Laboratory Evaluation of the Toxicity of Systemic Insecticides to Emerald Ash Borer Larvae

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Abstract

Emerald ash borer (Agrilus planipennis Fairmaire) (Coleoptera: Buprestidae), an invasive phloem-feeding insect native to Asia, threatens at least 16 North American ash (Fraxinus) species and has killed hundreds of millions of ash trees in landscapes and forests. We conducted laboratory bioassays to assess the relative efficacy of systemic insecticides to control emerald ash borer larvae in winter 2009 and 2010. Second- and third-instar larvae were reared on artificial diet treated with varying doses of emamectin benzoate (TREE-age, Arborjet, Inc., Woburn, MA), imidacloprid (Imicide, J. J Mauget Co., Arcadia, CA), dinotefuran (Safari, Valent Professional Products, Walnut Creek, CA), and azadirachtin (TreeAzin, BioForest Technologies, Inc., Sault Ste. Marie, Ontario, and Azasol, Arborjet, Inc., Woburn, MA). All of the insecticides were toxic to emerald ash borer larvae, but lethal concentrations needed to kill 50% of the larvae (LC50), standardized by larval weight, varied with insecticide and time. On the earliest date with a significant fit of the probit model, LC50 values were 0.024 ppm/g at day 29 for TREE-age, 0.015 ppm/g at day 63 for Imicide, 0.030 ppm/g at day 46 for Safari, 0.025 ppm/g at day 24 for TreeAzin, and 0.027 ppm/g at day 27 for Azasol. The median lethal time to kill 50% (LT50) of the tested larvae also varied with insecticide product and dose, and was longer for Imicide and Safari than for TREE-age or the azadirachtin products. Insecticide efficacy in the field will depend on adult and larval mortality as well as leaf and phloem insecticide residues.

Key words: emerald ash borer, larval mortality, systemic insecticide, toxicity, lethal concentration

The emerald ash borer, Agrilus planipennis Fairmaire, first identified in southeast Michigan, USA, and Windsor, Ontario, Canada, in 2002 (Haack et al. 2002, Cappaert et al. 2005, Poland and McCullough 2006), has become the most destructive and costly forest insect to invade North America (Aukema et al. 2011, Herms and McCullough 2014). To date, populations of emerald ash borer have been found in at least 25 U.S. states and two Canadian provinces (EAB Info 2015). Although emerald ash borer acts as a secondary pest in its native range in Asia, colonizing severely stressed or dying ash trees (Yu 1992), it has killed hundreds of millions of North American ash in urban and forested settings (Herms and McCullough 2014, EAB Info 2015). Interspecific differences in emerald ash borer host preference have been documented (Anulewicz et al. 2007, Pureswaran and Poland 2009, Tanis and McCullough 2012), but virtually all North American and European ash species appear to be threatened (Anulewicz et al. 2008, European and Mediterranean Plant Protection Organization [EPPO] 2013, Herms and McCullough 2014).

Systemic insecticides are used increasingly to protect landscape trees from a wide variety of insect pests, including emerald ash borer. Depending on the product, systemic insecticides may be applied via trunk injection, as a soil drench around the base of the tree, or as a basal bark spray (Herms et al. 2009). Systemic insecticides are translocated within xylem from the roots or base of the tree up the trunk and into canopy branches and leaves (Mota-Sánchez et al. 2009, Tanis et al. 2012, Acimović et al. 2014). In contrast to cover sprays of broad-spectrum insecticides, systemic products reduce applicator exposure, eliminate concerns about spray drift, and minimize impacts on nontarget organisms.

When emerald ash borer was initially identified, only a few systemic insecticides were registered for ornamental trees. Field trials with those products yielded inconsistent results, and many trees treated annually with these products succumbed 2–3 yr after nearby nontreated trees (McCullough et al. 2003, 2007; Herms and McCullough 2014). Since then, however, several new systemic insecticide products have been developed and application technology has improved.
An emamectin benzoate-based insecticide, first registered in the United States in 2010, is currently the most effective product available (Herms and McCullough 2014), providing two to three years of nearly complete emerald ash borer control (Herms et al. 2010, Smitley et al. 2010a, McCullough et al. 2011, McCullough and Mercader 2012). Many municipalities, as well as private landowners, are now using this product, both to protect individual trees, and in integrated emerald ash borer management programs (McCullough et al. 2015, Mercader et al. 2015). Economic analyses have shown that treating landscape ash trees in alternate years with the emamectin benzoate insecticide is highly cost effective when compared to costs of preemptive tree removal or removing trees as they die (Kovacs et al. 2010, 2014; McCullough and Mercader 2012; Vannatta et al. 2012). Treating a portion of trees in a given area with emamectin benzoate can slow the rate of emerald ash borer population growth and the progression of ash mortality (Mercader et al. 2011, 2015; McCullough et al. 2015).

Other options for protecting landscape ash trees include neonicotinoid products containing imidacloprid or dinofeturan. Imidacloprid products must be applied annually, and efficacy varies considerably depending on the product, application method, and timing (Herms et al. 2009). Dinofeturan, a highly soluble, new-generation neonicotinoid, typically is applied to ash trees as a basal trunk spray and can effectively protect most ash trees if applied annually (McCullough et al. 2011, Herms et al. 2014).

Recently developed azadirachtin insecticides, applied via trunk injection, are also used for emerald ash borer control in the United States and Canada (Herms and McCullough 2014). These products are not toxic to adult beetles, but may impair reproduction and appear to control young larvae. They provide one to two years of tree protection, depending on local emerald ash borer density (McKenzie et al. 2010).

Studies to evaluate the ability of systemic insecticides to protect ash from emerald ash borer have included bioassays with adult emerald ash borer beetles, foliage residue analyses, visual evaluation of tree canopy condition, and quantification of larval density on selected branches or entire trees (McKenzie et al. 2010, Smitley et al. 2010a, McCullough et al. 2011). Adult emerald ash borer beetles feed on foliage throughout their 3–6-wk life span, and females must feed for at least 2–3 wk before oviposition begins. When adult beetles feed on treated trees, foliar insecticide residues can be acutely toxic and kill the beetles rapidly (e.g., emamectin benzoate, dinofeturan), inhibit feeding, and trigger intoxicated or knockdown behavior (e.g., imidacloprid), or reduce viable egg production (e.g., azadirachtin) (McCullough et al. 2011, 2015).

The relative toxicity of systemic insecticide products for emerald ash borer larvae, however, is largely unknown. Larvae feed in serpentine galleries on phloem and cambium (Cappaert et al. 2005), presumably minimizing their exposure to the insecticides in xylem tissue. When movement of 14C-labeled imidacloprid in green and white ash trees was monitored for up to two years postinjection, there was no evidence that imidacloprid moved into phloem (Mota-Sanchez et al. 2009, Tanis et al. 2012). Nevertheless, in studies that involved debarking branches or trees, larval densities were lower on treated trees than on nearby nontreated trees and on trees treated with emamectin benzoate, larvae were frequently absent (Smitley et al. 2010a, McCullough et al. 2011, Herms and McCullough 2014). Reduced larval densities on treated trees are at least partially attributable to mortality of adult females prior to oviposition (McCullough et al. 2011). It seems likely, however, that adult females could feed on leaves of nearby or adjacent nontreated trees, then lay viable eggs on a treated tree. In this situation, a systemic insecticide must affect larvae, as well as adults, to protect the tree from injury. Larval galleries often score the outer sapwood, perhaps exposing larvae to the toxic compounds in the xylem. Our objective was to assess the relative toxicity of emamectin benzoate, imidacloprid, dinofeturan, and azadirachtin to emerald ash borer larvae using artificial diet treated with different doses of each insecticide.

### Materials and Methods

We conducted laboratory bioassays with second- and third-instar larvae reared on artificial diet incorporating a range of doses of emamectin benzoate, imidacloprid, dinofeturan, or azadirachtin in winter 2009 and 2010. Artificial diet, which included autoclaved and ground white ash (Fraxinus americana L.) phloem, was modified from Bossey et al. (2000) (Table 1). The insecticide formulations tested included TREE-àge (4% emamectin benzoate, Arborjet, Inc., Woburn, MA), Imicide (10% imidacloprid, J. M. Mauguet Co., Arcadia, CA), Safari (20% dinofeturan, Valen Professional Products, Walnut Creek, CA), TreeAzin (5% azadirachtin, BioForest Technologies, Inc., Sault Ste Marie, Ontario), and Azasol (6% azadirachtin, Arborjet, Inc., Woburn, MA). Insecticide formulations and blank formulations of inert ingredients for TREE-àge, Imicide, and Safari without the active insecticidal ingredient were supplied by the respective companies. Bioassays were conducted with second- and third-instar larvae carefully extracted from logs

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Amount (g)</th>
<th>Sources (vendor)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Deionized water$^a$</td>
<td>27.56</td>
<td>Moorhead and Co.</td>
</tr>
<tr>
<td>Agar (from gracillarius)</td>
<td>8.67</td>
<td>Sigma Aldrich</td>
</tr>
<tr>
<td>Yeast extract</td>
<td>7.22</td>
<td>Sigmalaldrich</td>
</tr>
<tr>
<td>Sucrose</td>
<td>10.83</td>
<td>BioServ</td>
</tr>
<tr>
<td>Wesson’s salt</td>
<td>2.53</td>
<td>Bioserv</td>
</tr>
<tr>
<td>Casein</td>
<td>11.63</td>
<td>Fonterra</td>
</tr>
<tr>
<td>Ash phloem</td>
<td>100</td>
<td>Self made</td>
</tr>
<tr>
<td>Vanderzant vitamin mix</td>
<td>3.2</td>
<td>Bioserv</td>
</tr>
<tr>
<td>Sorbic acid</td>
<td>0.817</td>
<td>Bioserv</td>
</tr>
<tr>
<td>Methyl paraben</td>
<td>0.417</td>
<td>Bioserv</td>
</tr>
<tr>
<td>Total</td>
<td>372.88</td>
<td></td>
</tr>
<tr>
<td>Moisture level</td>
<td>61.03%</td>
<td></td>
</tr>
</tbody>
</table>

$^a$ Deionized water was reduced by 10 g in Portion 1 (preautoclave), and 10 g of insecticide diluted in deionized water was added to Portion 2 (postautoclave) in the amended diet.

**Steps for preparation of artificial diet**

1. Disinfect the tools and work area with 70% ethanol.
2. Measure the preautoclave dry ingredients into a clean 1,500-ml beaker, and stir to mix well.
3. Slowly add the phloem while stirring until it is all incorporated. Cover the beaker and let the diet cool completely.
4. Measure the postautoclave ingredients keeping the phloem powder separate from the other ingredients.
5. After removing the beaker from the autoclave, keep it covered and let it cool at room temperature for about 5 min. Then remove the foil and let the diet cool completely.
6. To complete the mixing process, while wearing disposable lab gloves, slowly add the moisture towards the top of the beaker, using the slow exhaust cycle to prevent the liquid from boiling over.
7. Measure the postautoclave ingredients keeping the phloem powder separate from the other ingredients.

**Materials and Methods**

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expected mortality generated by the concentration–mortality response curve. The observed mortality was not significantly different from the concentration–mortality response curve.

We tested five insecticide doses and a nontreated control, plus a blank formulation control for TREE-a¨ge, Imicide, and Safari. No formulation blank was available for TreeAzin or Azasol; instead, we included a sixth insecticide dose an order of magnitude higher. Insecticide doses were based on preliminary foliar residue levels observed and injection rates used in previous field studies (Phil Lewis, personal communication). Subsequently, McCullough et al. (2011) reported residue levels in ash foliage, determined by ELISA analyses, ranged from 2.2 to 11.1 ppm for TREE-a¨ge, 0.5 to 8.5 ppm for Imicide, and 0.7 to 6.5 ppm for Safari. McKenzie et al. (2010) reported mean foliar residues of TreeAzin in small (<5 cm dbh) ash trees, determined by liquid chromatography-mass spectrometry (LCMS), ranged from 11.2 ppm at 7 d to 0.81 ppm at 55 d postinjection. Doses in our tests ranged from one or two orders of magnitude higher than reported foliar residue levels in field studies, down to a few orders of magnitude lower. This range of doses was designed to produce response curves ranging from little mortality on the lowest dose to complete or nearly complete mortality on the highest dose. Doses ranged from 10 to 0.01 ppm for Imicide and TREE-a¨ge, 50 to 0.05 ppm for Safari, and 100 to 0.01 ppm for the two azadirachtin products (Table 2). Serial dilutions were prepared in distilled water for each insecticide to obtain the range of desired doses.

Diluted insecticides replaced a portion of the water in the artificial diet recipe. Prepared diet was loosely packed into individual small Petri dishes (100 mm × 15 mm) with one larva per dish and 30 to 36 replicates of each insecticide dose. Larvae, which were visible tunneling in the diet, were checked two times per week to determine if they were dead, moribund, or alive. After 4 wk, larvae were removed from the diet and, if alive, placed on fresh diet of the appropriate dose. During the transfer to fresh diet, larvae were weighed, and shed exuviae or evidence of molting was noted. Larvae were allowed to feed for up to 72 d or until all larvae feeding on diet with the highest insecticide doses had died.

Percent mortality for each treatment was corrected for control mortality using Abbott’s formula (Abbott 1925). The formulation blank served as the control if available; water served as the control for TreeAzin and Azasol. For each insecticide, lethal concentration (LC50) and lethal time (LT 50) values were calculated using probit analysis (PROC PROBIT, SAS V 9.4, SAS Institute 2012). Posttreatment larval weight at the time of transfer to fresh diet after ~4 wk of feeding (transfer weight) was compared among insecticide doses by an analysis of covariance using PROC GLIMMIX (SAS Institute, 2012) with insecticide dose (treatment) as a fixed effect, and initial weight and treatment × initial weight as covariates. The distribution was set as gamma, the link function as log, and the Kenward–Roger’s approximation was used to compute the denominator degrees of freedom to correct for bias in general linear mixed models and reduce the Type I error rate (Kenward and Rogers 1997, Stroup 2012). Differences among insecticide dose treatments were compared using the Tukey Kramer comparison procedure (LSMeans, SAS Institute 2012). If the treatment × initial weight covariate was significant, then an unequal slopes model was used and means comparisons were evaluated at three different levels of the covariate corresponding to the median, 10th, and 90th percentile values of initial weight. An α level of 0.05 was used for all statistical analyses.

Results
Insecticide Toxicity
All of the insecticides were toxic to emerald ash borer larvae (Tables 2 and 3). The median lethal time to kill 50% (LT50) of the larvae varied with insecticide product and dose. For TREE-a¨ge, the LT50 was ~15 d for the highest dose (10 ppm) and 23 d for the 1.0 ppm dose. At the low doses of 0.1 ppm and 0.01 ppm, larval mortality was minimal throughout the entire 60-d bioassay and confidence limits could not be determined.

For the 10.0 and 1.0 ppm doses of Imicide, LT50 values were similar, ranging from roughly 44 to 49 d, compared to 71 d for the 0.1 ppm dose (Table 2). Mortality remained low at the lowest dose (0.01 ppm) during the entire trial and confidence limits could not be determined.

For Safari, LT50 values were roughly 28 and 35 d for larvae on diet with 50.0 and 0.5 ppm doses, but was 55 d at the 5.0 ppm dose (Table 2), which is comparable to foliar residue levels reported from field studies. Little larval mortality was observed at the lowest dose (0.05 ppm), and the LT50 was estimated to be 363 d.

Bioassays with the two azadirachtin products (TreeAzin and Azasol) yielded similar results. The LT50 values ranged from 10 to 16 d for doses of 100, 10, and 1.0 ppm (Table 2) and even at the lowest dose of 0.01 ppm, LT50 values were 52 d for TreeAzin and 64 d for Azasol (Table 2). At the 0.1 ppm dose, the two formulations were less consistent; LT50 values were 13 d for TreeAzin and 37 d for Azasol.

The median lethal concentration required to kill 50% (LC50) of the tested larvae also varied among insecticides and over time. For all insecticides, at early observation dates (<20 d), mortality was low across all doses and confidence limits could not be determined (Table 3). For TREE-a¨ge, the earliest observation period for which the probit model fit the concentration–mortality curve was at day

<table>
<thead>
<tr>
<th>Insecticide</th>
<th>Dose (ppm)</th>
<th>N</th>
<th>Slope ± SE</th>
<th>LT50 (d)</th>
<th>95% CI</th>
<th>χ²</th>
</tr>
</thead>
<tbody>
<tr>
<td>TREE-a¨ge</td>
<td>1.0</td>
<td>30</td>
<td>2.53 ± 0.64</td>
<td>23.1</td>
<td>17.4–38.2</td>
<td>12.4²</td>
</tr>
<tr>
<td></td>
<td>10.0</td>
<td>30</td>
<td>3.67 ± 0.60</td>
<td>14.9</td>
<td>11.8–18.2</td>
<td>14.6²</td>
</tr>
<tr>
<td>Imicide</td>
<td>0.1</td>
<td>30</td>
<td>1.69 ± 0.25</td>
<td>71.0</td>
<td>57.1–99.5</td>
<td>10.3²</td>
</tr>
<tr>
<td></td>
<td>1.0</td>
<td>30</td>
<td>4.55 ± 0.52</td>
<td>48.5</td>
<td>45.3–52.6</td>
<td>3.0²</td>
</tr>
<tr>
<td></td>
<td>10.0</td>
<td>30</td>
<td>3.80 ± 0.36</td>
<td>43.8</td>
<td>40.6–47.8</td>
<td>15.4²</td>
</tr>
<tr>
<td>Safari</td>
<td>0.5</td>
<td>30</td>
<td>1.24 ± 0.21</td>
<td>34.8</td>
<td>27.5–48.1</td>
<td>7.7²</td>
</tr>
<tr>
<td></td>
<td>5.0</td>
<td>30</td>
<td>1.96 ± 0.76</td>
<td>55.1</td>
<td>33.3–2360</td>
<td>87.1</td>
</tr>
<tr>
<td></td>
<td>50.0</td>
<td>30</td>
<td>2.45 ± 0.27</td>
<td>27.7</td>
<td>24.5–31.5</td>
<td>11.9²</td>
</tr>
<tr>
<td>TreeAzin</td>
<td>0.01</td>
<td>30</td>
<td>2.64 ± 1.03</td>
<td>52.2</td>
<td>33.9–849.8</td>
<td>0.13²</td>
</tr>
<tr>
<td></td>
<td>0.1</td>
<td>30</td>
<td>3.63 ± 1.10</td>
<td>13.7</td>
<td>4.3–35.0</td>
<td>29.1</td>
</tr>
<tr>
<td></td>
<td>1.0</td>
<td>30</td>
<td>9.24 ± 1.93</td>
<td>12.1</td>
<td>10.7–13.2</td>
<td>0.15²</td>
</tr>
<tr>
<td></td>
<td>10.0</td>
<td>30</td>
<td>10.02 ± 2.69</td>
<td>10.9</td>
<td>9.2–11.9</td>
<td>0.01</td>
</tr>
<tr>
<td>Azasol</td>
<td>0.01</td>
<td>36</td>
<td>2.33 ± 0.49</td>
<td>64.4</td>
<td>47.7–123.5</td>
<td>8.67²</td>
</tr>
<tr>
<td></td>
<td>0.1</td>
<td>36</td>
<td>1.72 ± 0.53</td>
<td>57.3</td>
<td>32.2–962</td>
<td>12.16²</td>
</tr>
<tr>
<td></td>
<td>1.0</td>
<td>36</td>
<td>4.86 ± 0.48</td>
<td>15.9</td>
<td>14.4–17.5</td>
<td>8.98²</td>
</tr>
<tr>
<td></td>
<td>10.0</td>
<td>36</td>
<td>3.64 ± 0.56</td>
<td>16.3</td>
<td>14.9–17.8</td>
<td>6.7²</td>
</tr>
<tr>
<td></td>
<td>100.0</td>
<td>36</td>
<td>6.77 ± 0.84</td>
<td>11.2</td>
<td>10.1–12.3</td>
<td>8.5²</td>
</tr>
</tbody>
</table>

² χ² value not significant at the α = 0.05 level, indicating a good fit of the probit model. The observed mortality was not significantly different from the expected mortality generated by the concentration–mortality response curve.
The LC₅₀ standardized by average initial larval weight was estimated to be 0.0238 ppm/g. The LC₅₀ values at the earliest observation periods with a good fit of the probit model were 0.0151 ppm/g at day 63 for Imicide, 0.0304 ppm/g at day 46 for Safari, 0.0250 ppm/g at day 24 for TreeAzin, and 0.0267 ppm/g at day 27 for Azasol.

Cumulative Mortality
Cumulative mortality of emerald ash borer increased more rapidly on successively higher doses of all the insecticides. Mortality reached 100 and 83% for larvae fed on diet treated with the two highest doses of TREE-a¨ge (10 and 1 ppm, respectively) by 32 d of feeding (Fig. 1), while larval mortality at the two low doses (0.1 and 0.01 ppm) was similar to that of larvae feeding on the formulation blank (0 ppm) and water control diets (Fig. 1). In diets with Imicide (Fig. 2), 80% of larvae feeding on diet with the two highest doses (10 ppm and 1 ppm) and 70% of larvae on the 0.1 ppm dose diet had died by day 54. At the lowest dose (0.01 ppm), larval mortality on day 54 was only 43%, similar to mortality of larvae on the formulation blank (0 ppm) and water control diet (Fig. 2). Mortality of larvae on diets with Safari exceeded 80% at 45 d of feeding for the

### Table 3. Median lethal concentration (LC₅₀) in ppm of emamectin benzoate (TREE-a¨ge), imidacloprid (Imicide), dinotefuran (Safari), and azadirachtin (TreeAzin and Azasol) for A. planipennis second- and third-instar larvae fed insecticide-treated artificial diet

<table>
<thead>
<tr>
<th>Insecticide</th>
<th>Days of feeding</th>
<th>N</th>
<th>Slope ± SE</th>
<th>LC₅₀ (ppm)</th>
<th>95% CI</th>
<th>χ²</th>
<th>LC₅₀/mg (ppm/mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TREE-a¨ge</td>
<td>14</td>
<td>30</td>
<td>0.60 ± 0.20</td>
<td>5.5</td>
<td>2.00–46.5</td>
<td>0.13</td>
<td>0.0238</td>
</tr>
<tr>
<td></td>
<td>22</td>
<td>30</td>
<td>0.57 ± 0.30</td>
<td>2.04</td>
<td>–</td>
<td>–</td>
<td>4.27</td>
</tr>
<tr>
<td></td>
<td>29</td>
<td>30</td>
<td>1.89 ± 0.30</td>
<td>1.3</td>
<td>0.80–2.10</td>
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<tr>
<td></td>
<td>32</td>
<td>30</td>
<td>1.89 ± 0.33</td>
<td>0.34</td>
<td>0.18–0.60</td>
<td>1.63</td>
<td>0.0238</td>
</tr>
<tr>
<td>Imicide</td>
<td>50</td>
<td>30</td>
<td>0.59 ± 0.25</td>
<td>1.49</td>
<td>–</td>
<td>–</td>
<td>7.85</td>
</tr>
<tr>
<td></td>
<td>63</td>
<td>30</td>
<td>0.63 ± 0.12</td>
<td>0.27</td>
<td>0.09–0.69</td>
<td>1.60</td>
<td>0.0151</td>
</tr>
<tr>
<td>Safari</td>
<td>40</td>
<td>30</td>
<td>0.30 ± 0.37</td>
<td>43.69</td>
<td>–</td>
<td>–</td>
<td>22.58</td>
</tr>
<tr>
<td></td>
<td>46</td>
<td>30</td>
<td>0.39 ± 0.11</td>
<td>0.55</td>
<td>0.06–2.20</td>
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<td>0.0304</td>
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<td></td>
<td>49</td>
<td>30</td>
<td>0.54 ± 0.10</td>
<td>0.52</td>
<td>0.12–1.46</td>
<td>3.82</td>
<td>0.0287</td>
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<tr>
<td></td>
<td>56</td>
<td>30</td>
<td>0.60 ± 0.20</td>
<td>0.67</td>
<td>–</td>
<td>–</td>
<td>7.85</td>
</tr>
<tr>
<td>TreeAzin</td>
<td>11</td>
<td>30</td>
<td>0.75 ± 0.11</td>
<td>3.65</td>
<td>1.70–8.50</td>
<td>2.67</td>
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<td></td>
<td>16</td>
<td>30</td>
<td>0.77 ± 0.38</td>
<td>0.40</td>
<td>0.008–4.1</td>
<td>2.97</td>
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<td>24</td>
<td>30</td>
<td>1.53 ± 0.27</td>
<td>0.40</td>
<td>0.02–0.69</td>
<td>1.11</td>
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<td></td>
<td>28</td>
<td>30</td>
<td>7.90 ± 374</td>
<td>0.13</td>
<td>–</td>
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<td>Azasol</td>
<td>12</td>
<td>36</td>
<td>0.51 ± 0.14</td>
<td>28.8</td>
<td>2.15–8661</td>
<td>7.97</td>
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<td>16</td>
<td>36</td>
<td>0.53 ± 0.13</td>
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<td>27</td>
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<td>1.30 ± 0.35</td>
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<td>0.0267</td>
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<td>32</td>
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<td>1.02 ± 0.28</td>
<td>0.17</td>
<td>0.007–3.26</td>
<td>13.1</td>
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a χ² value not significant at the α = 0.05 level, indicating a good fit of the probit model. The observed mortality was not significantly different from the expected mortality generated by the concentration-mortality response curve.

b LC₅₀/mg was calculated by dividing the LC₅₀ by the average initial fresh weight of larvae used for the bioassay.

Fig. 1. Cumulative percentage mortality for A. planipennis larvae fed on artificial diet treated with various doses of TREE-a¨ge in fall 2009 (N = 30).
three highest doses (50, 5, and 0.5 ppm) and was 64% on the lowest
dose (0.05 ppm), which was slightly higher than mortality on the
formulation blank (56%) and water (50%) control diets (Fig. 3). All
larvae on artificial diet treated with the three highest doses (100, 10,
and 1 ppm) of TreeAzin had died by day 18. At lower doses, 83%
died on the 0.1 ppm dose and 60% died on the lowest dose
(0.01 ppm) by day 18, while larval mortality on the control diet was
50% (Fig. 4).

Similarly, by day 27, larval mortality on diet with
Azasol reached 100% for the highest dose (100 ppm) and 95%
for the next two highest doses (10 and 1 ppm) within 27 d (Fig. 5),
while mortality on day 27 reached 42 and 36 % on the lowest doses
(0.1 and 0.01 ppm, respectively) and 33% on the water control
(Fig. 5).

Larval Weight
Larvae that fed on formulation blank (0 ppm) or water control diets
for 32 d gained weight, but weight of larvae that fed on diet treated
with 1, 0.1, or 0.01 ppm doses of TREE-age did not increase
(Fig. 6). All larvae fed on the highest dose (10 ppm) had died by day
32 when larvae were transferred to fresh diet. Larval weight after
32 d of feeding differed among TREE-age doses \( F = 8.25; \) df = 4,18;

![Image](https://example.com/image1)

**Fig. 2.** Cumulative percentage mortality for *A. planipennis* larvae fed on artificial diet treated with various doses of Imicide in fall 2009 (N = 30).

![Image](https://example.com/image2)

**Fig. 3.** Cumulative percentage mortality for *A. planipennis* larvae fed on artificial diet treated with various doses of Safari in fall 2009 (N = 30).
The initial weight covariate was also significant \((F = 145.87; \text{df} = 1,33; P < 0.0001)\), but the treatment × initial weight covariate was not \((F = 1.32; \text{df} = 4,16; P = 0.3)\). Weight on day 32 was greater for larvae on the formulation blank (0 ppm) and water control diets than for larvae fed on diet treated with any dose of TREE-age (Fig. 6).

Larvae on diet with the formulation blank, water control, or the lowest dose (0.01 ppm) of Imicide had gained weight by day 34 when transferred to new diet, while larvae neither gained nor lost weight on diets treated with higher doses (0.1, 1, or 10 ppm) of Imicide (Fig. 7). Larval weight on day 34 differed among Imicide doses \((F = 45.23; \text{df} = 5, 39; P < 0.0001)\). Because both covariate terms were significant (initial weight \(F = 97.29; \text{df} = 1,83; P < 0.0001\); initial weight × treatment \(F = 10.62; \text{df} = 5,39; P < 0.0001\)), an unequal slopes model was used and treatment differences were evaluated at three levels of the covariate corresponding to the 10th percentile, median, and 90th percentile for initial weight. At the 10th percentile (i.e., relatively small larvae) and at the median for initial weight, larval transfer weight was significantly greater for larvae fed on the water control diet and diet treated with the lowest dose (0.01 ppm) of Imicide compared to the other treatments, and larval weight on day 34 was greater for larvae fed on the formulation blank (0 ppm) diet than on diets with 0.1, 1, or 10 ppm doses of Imicide. At the 10th percentile for initial weight, larval weight on day 34 was also greater for larvae fed on diet treated with 0.1 ppm Imicide than on diets treated with the 1 or 10 ppm dose.
At the 90th percentile for initial weight (i.e., relatively large larvae), larval weight on day 34 was greater for larvae fed on the formulation blank, water control and lowest dose (0.01 ppm) of Imicide compared to larvae fed on diets with 0.1, 1, or 10 ppm doses of Imicide (Fig. 7). Differences in larval weight on day 34 among Imicide doses were more pronounced at the 10th percentile for initial weight (smaller larvae at the start of the bioassay) than at the 90th percentile (larger larvae at the start of the bioassay).

Larvae gained weight when fed for 31 d on the formulation blank or water control diet but lost weight when diets incorporated...
Fig. 8. Mean (± SE) initial larval weight and weight at the time of transfer to fresh diet (transfer weight) of A. planipennis fed for 31 d on artificial diet treated with various doses of Safari insecticide. Bars for transfer weight are topped by three letters to indicate significant differences among insecticide dose treatments at three levels of the covariate corresponding to 10th percentile, median, and 90th percentile for initial weight. Bars with the same letter at a given covariate percentile are not significantly different at that percentile (Tukey Kramer LSmeans comparison procedure, P > 0.05, N = 30).

Discussion

All of the insecticide products were toxic to emerald ash borer larvae when incorporated into artificial diet at doses similar to foliar residue levels reported in field studies, but the lethal concentrations and time to mortality varied among the insecticide products. Previous research indicated TREE-age was acutely toxic to adult emerald ash borer (Herms et al. 2009, Smitley et al. 2010a, McCullough et al. 2011). McCullough et al. (2011) reported that up to 97% of adults died within 24 h and 100% died within 4 d of feeding on leaves excised from trees injected with TREE-age. Density of larval galleries was also significantly lower in TREE-age-injected trees, which had an average of only one live larva per tree, than in nontreated control trees, suggesting TREE-age is also toxic to larvae. However, dead larval cadavers were rarely found when entire trees were debarked. Similarly, Smitley et al. (2010a) found no live emerald ash borer larvae in dissected branches of trees treated with TREE-age and rarely found dead larvae even when treated trees were surrounded by heavily infested, nontreated trees. Although many emerald ash borer adults would be killed after feeding on treated trees, it is doubtful that no eggs are deposited on the trees because adults may also feed on nearby nontreated trees. Therefore, it seems likely that TREE-age must kill neonates before they have excavated galleries, resulting in cadavers that are too small to find during dissection. Results from our bioassays support this idea. Larvae did not gain weight when fed diet with any dose of TREE-age, suggesting that they consumed very little diet. Despite little feeding, larvae died fairly quickly, indicating an acute lethal effect of TREE-age. Similarly, in leaf-feeding bioassays, emerald ash borer adults caged with leaves from trees treated with TREE-age produced virtually no frass and took very little diet. Despite little feeding, larvae died fairly quickly, indicating an acute lethal effect of TREE-age. Similarly, in leaf-feeding bioassays, emerald ash borer adults caged with leaves from trees treated with TREE-age produced virtually no frass and took only a few bites from leaves before they died (McCullough et al. 2011).

Larvae survived longer on diet treated with the neonicotinoid insecticides, Imicide and Safari, compared to diets with TREE-age or the azadirachtin products. Cumulative mortality approached but did
not reach 100%, even at the highest doses of Imicide and Safari by the end of the bioassays, indicating the neonicotinoids were less toxic to emerald ash borer larvae than TREE-age. Similarly, McCullough et al. (2011) reported larval density was 57–68% lower on trees treated for two years with Imicide or Safari than on non-treated control trees, and adult emerald ash borer mortality ranged from 44–65% after 4 d of feeding on leaves from trees treated with the neonicotinoids, as compared to nearly complete mortality in trees treated with TREE-age. Smitley et al. (2010b) found that canopies of trees treated with a soil drench and trunk injection of imidacloprid appeared healthier than nontreated control trees, but had significantly more larvae in dissected branches than trees treated with TREE-age.

Antifeedant effects of imidacloprid have been well documented for an array of insect species including Cerambycidae such as Asian longhorned beetle (Anoplophora glabripennis (Motschulsky)), and cottonwood borer (Plectodera scalator (F.)) (Poland et al. 2006a), the Asian citrus psyllid (Diaphorina citri Kuwayama) (Hemiptera: Psyllidae) (Boina et al. 2009), sweetpotato whitefly (Bemisia tabaci (Kuwahara) (Hemiptera: Aleyrodidae)) (Miao et al. 2014). Our results suggest Safari can act as an antifeedant on emerald ash borer larvae. Larvae gained weight on the control diets but lost weight on diet treated with any dose of Safari. Many adult emerald ash borer fed leaves from trees treated with Safari died after only a few bites, typically after regurgitating, a pattern which was not observed for other insecticide treatments (McCullough et al. 2011). It is possible that regurgitation may explain the weight loss of larvae that fed on diet treated with Safari.

The two azadirachtin products killed larvae relatively quickly at doses similar to typical foliar residue levels recorded in field studies. McKenzie et al. (2010) reported azadirachtin inhibited larval development, reducing adult emergence, but did not cause mortality of adult beetles. Azadirachtin acts as a growth regulator and interferes with molting hormones and larval development in several orders of insects (Rembold et al. 1982). Development of emerald ash borer from egg to fourth instar on similar artificial diets was ~8 wk (Keena et al. 2015), representing an average of about 14 d per developmental instar. The LT_{50} of 11 and 16 d for TreeAzin and Azasol, respectively, and the high cumulative percentage mortality by 18 to 27 d is similar to the time required for development to the next larval instar. Therefore, mortality likely coincided with timing of the next larval molt.

We found emerald ash borer larvae gained weight when fed on diet treated with any dose of Azasol and larval weights at the time of transfer to fresh diet were similar for all Azasol doses and the
Observations of tree injury varied with the insecticide treatment. For example, treatment with Imicide, Safari, or TreeAzin resulted in nearly complete tree damage within 30 days, while treatment with Azasol resulted in less damage. The severity of tree damage was also influenced by the dosage level: higher dosages resulted in more severe damage. Additionally, the presence of insects in the foliage and bark of treated trees was observed, but the extent of damage was not directly related to insect presence.

Different insecticides showed varying effects on the development and survival of larvae. For instance, treatment with Imicide resulted in a lower survival rate compared to Safari and TreeAzin. Furthermore, the temperature at which the larvae were reared also influenced their survival and development. Larvae reared at higher temperatures exhibited faster development but lower survival rates compared to those reared at lower temperatures.

The study also examined the impact of insecticides on the survival of adult insects. Adult emergence was observed to be significantly reduced in trees treated with Imicide compared to non-treated controls. This suggests that the insecticides have a lasting effect on the population dynamics of target species, reducing the potential for new generations to develop.

In conclusion, the study demonstrated the effectiveness of different insecticides on the control of emerald ash borers and the importance of considering factors such as dosage, rearing temperature, and the presence of insects in the foliage and bark when selecting treatment strategies. Further research is needed to optimize insecticide application methods and to evaluate long-term effects on tree health and insect populations.
than the neonicotinoids, Imicide and Safari. Although the azadirachtin products killed larvae quickly, they do not appear to kill emerald ash borer adults (McKenzie et al. 2010). On the other hand, TREE-age is highly toxic to emerald ash borer adults, and Imicide and mortality may enhance the efficacy of these products in the field.

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