EFFICACY OF THREE INSECTICIDES APPLIED TO BARK TO CONTROL AGRILUS PLANIPENNIS (COLEOPTERA: BUPRESTIDAE)

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ABSTRACT

Emerald ash borer (EAB), *Agrilus planipennis* Fairmaire (Coleoptera: Buprestidae), is a serious exotic pest of ash (*Fraxinus*) trees in North America. In 2003 and 2004, we tested the efficacy of different insecticides sprayed on the bark of cut ash logs for killing emerging EAB adults. Logs (means: length = 30 cm; diam. = 16 cm) were cut from infested ash trees and treated prior to adult emergence. In 2003, we applied imidacloprid at 2 times its label rate to logs 5 days before adults began emerging from logs stored indoors. No adults successfully emerged from the imidacloprid treated logs, while an average of 108 adults per m^2 emerged from untreated control logs. In 2004, we tested permethrin and bifenthrin at their label rates, and imidacloprid at its label rate and at 2 times its label rate. Logs (means: length = 25 cm; diam. = 12 cm) infested with EAB and stored outdoors were sprayed once or twice 4-5 wk prior to adult emergence; or once or twice 1-2 wk prior to adult emergence. Overall, mean percent adult mortality was higher for treated logs (90% mortality) compared to control logs (17%). Logs that received two applications had significantly higher mean percent mortality compared with logs that received one application. There was no significant difference in percent adult mortality among the three chemicals tested or between timing of application. In conclusion, permethrin, imidacloprid and bifenthrin were very effective at killing adult EAB emerging from cut logs, and were most effective when applied twice 1-5 wk prior to initial adult emergence.

The emerald ash borer (EAB), *Agrilus planipennis* Fairmaire (Coleoptera: Buprestidae), is native to Asia and was first discovered in North America in 2002 (Yu 1992, Haack et al. 2002). As of January 2007, EAB was established in the US states of Illinois, Indiana, Maryland, Michigan, and Ohio, and the Canadian province of Ontario (Haack 2006; http://www.emeraldashborer.info). EAB is a serious pest of ash (*Fraxinus*) in North America, where it has killed millions of ash trees (Liu et al. 2003, Cappaert et al. 2005).

Throughout EAB's current North American range, EAB adults emerge from trees between May and August, with most appearing in June and July (Cappaert et al. 2005). Adults feed on ash foliage before mating and periodically afterwards. After mating, EAB females lay eggs on the bark surface of trunks and branches of live ash trees. Eggs hatch in approximately 2-3 wk and young larvae tunnel to the cambial region of the host trees where they feed through the summer and early fall. Most larvae complete feeding by early fall, and then excavate pupal chambers in the sapwood or outer bark of trees where they spend the winter as prepupae. Larvae that have not completed feeding remain in the cambial region throughout the winter. Overwintered prepupae begin pupation in late spring to early summer and pupation lasts 2-3 wk. Adults form and then chew their way out of the tree in another 1-2 wk. EAB that overwintered as larvae complete development, form pupal cells, and become prepupae the following summer or fall. Some of these

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individuals may pupate and emerge as adults that same year, while others may overwinter a second year (Cappaert et al. 2005, Petrice and Haack 2006).

Because of the threat of EAB to ash trees throughout North America, effective control methods for this pest are critically needed. Research on control strategies in Asia is limited, primarily because EAB is rarely a significant pest in Asia (Yu 1992, Liu et al. 2003). A recent study by Nzokou et al. (2006) found that imidacloprid effectively prevented emergence of EAB from cut logs that were held indoors after treatment. Although previous research has been conducted on chemical control of native North American *Agrilus* spp., most studies assessed chemicals that are no longer registered or are highly toxic to mammals (Appleby et al. 1973, Haack and Benjamin 1980). We evaluated the efficacy of three currently available insecticides to kill EAB adults when applied to the bark surface of ash logs prior to EAB adult emergence.

METHODS

2003 Study. In March 2003, we cut 6 logs (means: length = 60 cm; diam. = 16 cm) from 2 infested ash trees near Livonia, Wayne County, Michigan. Logs were placed in a cold room at 4-5ºC to delay development and emergence of EAB adults. In August 2003, we removed the logs from cold storage and allowed EAB to develop indoors at 21-23ºC. Previous experience with EAB rearing had shown that infested logs cut in the winter can be held at 4-5ºC for several months to delay EAB development with little or no adverse effects on adult emergence (D. L. Miller, USDA Forest Service, East Lansing, MI, pers. comm.). On 11 September 2003, approximately 5 days before EAB adults were expected to emerge, we cut each log into two equal portions; one portion was randomly assigned to be sprayed with imidacloprid (Merit®2; Bayer Corporation, Kansas City, MO) at 2 times its label rate for foliar applications (Table 1) and the other portion was used as an untreated control. We doubled the label rate of imidacloprid because of a recent study that found higher concentrations of imidacloprid to be more effective than lower concentrations for killing *Anoplophora glabripennis* (Motschulsky) (Coleoptera: Cerambycidae) adults that fed on treated twig bark (Poland et al. 2006).

Existing exit holes on the logs were filled with white caulk to distinguish them from new exit holes made by future emerging adults. Imidacloprid was applied outdoors to all bark surfaces with a handheld sprayer until saturated to the point of run-off. The volume of insecticide spray per unit of log surface area was not recorded. Treated logs were allowed to dry indoors for 2 days and then treated and untreated logs were placed in separate plastic rearing containers with screened ventilation holes. The logs were maintained in the laboratory (approximately 21-23ºC) and checked every 2 days for EAB adults. After adult emergence was completed, we recorded length, diameter and number of new EAB exits holes for each log.

2004 Study. In 2004, we conducted a similar study with the following insecticides: permethrin (Astro®; FMC Corporation, Philadelphia, PA) at its label rate, imidacloprid at its label rate (Imidacloprid-1×) and two times its label rate (Imidacloprid-2×), and bifenthrin (Onyx™; FMC Corporation, Philadelphia, PA) at its label rate (Table 1). For each chemical, we compared four different spray schedules with respect to timing and number of applications prior to initial EAB adult emergence: A) one early application (Early-1); B) two early applications (Early-2); C) one late application (Late-1); and D) two late applications (Late-2). In March and April 2004, we cut 112 logs (means: length = 50 cm; diam. = 12 cm) from 10 EAB-infested ash trees in Ann Arbor, Washtenaw County, Michigan, and stored them in a shaded hardwood forest. As in 2003, just before treatment, each log was cut into two approximately equal portions, with one portion sprayed and the other left as an untreated control. Existing exit holes were filled with white caulk to distinguish them from new

Table 1. Active ingredients, label rates, LD_{50} (mg/kg, rats), and year tested for insecticide chemicals sprayed on bark of infested ash logs to kill emerging adult *Agrilus planipennis* in studies conducted in 2003 and 2004.

Chemical	Active ingredient	Label rate per 100 L of H_20	Ld50 (mg/kg, rats)	Year tested
Astro®	Permethrin (36.8 %)	1250 mL	998[2]	2004
Merit®2	Imidacloprid (21.4 %)	11.9 mL[1]	4870[3]	2003, 2004
Onyx™	Bifenthrin (23.4 %)	100 mL	153[4]	2004

[1]Label rate for tree and shrub foliar application.
[2]http://msds.fmcweb.com/files/03075tt0.htm.
[3]http://www.cdms.net/ldat/mp47D004.pdf.
[4]http://msds.fmcweb.com/files/8172nva1.htm.

exit holes. Logs were stood upright and insecticides were applied with a 5.7 liter handheld pump sprayer to all bark surfaces until saturated to the point of run-off. The volume of insecticide spray per unit of log surface area was not recorded. One half of the logs scheduled for treatment received the first early application (Early-1) on 14 May, approximately 35 days before EAB adults began emerging from the control logs. One wk later (21 May 2005), one half of the logs that received the early application were sprayed a second time (Early-2). On 3 June, approximately 15 days before EAB adults began emerging from the control logs, the second half of the logs were treated (Late-1). One wk later (10 June 2004), half of these logs received a second application (Late-2).

Immediately after the first early application (14 May 2004), before the insecticides had sufficient time to dry, a heavy rain occurred. We were concerned that the rain could have washed off some of the insecticides, thus reducing their efficacy. So thereafter, we suspended a tarp over the newly treated logs for one day to protect them from rain while insecticides dried.

All logs were stood upright and stored outdoors under a hardwood forest canopy until adults were expected to emerge. On 18 June, when emergence was expected to begin, a subset of treated and untreated control logs for each chemical and spray schedule were grouped separately in plastic rearing containers. Adults were collected approximately 3 times per wk from the containers and fed untreated ash foliage in petri dishes for 10 d to assess latent effects of the insecticides. EAB adults were allowed to freely exit the remainder of the logs that were not placed in containers.

After adult emergence was completed, all logs (including those not placed in containers) were measured to calculate bark surface area, inspected for new exit holes, and dissected to determine the number of EAB that died before emergence. The following formula was used to calculate percent mortality of EAB adults for each log: Percent mortality = (Total dead adults found in the bark and xylem) ÷ (Total dead adults found in the bark and xylem + Total adults that emerged) × 100. Mean EAB adult mortality of control logs was used to estimate actual percent EAB adult mortality of each treated log portion using Abbott's correction formula (Abbott 1925): Abbott's corrected mortality = (Treated log adult mortality – control adult mortality) ÷ (1 – control adult mortality). To more accurately calculate percent mortality, logs with less than 2 adults (dead or emerged) were not included in the data set.

Statistical analyses. In 2003, adult emergence density was compared among treatments using one-way ANOVA (Proc GLM; SAS 2001). In 2004, overall percent EAB mortality was compared between treated and untreated control logs using a one-way mixed model ANOVA (Proc MIXED; SAS 2001). Abbott's corrected percent mortality was compared among chemicals, timing of application, and number of sprays using a three-way mixed model ANOVA. The tree that individual logs were cut from was used as the random factor in the mixed model ANOVAs. Arcsine square root transformations were performed on percentage data prior to analysis to improve normality. Means that were significantly different at $P < 0.05$ were separated using Tukey's multiple comparison test.

RESULTS AND DISCUSSION

In 2003, after adult emergence was completed, the mean (± SE) density of new EAB exit holes per m^2 of bark surface area for logs treated with imidacloprid at two times its labeled rate (Imidacloprid-2×) was significantly lower (0 ± 0) compared to the untreated controls (108 ± 10; $F = 118.3$; df = 1, 11; $P = 0.0001$). Adults were first found in rearing containers of the control logs on 16 September, and the last live adult was collected on 26 September (47 days after logs were removed from cold storage). No adults successfully emerged from imidacloprid-2× treated logs, however, we did observe several dead, partially emerged adults in the bark of these logs.

Results of this indoor test suggest that trunk application of imidacloprid at 2x the label rate provides levels of control as good or better than other products, many of which have lost their registration. For example, Haack and Benjamin (1980) reported that Metasystox-R (a.i. oxydemeton-methyl) and chlorpyrofos, both restricted-use organophosphates, were 100% effective at preventing twolined chestnut borer, *Agrilus bilineatus* (Weber), emergence when bark was treated indoors. In another study, Dunbar and Stephens (1974) found dimethoate (organophosphate), chlorpyrofos, and lindane (organochlorine; registration cancelled) to be the most effective in killing *A. bilineatus* adults emerging from logs that were held indoors after treatment, however, none of the chemicals tested were 100% effective at preventing adult emergence. Nzokou et al. (2006) found similar results as our study for the imidacloprid product Preventol® (Bayer Corporation).

In 2004, total number of live adults plus dead adults averaged 54.4 ± 4.9 per m^2 bark surface area for treated logs compared with 52.4 ± 5.8 for untreated control logs. Overall, mean percent mortality of treated logs (89.9 ± 2.1 %) was significantly higher compared to control logs (16.6 ± 3.0 %; $F = 346.7$; df = 1,140; $P = .0001$). Overall mean percent mortality (Abbott's corrected) for logs that received two applications was 95.3 ± 2.3%, significantly higher compared with 81.5 ± 4.0% for logs that received one application (Table 2). No significant difference in percent mortality was found, however, among chemicals or timing of application (Table 2).

Adults were collected from rearing containers over the period of 23 June – 9 July 2004. All live adults emerged from untreated control logs with the exception of one adult that emerged from a log treated with an early single application of Imidacloprid-1×. This adult died within 3 days after it was collected. Adults that emerged from the untreated control logs lived a mean of 7 days; adults were reared for a maximum of 10 days (N = 56 adults reared). By contrast, dead adults were found in the bottom of each rearing container (135 adults in 4 control containers, and 10 adults in 16 treatment containers). Some or all of the dead adults in the containers holding treated logs may have perished from latent effects of the insecticides; however, we do not know why the adults that died in the containers of control logs did not make it to the collection cups as expected from previous rearing experiences (D. L. Miller, pers. comm.). Rearing

Table 2. Mixed model ANOVA results for *Agrilus planipennis* adult mortality corrected using Abbott's formula (see text) in *A. planipennis* infested bolts comparing chemicals, timing of application and number of applications (Proc Mixed, SAS 2001).

Source[1]	df[2]	*F* value	*P*
Chemical	3,49	1.56	0.2126
Timing	1,49	0.18	0.6746
Chemical × Timing	3,49	0.15	0.9312
No. of applications	1,49	7.89	0.0071
Chemical × No. of applications	3,49	0.76	0.5202
Timing × No. of applications	1,49	0.51	0.4789
Chemical × Timing × No. of applications	3,49	2.06	0.1181

[1]Analysis performed on arcsine square root transformed data.
[2]Degrees of freedom.

containers were stored in the shade and perhaps there was not enough sunlight to attract the insects to the transparent collection cups (containers themselves were either black or blue in color).

Rainfall recorded at Ann Arbor Municipal Airport (approximately 11.3 km southwest of our study site) for the months of May-July 2004 totaled 29.7 cm, which is above the 30 yr average of 23.9 cm for that location (http://climvis.ncdc.noaa.gov/oa/climate/climatedata.html). In our study, timing of application did not significantly affect percent mortality, which illustrates that despite higher than normal rainfall, all three insecticides persisted in their ability to kill EAB adults that emerged through the treated bark at least one month after application. The fact that logs that received two applications had significantly higher mortality compared with one application, suggests when two applications were made the insecticide concentration was increased which enhanced insecticide efficacy. In absolute terms, the logs treated "late" with two applications consistently had the highest percent EAB mortality for all chemicals (Table 3).

Insecticides tested in our study gave similar control compared to dimethoate 2EC (over 95% fewer adults emerged from treated trees compared to controls), which was tested by Appleby et al. (1973) for killing bronze birch borer, *Agrilus anxius* Gory. In their study, treatments were applied when adults were active (early- and mid-June) to target current-year ovipositing adults, eggs and young larvae, as well as emerging adults the following year. In their study, however, no tree dissections were made to evaluate what *A. anxius* life stages were affected by treatments.

Our log dissections revealed that the insecticides we tested did not penetrate the bark very deeply, given that almost all of the dead adults found on the treated logs had died while chewing through the outer bark. Moreover, we found similar densities of dead larvae and prepupae on treated (26.3 ± 2.4 dead larvae and pupae per m^2 of bark surface) and control logs (27.3 ± 3.3; $F = 0.08$, df = 1, 177, $P = 0.78$). These larvae likely perished from log desiccation or intraspecific larval competition. Adult mortality was a result of contact or consumption of the insecticide as adults emerged. Therefore, thicker barked logs should not reduce the efficacy of these treatments. It is possible that corky bark would absorb more insecticide as compared to smooth bark and would require more insecticide to be applied to saturate bark surfaces to the point of run-off.

Overall, the permethrin, imidacloprid and bifenthrin products were very effective at killing EAB adults in our study and their efficacy was increased when logs received two applications in late spring prior to initial adult emergence.

Table 3. Mean (± SE) Abbott's corrected percent *Agrilus planipennis* adult mortality and number (N) of *A. planipennis* infested ash bolts treated with one of three different chemicals at the label rate or twice the label rate in spring and stored outdoors by timing and number of applications. Logs were sprayed to the point of runoff using handheld sprayers. Mean percent mortality was significantly ($P = 0.0071$) higher for one application versus two; no significant difference was found among chemicals or timing of application (Proc Mixed, SAS 2001).

		Mean percent[3] adult EAB mortality and number of replicates (N)							
		Permethrin		Imidacloprid-1×[4]		Imidacloprid-2×[5]		Bifenthrin	
Timing[1]	No. of sprays	$\bar{x}$ ± SE	N	$\bar{x}$ ± SE	N	$\bar{x}$ ± SE	N	$\bar{x}$ ± SE	N
Early	1	75 ± 10	5	90 ± 6	4	80 ± 20	3	87 ± 6	7
Early	2[2]	100 ± 0	4	95 ± 5	4	100 ± 0	3	76 ± 13	4
Late	1	72 ± 20	5	84 ± 10	5	100 ± 0	3	67 ± 6	4
Late	2	100 ± 0	3	96 ± 4	5	100 ± 0	3	100 ± 0	3

[1]Early = sprayed 4-5 wk before EAB adult emergence; Late = sprayed 1-2 wk prior to initial EAB adult emergence.
[2]Second spray applied one wk after first spray.
[3]Arcsine square root transformation performed on data prior to analysis; means and SE's of actual data are shown.
[4]Merit®2 tested at its label rate.
[5]Merit®2 tested at two times its label rate

All of these insecticides would be very effective at reducing the number of EAB adults emerging from cut logs or the lower stems of trees that can be easily accessed with a sprayer. Doubling the label rate of imidacloprid consistently gave 100% mortality except for the earliest application in 2004 and similar mortality would be expected if the label rate was doubled for permethrin or bifenthrin. The increased application rates we tested were performed under research conditions. The pesticide manufacturer would have to modify their labels before higher rates of these insecticides could be used operationally.

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