasticity. For both 2000 and 2001, density of live *A. glabripennis* was transformed by log $(x + 1)$ before analysis. For all experiments, the number of dead adults found beneath each tree was analyzed by treatment using one-way ANOVA followed by the REGW test. For the 2002 field experiment, differences in residue levels for each treatment and tree tissue (leaves or twigs) were compared between the first sample collection and the second sample collection using a *t*-test (PROC TTEST, SAS Institute 1996). An α-level of 0.05 was used for all statistical tests.

Results

2000 Field Experiment. Poplar and willow trees injected with imidacloprid and willow trees injected with emamectin benzoate had significantly lower densities of live *A. glabripennis* compared with controls at 4 mo after injection (Table 1). At 12 mo after injection, density of live *A. glabripennis* was significantly lower in willow trees injected with imidacloprid. Poplar trees injected with imidacloprid had significantly higher mortality of all *A. glabripennis* life stages compared with uninjected control trees at 4 mo after injection. *A. glabripennis* mortality levels in poplar trees injected with azadirachtin or emamectin benzoate were intermediate between imidacloprid-injected trees and control trees at 4 mo postinjection. By 12 mo postinjection, the mortality level in the imidacloprid-injected poplar trees was no longer significantly different from control trees. There were no significant differences in *A. glabripennis* mortality among treatments for either elm or willow trees at 4 or 12 mo postinjection (Table 1).

Significantly more dead *A. glabripennis* adults were found beneath poplar and elm trees injected with imidacloprid compared with uninjected control trees (Table 2). The number of dead *A. glabripennis* adults found beneath trees injected with emamectin benzo-

Table 1. Mean density of live larvae, pupae, adults, and fresh exit holes and percentage mortality (mean ± SE) of all life stages of *A. glabripennis* in elm, poplar, and willow trees injected on 11–14 June 2000 with imidacloprid (0.06 g/cm dbh), emamectin benzoate (0.2–0.6 g/cm dbh), or azadirachtin (0.165 g/cm dbh) in Gansu Province, China

Insecticide treatment	Live *A. glabripennis*/m² surface area (% reduction relative to control)			% mortality of all life stages of *A. glabripennis*		
	Elm	Poplar	Willow	Elm	Poplar	Willow
4 mo postinjection						
Imidacloprid	8.2 ± 2.2a (30)	4.1 ± 1.9b (90)	3.8 ± 1.8c (83)	6.8 ± 3.3a	64.6 ± 11.6a	34.7 ± 13.4a
Emamectin benzoate	3.4 ± 1.2a (71)	33.6 ± 11.5a (16)	6.4 ± 1.7bc (71)	21.5 ± 15.8a	9.9 ± 1.7ab	18.1 ± 6.2a
Azadirachtin	7.9 ± 1.7a (34)	34.3 ± 6.4a (14)	16.3 ± 2.9ab (26)	8.3 ± 2.3a	12.6 ± 3.6ab	11.0 ± 3.1a
Control	11.7 ± 3.5a	39.9 ± 6.9a	21.9 ± 4.7a	18.7 ± 14.3a	0.5 ± 0.5b	13.3 ± 3.3a
ANOVA results						
F; df; *P*	2.5; 3, 19; 0.09	16.1; 3, 19; 0.0001	8.5; 3, 19; 0.001	0.4; 3, 19; 0.74	25.9; 3, 19; 0.0001	1.9; 3, 19; 0.15
12 mo postinjection						
Imidacloprid	3.2 ± 1.6a (73)	15.5 ± 4.9a (36)	1.9 ± 0.9c (90)	12.8 ± 11.2a	30.3 ± 14.8a	25.0 ± 25.0a
Emamectin benzoate	4.8 ± 1.9a (58)	21.2 ± 2.2a (13)	4.0 ± 0.6bc (79)	19.3 ± 5.7a	14.5 ± 5.4a	16.7 ± 6.8a
Azadirachtin	3.8 ± 1.3a (67)	29.1 ± 5.2a	36.7 ± 13.5a	13.9 ± 10.0a	12.2 ± 4.4a	8.4 ± 3.9a
Control	11.5 ± 3.4a	24.2 ± 2.1a	19.1 ± 13.6ab	7.1 ± 4.1a	11.6 ± 3.4a	4.7 ± 2.0a
ANOVA results						
F; df; *P*	2.6; 3, 18; 0.09	2.4; 3, 18; 0.10	10.6; 3, 11; 0.002	0.5; 3, 16; 0.70.7	1.2; 3, 18; 0.320.32	1.3; 3, 11; 0.320.32

Half of the trees were dissected 22–27 October 2000 (4 mo postinjection) and the remaining trees were dissected 25–30 June 2001 (12 mo postinjection) ($n = 6$ trees per treatment for each species and dissection date). Means within a column and dissection date followed by the same letter are not significantly different, REGW multiple comparison test, $P < 0.05$.

Density of live *A. glabripennis* was transformed by log $(x + 1)$ before analysis.

Trees with no *A. glabripennis* of any life stage were deleted from the analysis.

ate or azadirachtin were either intermediate or not significantly different from the untreated controls.

Imidacloprid residue levels in leaves and twigs 1 mo postinjection were found to be very similar among tree species. Residue levels in leaves and twigs, respectively, were found to be 1.49 ± 0.22 and 1.54 ± 0.12 ppm in elm, 1.46 ± 0.43 and 1.49 ± 0.03 ppm in poplar, and 1.15 ± 0.07 and 1.12 ± 0.13 ppm in Willow. Azadirachtin residue levels were much higher in elm and willow leaves than in poplar leaves or in twigs of any of the tree species. Azadirachtin residues levels in leaves and twigs, respectively, were found to be 30.64 ± 1.7 and 1.70 ± 0.28 ppm in elm, 3.94 ± 0.57 and 2.31 ± 0.25 ppm in poplar, and 9.21 ± 0.56 and 1.21 ± 0.18 ppm in willow.

2001 Field Experiment. The density of live *A. glabripennis* was significantly reduced in poplar trees and willow branches injected with imidacloprid compared with controls (Table 3). Percentage mortality of all *A. glabripennis* life stages was significantly higher within imidacloprid-injected trees than within uninjected control trees for both elms and poplars (Table 3). Poplar trees injected with low- or high-dose thiacloprid or azadirachtin had *A. glabripennis* mortality levels that were intermediate between imidacloprid-injected trees and control trees. Mortality of *A. gla-*

Table 2. Mean ± SE number of dead *A. glabripennis* adults beneath elm and poplar trees in Gansu Province and willow trees in Hebei province, China

Insecticide treatment	Mean no. of dead *A. glabripennis*/tree		
	Elm	Poplar	Willow
2000			
Imidacloprid	5.0 ± 0.9a	17.3 ± 6.8a	
Emamectin benzoate	0.3 ± 0.2b	4.7 ± 1.7ab	
Azadirachtin	0.3 ± 0.2b	2.8 ± 0.8b	
Control	1.2 ± 0.5b	0.2 ± 0.2b	
ANOVA results			
F; df; *P*	15.1; 3, 20; 0.0001	4.4; 3, 19; 0.016	
2001			
Imidacloprid	70.5 ± 12.4a	47.0 ± 10.1a	31.2 ± 6.9a
Low thiacloprid	35.9 ± 6.2b	54.6 ± 8.0a	15.7 ± 3.9ab
High thiacloprid	31.5 ± 5.6bc	45.0 ± 8.9a	18.5 ± 4.8ab
Azadirachtin	13.6 ± 3.5c	5.8 ± 1.0b	3.2 ± 0.6b
Control	8.8 ± 2.9c	9.5 ± 2.1b	
ANOVA results			
F; df; *P*	12.2; 4, 45; 0.0001	10.5; 4, 45; 0.0001	6.1; 3, 36; 0.002

In 2000, trees were injected with imidacloprid (0.06 g/cm dbh), emamectin benzoate (0.2–0.6 g/cm dbh), or azadirachtin (0.165 g/cm dbh on 11–14 June 2000 and dead adults were collected on 2 September 2000 ($n = 6$ trees per treatment within each species). In 2001, trees were injected with imidacloprid (0. 9 g/cm dbh), low-dose thiacloprid (0.45 g/cm dbh), high-dose thiacloprid (0.9 g/cm dbh), or azadirachtin (0.25 g/cm dbh) on 2–5 July 2001, and dead adults were collected every other day until 16 August 2001 ($n = 10$ trees per treatment within each species).

Dead adults were not collected from beneath any willow trees in 2000 or from beneath the control willow trees in 2001.

Means within a column and year followed by the same letter are not significantly different, REGW multiple comparison test, $P < 0.05$.

Table 3. Density of live larvae, pupae, adults, and fresh exit holes and percent mortality (mean ± SE) of all life stages of *A. glabripennis* in elm and poplar trees in Gansu Province and willow branches in Wu Ji Province, China

Insecticide treatment	Live *A. glabripennis*/m^2 surface area (% reduction relative to control)			% mortality of all life stages of *A. glabripennis*		
	Elm	Poplar	Willow	Elm	Poplar	Willow
Imidacloprid	2.0 ± 0.4a	3.5 ± 1.2b (76)	2.4 ± 1.5b (45)	54.9 ± 9.5a	63.1 ± 9.8a	28.1 ± 16.0ab
Low thiacloprid	5.5 ± 2.7a	11.4 ± 4.1ab (21)	2.5 ± 0.5ab (43)	0 ± 0b	34.3 ± 5.5b	35.5 ± 10.2a
High thiacloprid	3.4 ± 1.9a	16.7 ± 4.5a	3.6 ± 1.9ab (18)	8.1 ± 6.0b	19.7 ± 3.6bc	25.1 ± 11.6ab
Azadirachtin	1.1 ± 0.2a	9.5 ± 3.1ab (33)	7.9 ± 2.3a	0 ± 0b	37.7 ± 9.9b	2.3 ± 1.5b
Control	0.3 ± 0.3a	20.1 ± 2.1a	4.4 ± 1.3a	0 ± 0b	5.5 ± 1.9c	0 ± 0b
ANOVA results						
F; df; *P*	0.9; 4, 40; 0.5	4.2; 4, 40; 0.006	3.3; 4, 42; 0.01	9.8; 4, 40; 0.01	6.1; 4, 40; 0.001	3.98; 4, 42; 0.009

Trees were injected with imidacloprid (0.9 g/cm dbh), low-dose thiacloprid (0.45 g/cm dbh), high-dose thiacloprid (0.9 g/cm dbh), or azadirachtin (0.25 g/cm dbh) on 2–5 July 2001. Trees and branches were dissected on 12–15 April 2002 ($n = 10$ trees/treatment for each species). Means within a column followed by the same letter are not significantly different, REGW multiple comparison test, $P < 0.05$.

Density of live *A. glabripennis* was transformed by log $(x + 1)$ before analysis.

Percentage mortality in willow branches was transformed by arcsine square root before analysis.

Trees with no *A. glabripennis* of any life stage were deleted from the analysis.

bripennis was higher in branches of willow trees injected with low-dose thiacloprid compared with trees injected with azadirachtin or untreated control trees. Mortality of *A. glabripennis* in branches of willow trees injected with imidacloprid or high-dose thiacloprid was intermediate between mortality in trees injected with low-dose thiacloprid and untreated controls.

Significantly more dead *A. glabripennis* adults were found beneath elm and poplar trees injected with imidacloprid or low-dose thiacloprid compared with uninjected control trees (Table 2). Poplar trees injected with high-dose thiacloprid also had significantly more dead *A. glabripennis* adults beneath them than did uninjected control poplars. Control willow trees were not checked for dead *A. glabripennis* adults; however, significantly more dead beetles were found beneath the imidacloprid-injected willows compared with willows injected with azadirachtin. The number of dead *A. glabripennis* adults found beneath willow trees injected with thiacloprid (low or high dose) was intermediate between willows injected with imidacloprid and willows injected with azadirachtin.

The number of dead *A. glabripennis* adults found beneath poplar and elm trees increased over the first 3 wk after injection and then stabilized (elm trees injected with low- or high-dose thiacloprid) or declined (elm trees injected with imidacloprid, poplar trees injected with imidacloprid or low- or high-dose thiacloprid) (Fig. 1). Few dead adults were found beneath poplar or elm trees injected with azadirachtin or uninjected control trees. The number of dead *A. glabripennis* adults found beneath the willow trees peaked 4 d after injection and then declined for imidacloprid and both low- and high-dose thiacloprid. Very few dead adults were found beneath the willow trees injected with azadirachtin (Table 2).

Imidacloprid residue levels at 9 mo postinjection were found to be 0.29 ± 0.04, 0.44 ± 0.02, and 0.35 ± 0.03 ppm in elm, poplar, and willow twigs, respectively. The residue level of imidacloprid was 0.44 ± 0.03 in willow leaves; elm and poplar leaves had not flushed at the time samples were collected. Azadirachtin residue levels were found to be 0.73 ± 0.06, 3.58 ± 1.1, and 0.99 ± 0.1 ppm in elm, poplar, and willow twigs, respectively.

2002 Field Experiment. Significantly more dead *A. glabripennis* adults were found beneath willow trees injected with low- or high-dose imidacloprid (15.7 ± 2.0 and 15.2 ± 1.3, respectively) or thiacloprid (15.9 ± 1.7) compared with uninjected control trees (7.5 ± 1.2) ($F = 6.65$, df = 3, $P = 0.0006$). The number of dead *A. glabripennis* adults found beneath the treated willow trees was highest during the first week after injection and decreased steadily over the 5 wk the trees were inspected (Fig. 2). Residue levels of low-dose imidacloprid were similar between leaves and twigs and were higher 1 mo after injection than 1 d after injection (Table 4). Similarly, levels of high-dose imidacloprid were similar between leaves and twigs and were higher in leaves 1 mo after injection than 1 d after injection. Residue levels of thiacloprid in twigs were somewhat lower than residue levels for low- or high-dose imidacloprid and were higher 1 d after injection than 1 mo after injection.

Discussion

Of the insecticides tested, imidacloprid resulted in the highest and most consistent mortality levels of *A. glabripennis* adults and all life stages inside trees in 2000 (Tables 1 and 2) and in 2001 (Tables 2 and 3). *A. glabripennis* adult mortality was similar for imidacloprid and thiacloprid in 2002. Mortality of *A. glabripennis* adults feeding on leaves and twigs and of all life stages feeding within trees was not significantly increased in trees injected with either azadirachtin or emamectin benzoate (Tables 1–3). Reduced density of live *A. glabripennis* in poplar and willow trees injected with imidacloprid and willow trees injected with emamectin benzoate (Table 1) may have been because of a combination of mortality of within-tree life stages and mortality of adults resulting in less oviposition.

In the 2000 field experiment, mortality of all *A. glabripennis* life stages within poplar trees injected with imidacloprid was significantly increased com-

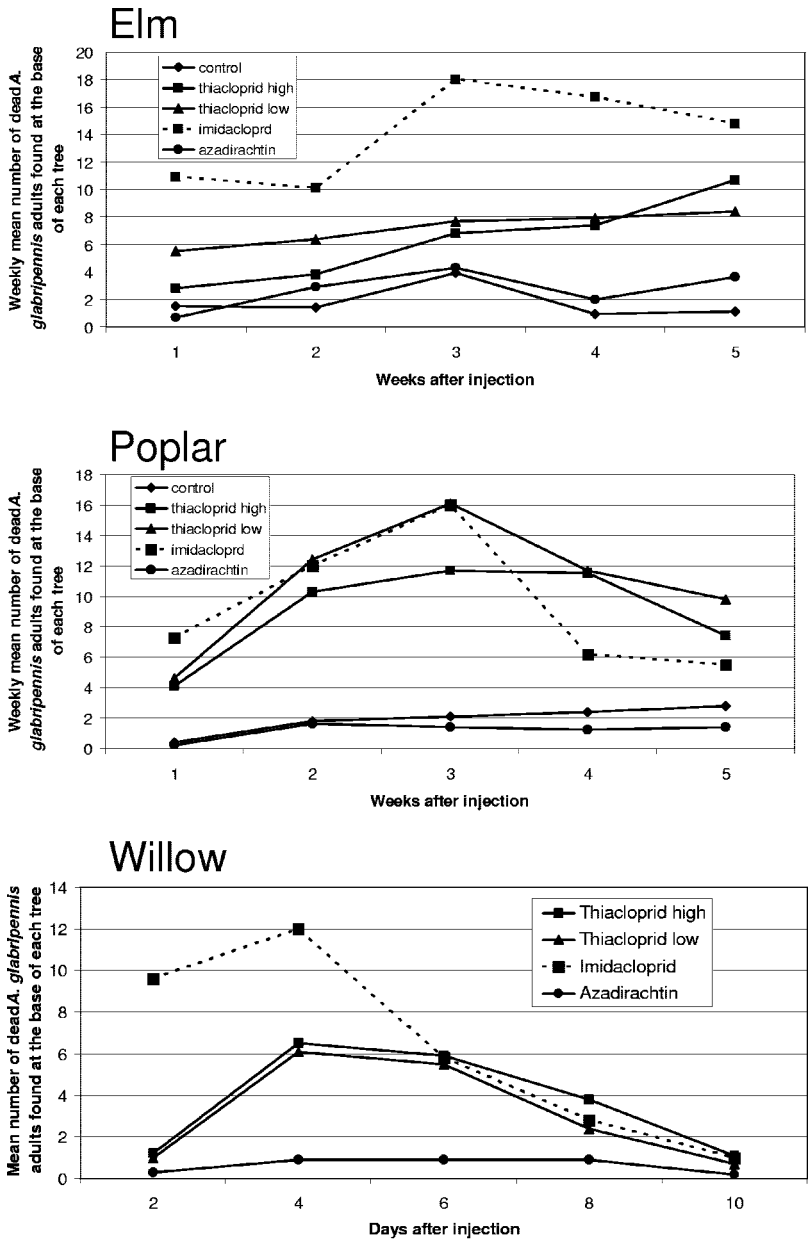

Fig. 1. Mean number of dead adult *A. glabripennis* found per week under elm and poplar trees in Gansu Province, China, or per every other day under willow trees in Hebei Province China. Trees were injected with imidacloprid (0.9 g/cm dbh), low-dose thiacloprid (0.45 g/cm dbh), high-dose thiacloprid (0.9 g/cm dbh), or azadirachtin (0.25 g/cm dbh) on 2–5 July 2001, and dead adults were collected every other day until 16 August 2002 (poplar and elm) or daily until 15 July 2001 (willow). Two pairs of field-collected *A. glabripennis* adults were released on each willow tree 2 d after injection, and an additional pair was released on each willow tree both 4 d and 6 d after injection (n= 10 trees per treatment).

pared with control trees 4 mo after injection; however, differences in mortality were not significant 12 mo after injection (Table 1). It is possible that some individuals that died soon after tree injection had decomposed by the time trees were dissected 12 mo later. Fewer large larvae were observed during dissection 12 mo after injection compared with dissection at 4 mo after injection (data not shown). Overall, mortality of large larvae was higher than for other life stages. It is also possible that imidacloprid residue levels may have declined over time and that *A. glabripennis* eggs laid on the tree several months after injection resulted in larvae that were not exposed to lethal levels of insecticide.

Analyses of imidacloprid residues in leaf and twig samples suggest that imidacloprid levels may increase

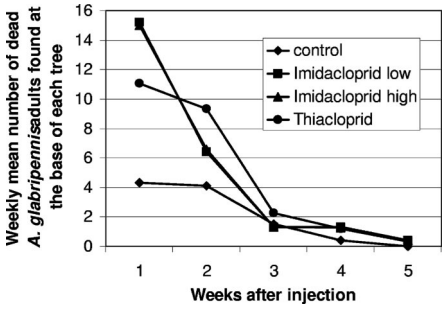

Fig. 2. Mean number of dead adult *A. glabripennis* found per week under willow trees in Tianjin Province, China. Trees were injected with low-dose imidacloprid (0.135 g/cm dbh), high-dose imidacloprid (0.225 g/cm dbh), or thiacloprid (0.135 g/cm dbh) on 28 June 2002, and dead adults were collected daily until 28 July 2002 ($n = 15$ trees per treatment).

over a short period after injection but then decline to nonlethal levels over prolonged periods. Imidacloprid residue levels increased during the first month of injection for the 2002 field experiment (Table 4). Samples of leaves and twigs collected for the 2000 field experiment (1 mo after injection) contained ≈1.3 ppm imidacloprid, whereas samples collected for the 2001 field experiment (9 mo after injection) contained ≈0.4 ppm imidacloprid, despite that in 2001 trees were injected with a higher concentration and dose of imidacloprid. This finding suggests that residue levels declined over several months. It is also possible that the translocation capability of imidacloprid formulations actually declines as the concentration is increased. Wang et al. (2002) found that imidacloprid levels detected in leaves, twigs, and bark or xylem of tree trunks exceeded the LC_{50} level of 1.9 ppm (determined at 72 h for *A. glabripennis* adults fed treated twigs) within 1 mo postapplication and remained above the LC_{50} level for several months. However, they found levels of imidacloprid in leaves, twigs, and bark or xylem of tree trunks ranged from undetectable

Table 4. Residue levels (mean ± SE) of imidacloprid and thiacloprid in leaves and twigs of willow trees in Tian Jin Province, China

Insecticide treatment	Mean residue level (ppm)		Test of difference in residue level between 29 June and 27 July	
	29 June	27 July	T value	*P*
Low imidacloprid				
Leaves	0.27 ± 0.02	0.34 ± 0.02	2.25	0.04
Twigs	0.28 ± 0.02	0.33 ± 0.01	2.27	0.04
High imidacloprid				
Leaves	0.37 ± 0.02	0.46 ± 0.01	3.94	0.001
Twigs	0.39 ± 0.02	0.46 ± 0.02	1.98	0.06
Thiacloprid				
Twigs	0.10 ± 0.01	0.04 ± 0.002	5.50	0.0001

Trees were injected with low dose imidacloprid (0.135 g/cm dbh), high-dose imidacloprid (0.225 g/cm dbh), or thiacloprid (0.135 g/cm dbh) on 28 June 2002. Leaf and twig samples were collected on 29 June and 27 July 2002 ($n = 10$ trees sampled per treatment).

to <0.5 ppm 2 yr after application. Imidacloprid residue levels found in trees in our experiments were similar to the LC_{50} values determined by Wang et al. (2002, adult $LC_{50} = 1.9$ ppm) and values found for *A. glabripennis* and cottonwood borer, *Plectrodera scalator* (F.) (Coleoptera: Cerambycidae), in laboratory studies using insecticide-treated artificial diet (larval $LC_{50} = 1.6$ ppm; Poland et al. 2006). Because we did not evaluate long-term efficacy of treatments, additional studies should be conducted to determine whether lethal levels of insecticide can be maintained for multiple years by repeated injections.

The number of dead *A. glabripennis* adults found beneath elm and poplar trees was highest 3 wk after injection in 2001 (Fig. 1). In contrast, the peak in number of dead *A. glabripennis* adults occurred 4 d after injection for willow trees (Fig. 1). Similarly, in 2002, the number of dead *A. glabripennis* adults found beneath willow trees was highest during the first week after injection and then declined steadily over the next month (Fig. 2). It is possible that translocation is faster in willows than in poplars or elms. Another reason for this trend could be the local temperature conditions at the study sites. The poplar and elm field sites were located in the north central China province of Gansu, whereas the willows were located in the eastern China provinces of Hebei in 2001 and Tianjin in 2002. Based on historical weather records (Chinese Meteorology Administration 1974), average temperatures in Hebei and Tianjin are significantly warmer than in Gansu during all months except March (T-test of mean monthly temperatures from 1951 to 1970; data not shown); thus, insect and tree phenology are likely accelerated in Hebei and Tianjin compared with Gansu. For instance, leaves had flushed on willow trees in Hebei province in April 2002, whereas they had not flushed on poplar and elm trees in Gansu province during the same week. The period of *A. glabripennis* adult activity likely occurs earlier in Hebei and Tianjin provinces than in Gansu province, thus fewer beetles were found beneath willows later in the season because the flight period was already ending at the willow field sites. It is also possible that the beetle population at the willow site declined over time because of mortality.

Imidacloprid and thiacloprid were translocated very rapidly in the willows after injection, and residue levels in leaves and twigs were significant. Dead *A. glabripennis* adults were observed beneath injected trees within 4 h (2001 and 2002; unpublished data) and were collected and tallied 1 to 2 d after injection in the 2001 and 2002 field experiments (Table 2; Figs. 1 and 2). Similarly, Tattar et al. (1998) found that imidacloprid reached 0.15 ppm, a concentration found to be generally lethal to tree-sucking pests (Elbert et al. 1991), within 1 wk of trunk injection into pin oak, *Quercus palustris* Muenchh. Imidacloprid residue levels increased in leaves and twigs between 1 d and 1 mo after injection (Table 4). However, the number of dead *A. glabripennis* adults found beneath the treated willow trees was highest during the first week after injection and declined over the next month. The de-

cline in numbers of dead beetles found beneath the willow trees was likely because of a decline in beetle population or activity as the flight period ended rather than because of reduced levels of insecticide over time.

Although *A. glabripennis* mortality was significantly increased in poplar (2000 and 2001) and elm (2001) trees injected with imidacloprid (Tables 1 and 3), mortality rates tended to be somewhat low with the highest rate being ≈64% in poplar trees 4 mo after injection (Table 1). Mortality was fairly consistent but lower in imidacloprid-injected willows and was variable in the elms (Tables 1 and 3). Variation in mortality may have been because of differential within-tree distribution of imidacloprid in relation to the distribution of *A. glabripennis* within the trees. Systemic chemicals generally move within stems following water ascent in the sapwood. Movement, both upward and downward, is most evident in the outer growth rings, particularly in ring porous hardwood tree species (Kozlowski and Winget 1962, 1963). Pronounced lateral transfer can occur in diffuse porous trees when moisture content is high (Greenidge 1958). Although imidacloprid residue levels were similar in the leaves and twigs of all species, these levels may not reflect residue levels in the trunk or deeper in the sapwood where larvae actively feed. Elm trees are ring porous, whereas poplars and willows are diffuse porous; therefore, it is possible that in elm trees only life stages that feed in the outer rings of sapwood, where translocation is active, would be exposed to systemic insecticides.

Patterns of systemic compound movement within trees may be influenced by many factors, including sun exposure, canopy architecture, branch and trunk injury, presence of reaction and compression wood, sources of underground water, and root injury. Thus the distribution of systemic compounds within trees is extraordinarily complex and variable. The location of injection has been found to be an important factor in determining the distribution of dye within trees. When dye was injected in eight deciduous tree species at locations directly below the center of a branch, it moved distally into the branch. When injected below, but not directly below the center of a branch, the dye moved into the sides or top of the branch and into the main stem. When injected below and to the side of a branch, it moved around the branch and remained in the main stem (Neely 1991). Therefore, distribution of insecticide throughout the trunk and branches of a tree depends on the position of the injection sites relative to branches and on other factors such as tree injury or damage that may alter the pattern of systemic movement within the tree. Increasing the number of injection points may improve the uniformity of within-tree distribution of systemic compounds. *A. glabripennis* larvae bore deeply in trees, tunneling as much as 30 cm in both the sapwood and heartwood (Haack et al. 1997). If *A. glabripennis* larvae have already begun to tunnel in the sapwood at the time an infested tree is injected, and the insecticide remains in the outer growth layers, the larvae would not be exposed to the insecticide. Insecticide residues in the outer growth layers may decline to sublethal levels by the time *A. glabripennis* larvae complete development, pupate under the bark, and chew their way out as adults. Larval tunnels also disrupt translocation. *A. glabripennis* adults typically lay eggs first in the upper trunk and along major branches and then along the entire trunk as the crown begins to die (Haack et al. 1997). Depending on the point of injection, insecticides may move around branches, remaining in the main stem, and thus would have no effect on larvae tunneling within branches.

Our results, along with the findings of others (Wang et al. 2002), suggest that injecting trees with systemic insecticides could aid in eradication and management of *A. glabripennis* by reducing populations through adult and larval mortality. For eradication to be successful, all *A. glabripennis* must be destroyed and thus insecticide treatment would have to be 100% effective if it were the only tool used in the program. Our results demonstrate that *A. glabripennis* adult and larval mortality can be significantly increased in injected trees; however, complete within-tree mortality was never achieved. Nevertheless, insecticide injection may complement other tools in an eradication program by protecting uninfested trees in areas surrounding removed infested trees. If very low residual populations remain in the tree-removal area and are below the detection threshold, individuals would encounter insecticide-treated hosts and significant numbers would die. This could help to reduce the residual population to a level below a minimum viable population size and thus lead to ultimate eradication (Liebhold and Bascompte 2003). Widespread use of insecticides enables the opportunity for development of tolerance or resistance within a population; therefore, efficacy must be monitored over time.

Injecting trees with systemic insecticides would be one tool in a comprehensive program for managing *A. glabripennis* populations should the eradication program fail. Mortality of *A. glabripennis* adults feeding on insecticide-treated trees and of all life stages within the trees would reduce *A. glabripennis* populations and damage. Furthermore, it is possible that mortality of a significant percentage of the *A. glabripennis* within a tree could reduce damage to levels that the tree could withstand.

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