Forest Ecology and Management 511 (2022) 120144

Contents lists available at ScienceDirect

Forest Ecology and Management

journal homepage: www.elsevier.com/locate/foreco

Effects of systemic insecticides against emerald ash borer on ash seed resources

Dorah M. Mwangola [a,*], Aubree M. Kees [a], Donald M. Grosman [b], Brian H. Aukema [a]

[a] *Department of Entomology, University of Minnesota, St. Paul, MN, United States*
[b] *Arborjet Inc., Woburn, MA, United States*

ARTICLE INFO

Keywords:
Emerald ash borer
Agrilus planipennis
Ash tree
Fraxinus
Ash seed weevil
Lignyodes spp.
Systemic insecticides
Conservation of genetic resources

ABSTRACT

Emerald ash borer (EAB), *Agrilus planipennis*, is an invasive insect that was accidentally introduced to North America from Asia. It continues to spread rapidly across North America and is responsible for the death of tens of millions of ash trees (*Fraxinus* spp.). All North American species of ash are susceptible to EAB infestation threatening the ash resource and diversity. Measures such as systemic insecticide treatments in urban forests and collection of ash seeds provide a means of conserving genetic resources. Understanding the effect these insecticides could have on ash seed viability is therefore important to informing conservation efforts. Another potential concern for effective conservation of ash seeds is the ash seed weevil (*Lignyodes* spp.) whose larvae develop in and feed on ash seeds. However, the effect of EAB insecticides on weevil infestation levels in ash seeds has not been investigated to date. Our study investigated the effect of two systemic insecticide treatments, azadirachtin and emamectin benzoate, on levels of ash seed weevil infestation, seed germination ability, and seed germination time of seeds collected from boulevard trees of green ash (*F. pennsylvanica* Marsh.) in cities in Minnesota from 2017 to 2019. Weevil infestation levels were similar between untreated and treated trees in 2017 and 2018. In 2019, the weevil prevalence in untreated trees was on average 17% and 30% higher than in azadirachtin and emamectin benzoate-treated trees respectively. Weevil infestation data suggests that repeated insecticide treatments at labelled rates can reduce seed weevils that target germplasm. Additionally, insecticide treatments did not affect ash seed germination rates between treatments. These results suggest that systemic insecticides may be effective at conserving the seed resource in addition to known benefits such as canopy preservation.

1. Introduction

The emerald ash borer, *Agrilus planipennis* Fairmaire (Coleoptera: Buprestidae) is a wood boring beetle accidentally introduced to North America from Asia on wood packing material (Cappaert et al., 2005). Hundreds of millions of ash trees (*Fraxinus* spp.) have died from emerald ash borer infestation (USDA Forest Service and Michigan State University, 2022). Ash trees are of cultural importance to indigenous peoples, provide homes for wildlife, act as nutrient sources for other arthropods, and are used for wood products such as baseball bats and furniture. In urban centers, *Fraxinus* species are popular street trees that foster increased property value and human health benefits (Gandhi and Herms, 2010a, 2010b; Donovan et al., 2013; Herms and McCullough, 2014). In natural forests, emerald ash borer causes reproductive isolation of surviving ash trees, reduces seed production in infested trees, and slows regeneration rates of new saplings (Kashian and Witter, 2011; Klooster et al., 2014; O'Brien et al., 2017).

A combination of both *in situ* and *ex situ* measures have been employed in conserving ash genetic resource in the wake of the invasion of emerald ash borer to North America. The *in situ* approach is the use of systemic insecticide treatments that kill larvae feeding underneath the bark and adults that feed on leaves after emergence. This approach

---

* Corresponding author at: Department of Entomology, University of Minnesota, St. Paul, MN, 219 Hodson Hall, 1980 Folwell Ave, Falcon Heights, MN 55108, United States.

E-mail address: mwang022@umn.edu (D.M. Mwangola).

https://doi.org/10.1016/j.foreco.2022.120144
Received 24 December 2021; Received in revised form 25 February 2022; Accepted 3 March 2022

maintains mature trees on the landscape, reducing the impacts of the loss of ash trees (O'Brien et al., 2017; Flower et al., 2018). The *ex situ* approach entails the collection of ash seeds for seed banks and breeding material (Widrlechner, 2010; Koch et al., 2011).

The conservation of genetic resources is an important component of sustainable management of forests especially in the face of environmental, pest and disease challenges facilitated by climate change and globalization (Rajora and Mosseler, 2001; St.Clair and Howe, 2011; Ramsfield et al., 2016). A primary goal in the genetic conservation of forest trees is the preservation of genetic diversity among populations. Genetic diversity enables the continued survival and success of a population by providing a population the ability to adapt and evolve in response to changes in the environment or biotic threats such as pests and diseases (Rajora and Mosseler, 2001; Šijačić-Nikolić et al., 2014). Furthermore, genetic variation in forest tree populations is a cumulation of years of different evolutionary processes and can be difficult to recreate if lost (Rajora and Mosseler, 2001). Genetic resources are also important in future breeding efforts for desirable/valuable traits such as resistance to diseases and pests (St.Clair and Howe, 2011; Woodcock et al., 2019).

The types of conservation measures for genetic resources can be grouped into two types: *in situ* and *ex situ* conservation. *In situ* (in site) involves conserving a species in its native habitat, e.g., conserving mature trees in natural populations or in protected parks or setting up artificial populations without controlled selection. *Ex situ* (out of site) conservation involves conserving the species away from its native habitat; e.g. seed bank collections, pollen bank collections and seedlings maintained in plantations (Ledig et al., 1998; Šijačić-Nikolić et al., 2014). The benefits of using *in situ* measures include maintaining a species' role in its environment, allowing for natural evolutionary forces to continue to act on the species' population. Such measures can be more affordable than maintaining *ex situ* populations. *Ex situ* measures provide protection for populations at risk in their natural habitat until resistance or tolerance is obtained from a pest or disease, but may limit the amount of diversity that can be conserved. Given both the pros and cons of each strategy, a cautious conservation strategy would implement both measures (Ledig, 1986; Rajora and Mosseler, 2001; St.Clair and Howe, 2011; Pritchard et al., 2014).

While much focus continues to be on emerald ash borer as a primary tree killer, ash seed weevils, *Lignyodes* spp. (Coleoptera: Curculionidae), feed on seeds of trees belonging to the Oleaceae such as *Fraxinus* spp. and *Syringa* (lilac) (Barger and Davidson, 1967; Clark, 1980; Dix, 1986). These insects are found in North America and Eurasia (Clark et al., 1977; Clark, 1980, 1982; Haran et al., 2019) and could affect the effective conservation of ash seed resources (Solomon et al., 1993; Knight et al., 2010). *Lignyodes bischoffi* (Blatchley), for example, is widespread in North America, including the Great Lakes region, and is known to infest seeds of green ash (*F. pennsylvanica* Marsh.), black ash (*F. nigra* Marsh.) and white ash (*F. americana* Linnaeus) (Clark, 1980). Additionally, *L. bischoffi* weevils are invasive in Europe and have been observed infesting introduced green ash, European ash (*F. excelsior* Linnaeus), manna ash (*F. Ornus* Linnaeus) and the narrow-leaved ash (*F. angustifolia* subsp. *oxycarpa* Wild.) (Poiras, 1998; Wanat and Mocarski, 2008; Arzanov, 2013). Female ash seed weevils oviposit a single egg into a single ash seed encased in its fruiting body (samara) in the summer or fall, whereupon larvae hatch and feed on the seed contents. Larvae emerge from the seed in the summer, fall or spring and burrow into the soil. There, they pupate and emerge as adults in the subsequent summer (Barger and Davidson, 1967; Solomon et al., 1993).

The effect of merging the two *in situ* and *ex situ* measures, i.e. insecticide treatment of ash trees and seed collection, has not been investigated. The primary aim of this study was to analyze the effect of systemic insecticides on ash seed resource collected in cities in Minnesota. The specific objectives were to analyze the effect of two systemic insecticides against emerald ash borer, i.e. emamectin benzoate and azadirachtin, on (i) ash seed weevil prevalence and (ii) ash seedling germination time and ability.

2. Materials and methods

2.1. Sites

In July 2017, twelve urban sites with trees with visible signs and symptoms of early-stage infestation of emerald ash borer such as crown thinning and epicormic shoot growth were selected in Minnesota, USA (Table 1). At each site, we selected mature boulevard green ash trees of *F. pennsylvanica* with more than 70% crown present and administered one of two different systemic insecticides; emamectin benzoate (n=395 trees; Tree-äge G4®, Arborjet, Woburn, MA) or azadirachtin (n=200 trees; AzaSol®, Arborjet, Woburn, MA). A total of 678 other trees within these sites were left as untreated controls. Treated trees were assigned according to a gradient, but trees assigned to this study were randomly selected. The ratio of treated: untreated trees in each site was 50:50, and each site contained approximately 100 trees (Table 1). The insecticides were administered using a pressurized injection system (the QUIK-jet AIR® tree injection system, Arborjet, Woburn, MA). Each treated tree received a dose of 0.2 g AI per 2.54 cm diameter at breast height (DBH). The mean (±SE) DBH of all trees in the study sites from which seeds were collected from was 44 ±1.0 cm and was similar across the three treatments (ANOVA$F_{2,178}$=1.27, P=0.28).

Insecticide treatments were carried out from 26 to 30 June 2017 and 31 July to 8 August 2017 for emamectin benzoate and azadirachtin respectively. In subsequent years treatments were reapplied according to the manufacturer's instructions. The trees receiving emamectin benzoate were re-treated 26 to 28 June 2019 on an every other year schedule and the trees receiving azadirachtin were re-treated 4 to 8 June 2018, 10 to 17 June 2019 and 15 to 25 June 2020 on an every-year schedule.

Table 1
Cities in central and southeastern Minnesota, USA with experimental sites, 2017–2020, with the number of emamectin benzoate-treated, azadirachtin-treated and untreated ash trees.

Insecticide	City	Number of trees	
		Treated	Untreated
Emamectin benzoate	St. Paul	50	65
	Roseville	24	33
	Rochester	48	60
	Eagan	63	50
	Mendota Heights	40	44
	Maple Grove	65	58
	Lake City	60	65
	Coon Rapids	45	41
Azadirachtin	St. Paul	50	91
	Eagan	50	55
	Maple Grove	50	54
	Lake City	50	53

Table 2
The number of ash trees from which seeds were sampled for each treatment in October 2017, 2018 and 2019 from across eight cities in Minnesota, USA.

Year	Azadirachtin	Emamectin benzoate	Untreated	Total
2017	15	7	20	42
2018	31	45	52	128
2019	29	48	58	135

2.2. *Ash seed collection*

Ash seeds are contained in fruiting bodies called samaras that develop from fertilized female ash flowers. Ash seeds were collected in fall from ash trees when the samaras had changed from green to yellow to brown from 28 September to 26 October 2017(n=42 trees), 12 to 28 October 2018 (n=128 trees) and 10 to 28 October 2019 (n=135 trees). All the green ash trees in the 12 study sites were surveyed for seeds and seeds were sampled from all trees that had seeds in that year. The number of trees sampled for each treatment are shown in Table 2. A pole pruner was used to cut branches with seeds and then seeds were placed in brown paper bags and stored at room temperature.

2.3. *Ash seed weevil infestation level in ash seeds*

In December 2017, 2018, and 2019, a random collection of n=10 seeds for each of six trees treated with azadirachtin, emamectin benzoate, or untreated were peeled to calculate the frequency of seeds with ash seed weevil larvae. We did not try to determine if the larvae were alive (and potentially in diapause) or dead, as it was difficult to remove the seed casing without injuring or killing the insects. Generalized linear mixed effect models were used to analyze the effect of insecticide treatment, a categorical variable, weevil prevalence (i.e., the proportion of seeds that contained ash weevil larvae) as the binomial response variable on a per seed basis. A term for individual trees was incorporated as a random effect with a logit link function and binomial family specified in the model. The effect of treatment on weevil prevalence was analyzed with ANOVAs and, where treatment effects were observed, pairwise differences between treatments were explored using Tukey's means comparison test using the multcomp package in R (Hothorn et al., 2008). All data analysis was conducted in R (R Core Team, 2018).

2.4. *Ash seed germination assay*

Seeds were randomly selected from trees from each treatment (n=15 trees) and peeled in December 2017, 2018 & 2019 and stored in brown envelopes (2.54 cm L by 2.54 cm W) at room temperature until spring the subsequent year. On 27 March 2018, 2 April 2019 and 2 April 2020 the peeled seeds were placed in small (5.08 cm W by 7.62 cm H) clear Ziploc bags containing moist soil (Sun Gro® Horticulture, growing mix OMRI, Agawam, MA), which contains Canadian sphagnum peat moss, coarse perlite and dolomite lime. The Ziploc bags were left in refrigerator at 4 degrees Celsius for 90 days to allow the seeds to undergo cold stratification. In July, a subset of the seeds (n=6/tree in 2018 and n=5/tree in 2019 and 2020) were planted in 225-cell seedling starter trays filled with soil (Sun Gro® Horticulture, growing mix OMRI, Agawam, MA) to ¾ the height of each cell. Cell dimensions were 3.81 cm W by 5.72 cm H. The seedlings were watered once a week and covered with clear plastic bags to maintain a relative humidity of 50–60%. The trays were placed on a laboratory bench at room temperature and florescent light was used as a source of light. The first day of the germination assay was the day the seeds were planted, and the end date of the germination assay was the last day germination occurred before no new germination was observed for five consecutive days. The germination assays were concluded over a period of 30 to 34 days in the three years they were carried out; in 2018 from 10th July to 10th August (33 days), in 2019 from 5th July to 7th August (34 days) and in 2020 from 17th July to 15th August (30 days). Two events were recorded throughout the assay; germination ability, defined as whether a seedling germinated, and germination time defined as the day the cotyledon emerged above the surface of the soil.

Separate generalized linear mixed effect ANOVA models were used to analyze the effects of insecticide treatment on the response variables of seedling germination ability and time to germination. For seedling germination ability, we used a logit link function for this binomial response variable (i.e. did or did not germinate) and also incorporated a term for individual trees as a random effect. For seedling germination time, we again incorporated individual trees as a random effect and used a normal data distribution family. Analytical assumptions of linear models were examined through visual examination of residual plots. All data analysis was conducted in R (R Core Team, 2018).Where treatment effects were observed, pairwise differences between treatments were explored using Tukey's means comparison test using the multcomp package in R (Hothorn et al., 2008).

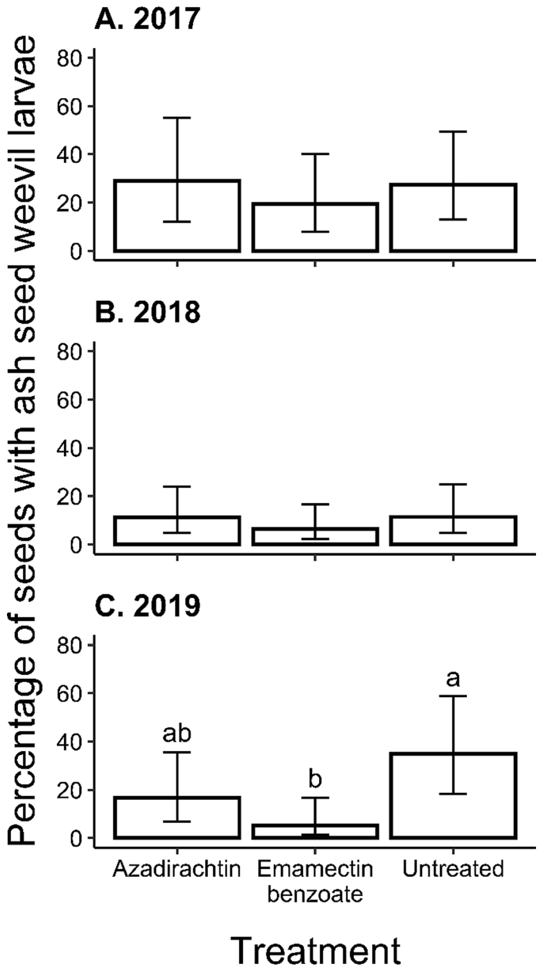

Fig. 1. Bar graphs of percentage of ash seeds with ash seed weevil larvae, i.e. weevil prevalence observed across seeds collected from ash trees treated with one of two insecticides, i.e. azadirachtin or emamectin benzoate and untreated trees in **A.** 2017 **B.** 2018 and **C.** 2019 in 8 cities in central and south east Minnesota (n=10 seeds per tree, from n=6 trees for each treatment). Error bars show 95% confidence intervals about means. Different letters indicate significant treatment differences (Tukey's means comparison test); ANOVA summaries provided in Table 3.

Table 3
Effects of insecticide treatment (azadirachtin, emamectin benzoate, or untreated ash trees) on weevil prevalence, seedling germination time, and germination ability. Seeds were examined or collected from trees in October 2017, 2018 and 2019 from across eight cities in Minnesota, USA.

Response	Figure	Year	Treatment Effect	
			χ^2_2	*P*
Weevil prevalence	1	2017	0.70	0.71
		2018	1.03	0.60
		2019	8.44	0.02
Seedling germination time	2	2018	4.41	0.11
		2019	0.82	0.66
		2020	0.99	0.61
Seedling germination ability	3	2018	1.18	0.55
		2019	0.61	0.74
		2020	5.32	0.07

3. Results

3.1. Ash seed weevil larvae prevalence in ash seeds

The ash seed weevil larval prevalence across all trees ranged from 0% to 70% in 2017, 0% to 30% in 2018 and 0% to 70% in 2019. The average weevil prevalence across all treatments in 2017 was 28% (±3.0 SE). The mean prevalence across all treatments dropped to10% (±2.0) in 2018, but then rose to 21% (±9.0) in 2019. Weevil prevalence was similar across all treatments; i.e. azadirachtin-treated trees, emamectin benzoate-treated trees and untreated trees in 2017 (Fig. 1A; Table 3) and 2018 (Fig. 1B). In 2019, the average weevil prevalence was highest in seeds collected from untreated trees (37%), followed by the trees treated with azadirachtin (20%) and then those treated with emamectin benzoate, where only 7% of the seeds contained a weevil larva. The untreated trees had a comparable weevil prevalence to azadirachtin-treated trees but were significantly greater than trees injected with emamectin benzoate (Fig. 1C).

We reared a weevil larva to an adult to aid in confirming species identity. We covered a peeled seed from an untreated tree containing a live larva with a thin layer of soil in a petri dish (50 by 9 mm, Falcon® Corning®, Corning, NY). The petri dish was placed in a 4 degrees Celsius fridge for 3 months. The petri dish was then removed from the fridge and placed at room temperature for 21 days until an adult weevil emerged. The weevil was confirmed as *Lignyodes bischoffi* (Blatchley).

3.2. Ash seed germination assay

Although we treated the seeds similarly each year, germination time was similar but percentage of seeds that germinated was variable. The germination time across all treatments, i.e. azadirachtin-treated trees, emamectin benzoate-treated trees and untreated trees was similar in all three years (Fig. 2; Table 3). Across all treatments seeds germinated in a mean time of 32 ±1 days, after planting if they were going to germinate at all.

The percentage of ash seeds that germinated (germination ability) across all treatments collected from an individual tree ranged from 0% to 100% in all three years (2018 to 2020). The average percentage of seeds that germinated across all treatments was 53% (±3.0) in 2018, it was 39% (±1.0) in 2019 and it was 81% (±4.0) in 2020. Germination ability was similar across all treatments i.e. azadirachtin-treated trees, emamectin-benzoate treated trees and untreated trees in 2018, 2019, and 2020 (Fig. 3; Table 3).

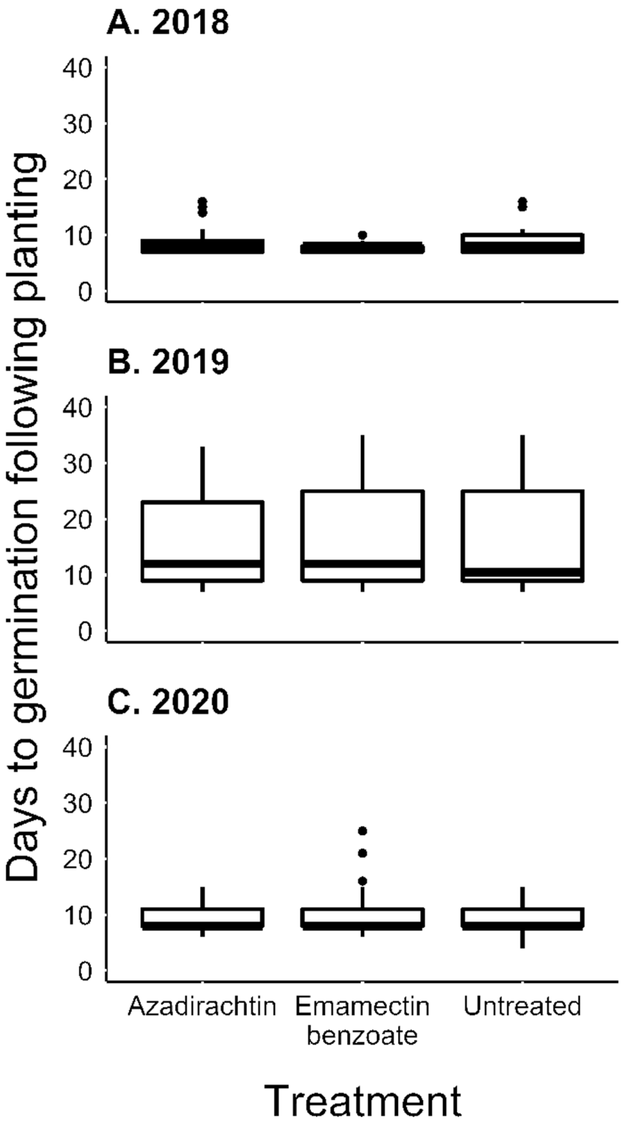

Fig. 2. Box and whisker plots of number of days to germination (i.e, the day the cotyledon emerged above surface of the soil) after cold treatment and planting. Ash seeds were collected in 8 cities in central and southeast Minnesota and planted in **A.** 2018 **B.** 2019 and **C.** 2020. The upper whisker indicates the maximum value as the third quartile added to 1.5 times the interquartile range. The lower whisker represents the minimum value as the first quartile minus 1.5 times the interquartile range. The middle line of each box and whisker plot represents the median of the data set. Dots represent data points beyond plus or minus 1.5 times the interquartile range. In 2018, $n=6$ seeds were planted per tree and in 2019 and 2020 $n=5$ seeds were planted per tree.

4. Discussion

Our data suggests that repeated years of the application of insecticide treatments against EAB can reduce the prevalence of seed-feeding weevils in ash trees, thus assisting in additional preservation of ash seed germplasm (Fig. 1). After three years of insecticide treatments the average weevil prevalence in 2019 was 30% and 17% higher in untreated trees than in trees treated with emamectin benzoate and azadirachtin respectively, although the reduction in weevil prevalence among trees treated with azadirachtin was not statistically significant. We postulate that this sink effect emerges over several years due to localized mortality of adult weevils that feed on treated ash leaves and limited dispersal capacity of the species. While feeding behaviour of ash weevil adults is not well elucidated in literature, the ligustrum weevil *(Ochyromera ligustri* Warner), a close relative that belongs to the same tribe (Tychiini), feeds on the seeds of the Chinese or hedge privet (*Ligustrum sinense* Lour., Oleaceae) during its larval stage and the leaves during its adult stage (Cuda and Zeller, 1998; Cuda et al., 2005). If ash seed weevils generally re-infest the same trees from year to year, a lower weevil prevalence would emerge where adult mortality accrues on trees

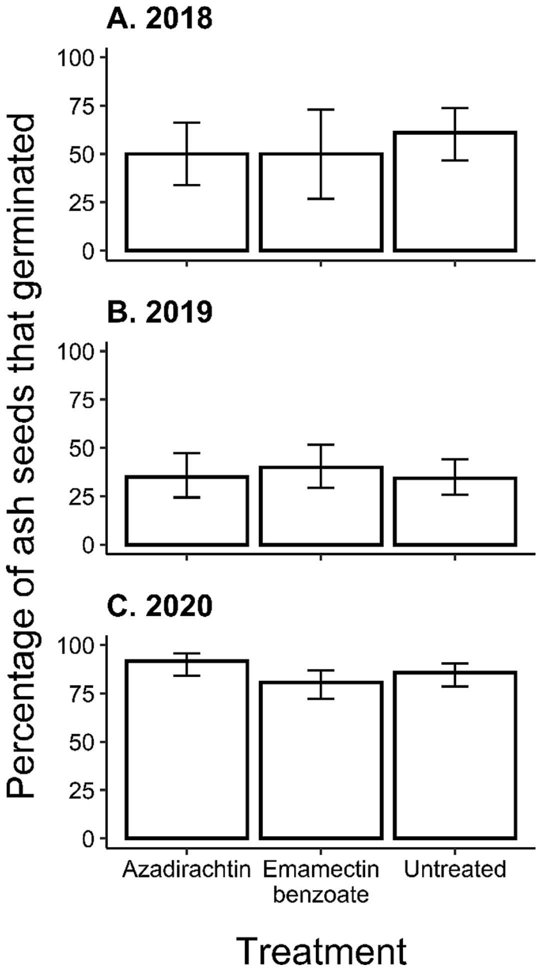

Fig. 3. Bar graphs of percentage of ash seeds that germinated from ash seeds collected from trees in 8 cities in central and southeast Minnesota and planted in **A.** 2018 **B.** 2019 and **C.** 2020. In 2018, $n=6$ seeds were planted per tree and in 2019 and 2020 $n=5$ seeds were planted per tree. Error bars show 95% confidence intervals about mean.

treated with insecticide *versus* weevil populations in untreated trees where they continue to proliferate. Localized feeding and restricted dispersal is not uncommon among seed-feeding weevils. Adults of the mango seed weevil (*Sternochetus mangiferae* Fabricius), which is a pest of mangoes in Africa, Asia, North America, Oceania, Europe and South America (EPPO, 2021), for example, usually remain on the same tree from which they had emerged such that dispersal and movement relies on transportation of seed and fruit (Subramanyam, 1926; Jarvis, 1946; Balock and Kozuma, 1964). The higher mortality noted in the emamectin benzoate vs. azadirachtin treatments could due to lower toxicity of azadirachtin, or perhaps differences in the mode of action of the two insecticides. Emamectin benzoate is a neurotoxin while azadirachtin can act as an antifeedant, growth disruptor, and/or reduce reproduction (Jansson et al., 1997; Mordue and Nisbet, 2000; Shawir et al., 2014).

Our study shows that the two systemic insecticides used against emerald ash borer, emamectin benzoate and azadirachtin, did not affect germination time and ability of ash seeds (Fig. 2; Fig. 3). Most research on phytotoxic effects of insecticides on seed germplasm have focused on direct contact of insecticides with seeds (Parween et al., 2016). In a laboratory germination study conducted by Olofinboba and Kozlowski (1982), for example, germination (i.e. cotyledon emergence) was either inhibited or completely prevented by direct application of three trunk systemic insecticides to seeds of Aleppo pine (*Pinus halepensis* Mill.). Potential mechanism for the limitation or inhibition of seed germination by insecticides include prevention of the germination of pollen that, aside from preventing seed development, could result in development of seeds void of endosperms that are thus inviable (Meyer et al., 1973; Sutherland et al., 1984; Annila and Heliövaara, 1991; Kimura et al., 1996). Furthermore, insecticides could affect the production of and activity of hormones and enzymes involved in seed germination (Singh et al., 1982; Bashir et al., 2014).

Previous work investigating the translocation of trunk injected systemic insecticides has focused on detecting insecticide presence in the bark, vascular system, roots and leaves of trees (Mota-Sanchez et al., 2009; Cevenini and Minelli, 2010; Tanis et al., 2012). In the absence of published data that systemic insecticides are translocated to the seed germplasm, we conclude that the insecticide either does not reach the seed germplasm or is present only at trace levels. Insecticide concentration can be a proponent of phytotoxic effects observed in seed germination (Werner, 1974) but we did not observe phytotoxic effects on germination time and ability between seeds from treated and untreated trees after three years of insecticide application.

Our work suggests a number of avenues for future research. First, feeding bioassays in the laboratory with adult weevils on treated foliage could ascertain mechanisms behind the field patterns of decreasing weevil prevalence observed over three years (e.g., toxicity vs. aversion). Second, little is known about dispersal capacity of the adults, and/or whether they could feed on other members of the Olaceae prior to oviposition on *Fraxinus* spp. Both of these factors could affect long-term population reduction strategies. Finally, there is little known about mortality factors of these weevils that affect their population dynamics. We noted a marked decrease in weevil abundance across the three treatments in the second year of the study (Fig. 1), but are unsure whether overwintering mortality or other causes were responsible for this pattern.

As emerald ash borer continues to kill ash trees in North America, there is a need to conserve the ash genetic resource until a time when the insect is no longer a threat or more permanent measures have been put in place to re-introduce ash into the landscape. Seed bank collections allow for the ash genetic variation to be conserved, while systemic insecticides treatments allow ash trees to remain on the landscape where they can continue to provide key ecological services. Our results show that there is potential in combining both genetic conservation measures. The insecticide treatments against emerald ash borer reduced potential loss of seed germplasm by curtailing the presence of seed feeding weevils after multiple years of insecticide application. Moreover, it did not appear to have an adverse effect on ash seed germination time and ability.

CRediT authorship contribution statement

Dorah M. Mwangola: Conceptualization, Methodology, Formal analysis, Investigation, Writing – original draft, Visualization. **Aubree M. Kees:** Methodology, Investigation, Writing – review & editing, Visualization. **Donald M. Grosman:** Methodology, Writing – review & editing. **Brian H. Aukema:** Conceptualization, Methodology, Writing – review & editing, Supervision, Project administration, Funding acquisition.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

We thank Bade Turgut, Joe Ure, Elgin Lee, Mara Short, Kristine Jecha, Tenzin Dothar, Nicole Witt, Piper Haynes, Stephanie Gunter and Cole Doolittle for technical and field help (UMN). Jennifer Burington with the Minnesota Department of Agriculture (MDA) and city foresters of the city of Saint Paul, Rochester, Maple Grove, Coon Rapids, Maple Grove, Roseville, Lake City and Eagan for continued support and correspondence throughout the study. Funding for this project was provided by the Minnesota Invasive Terrestrial Plants and Pests Center through the Minnesota Environment and Natural Resources Trust Fund appropriation ML 2015, Ch. 76, Art. 2, Sec. 6a. We appreciate the comments of two anonymous reviewers who improved earlier drafts of this work.

References

Annila, E., Heliövaara, K., 1991. Chemical control of cone pests in a Norway spruce seed orchard. Silva Fenn. 25, 56–67.

Arzanov, Y.G., 2013. *Lignyodes bischoffi* Blatchley, 1916 (Curculionidae)-A new species of invasive weevils for Russia. Russ. J. Biol. Invasions 4 (4), 209–211. https://doi.org/10.1134/S2075111713040024.

Balock, J.W., Kozuma, T.T., 1964. Notes on the Biology and Economic Importance of the Mango Weevil, Sternochetus mangiferae (Fabricius), in Hawaii (Coleoptera: Curculionidae).

Barger, J., Davidson, R., 1967. A life history study of the ash seed weevils, *Thysanocnemis bischoffi* Blatchley and *T. helvola* LeConte (Coleoptera: Curculionidae). Ohio J. Sci. 67, 123–127.

Bashir, F., Zahid, F., Iqbal, M., 2014. Growth performance, photosynthetic efficiency and pigment concentration of Glycine max (L.) Merr., as affected by alphamethrin, a synthetic pyrethroid insecticide. Trends Biotechnol. Biol. Sci 1, 29–35.

Cappaert, D., McCullough, D.G., Poland, T.M., Siegert, N.W., 2005. Emerald ash borer in North America: A research and regulatory challenge. Am. Entomol. 51, 152–165. https://doi.org/10.1093/ae/51.3.152.

Cevenini, L., Minelli, A., 2010. Translocation of active ingredient using three trunk injection methods. Acta Hortic. (881), 409–412. https://doi.org/10.17660/ActaHortic.2010.881.64.

Clark, W.E., 1982. Classification of the weevil tribe Lignyodini (Coleoptera, Curculionidae, Tychiinae), with revision of the genus Plocetes. Trans. Am. Entomol. Soc. 108, 11–151.

Clark, W.E., 1980. Revision of Nearctic weevils of the genus *Lignyodes* DEJEAN (Coleoptera: Curculionidae). Trans. Am. Entomol. Soc. 103, 273–326.

Clark, W.E., Whitehead, D.R., Warner, R.E., 1977. Classification of the weevil subfamily Tychiinae, with a new genus and species, new combinations, and new synonymy in Lignyodini (Coleoptera: Curculionidae). Coleopt. Bull. 31, 1–18.

Cuda, J.P., Zellar, M.C., Thomas, M.C., 2005. Ligustrum Weevil (suggested common name), *Ochyromera ligustri* Warner (Insecta: Coleoptera: Curculionidae: Tychiinae: Tychiini: Endaeina). EDIS. https://doi.org/10.32473/edis-in620-2005.

Cuda, J.P., Zeller, M.C., 1998. First record of *Ochyromera ligustri* (Coleoptera: Curculionidae) from Chinese privet in Florida. Florida Entomol. 81, 582–584. https://doi.org/10.2307/3495965.

Dix, M.E., 1986. Lilac seed, an alternate host for the ash seed weevil, *Lignyodes bischoffi* (Blatchley) (Coleoptera: Curculionidae). J. Kansas Entomol. Soc. 59, 389–390.

Donovan, G.H., Butry, D.T., Michael, Y.L., Prestemon, J.P., Liebhold, A.M., Gatziolis, D., Mao, M.Y., 2013. The relationship between trees and human health: Evidence from the spread of the emerald ash borer. Am. J. Prev. Med. 44 (2), 139–145. https://doi.org/10.1016/j.amepre.2012.09.066.

EPPO, 2021. *Sternochetus mangiferae* (World distribution) EPPO Global Database [WWW Document]. URL https://gd.eppo.int/taxon/CRYPMA/distribution (Accessed June 17, 2021).

Flower, C.E., Fant, J.B., Hoban, S., Knight, K.S., Steger, L., Aubihl, E., Gonzalez-Meler, M. A., Forry, S., Hille, A., Royo, A.A., 2018. Optimizing conservation strategies for a threatened tree species: In situ conservation of white ash (*Fraxinus americana* L.) genetic diversity through insecticide treatment. Forests 9, 202. https://doi.org/10.3390/f9040202.

Gandhi, K.J.K., Herms, D.A., 2010a. North American arthropods at risk due to widespread Fraxinus mortality caused by the alien Emerald ash borer. Biol. Invasions 12 (6), 1839–1846. https://doi.org/10.1007/s10530-009-9594-1.

Gandhi, K.J.K., Herms, D.A., 2010b. Direct and indirect effects of alien insect herbivores on ecological processes and interactions in forests of eastern North America. Biol. Invasions 12 (2), 389–405. https://doi.org/10.1007/s10530-009-9627-9.

Haran, J., Cocquempot, C., Anderson, R., 2019. *Lignyodes helvolus* (LeConte, 1876), une espèce associée aux Frênes nouvellement établie en France continentale (Coleoptera Curculionidae). L'Entomologiste 75, 309–311.

Herms, D.A., McCullough, D.G., 2014. Emerald Ash Borer Invasion of North America: History, Biology, Ecology, Impacts, and Management. Annu. Rev. Entomol. 59 (1), 13–30. https://doi.org/10.1146/annurev-ento-011613-162051.

Hothorn, T., Bretz, F., Westfall, P., 2008. Simultaneous inference in general parametric models. Biometrical J 50 (3), 346–363. https://doi.org/10.1002/bimj.200810425.

Jansson, R.K., Brown, R., Cartwright, B., Cox, D., Dunbar, D.M., Dybas, R.A., Eckel, C., Lasota, J.A., Mookerjee, P.K., Norton, J.A., Peterson, R.F., Starner, V.R., White, S., 1997. Emamectin benzoate: a novel avermectin derivative for control of lepidopterous pests. Chem. Control 171–177.

Jarvis, H., 1946. Pests of the mango. Queensl. Agric Jour 62, 10–14.

Kashian, D.M., Witter, J.A., 2011. Assessing the potential for ash canopy tree replacement via current regeneration following emerald ash borer-caused mortality on southeastern Michigan landscapes. For. Ecol. Manage. 261 (3), 480–488. https://doi.org/10.1016/j.foreco.2010.10.033.

Kimura, P.H., Okamoto, G., Hirano, K., 1996. Effects of gibberellic acid and streptomycin on pollen germination and ovule and seed development in muscat bailey A. Am. J. Enol. Vitic. 47, 152–156.

Klooster, W.S., Herms, D.A., Knight, K.S., Herms, C.P., McCullough, D.G., Smith, A., Gandhi, K.J.K., Cardina, J., 2014. Ash (*Fraxinus* spp.) mortality, regeneration, and seed bank dynamics in mixed hardwood forests following invasion by emerald ash borer (Agrilus planipennis). Biol. Invasions 16 (4), 859–873. https://doi.org/10.1007/s10530-013-0543-7.

Knight, K.S., Karrfalt, R.P., Mason, M.E., 2010. United States Department of Agriculture Methods for Collecting Ash (*Fraxinus* spp.) Seeds.

Koch, J.L., Carey, D.W., Knight, K.S., Poland, T., Herms, D.A., Mason, M.E., 2011. Breeding strategies for the development of emerald ash borer-resistant North American ash, in: Proceedings of the 4th International Workshop on Genetics of Host-Parasite Interactions in Forestry. pp. 235–239.

Thomas Ledig, F., 1986. Conservation strategies for forest gene resources. For. Ecol. Manage. 14 (2), 77–90. https://doi.org/10.1016/0378-1127(86)90093-9.

Ledig, F.T., Vargas-Hernández, J.J., Johnsen, K.H., 1998. The conservation of forest genetic resources: Case histories from Canada, Mexico, and the United States. J. For. 96, 32–41. https://doi.org/10.1093/jof/96.1.32.

Meyer, B.S., Anderson, D.B., Bohning, R.H., Fratianne, D.G., 1973. Introduction to Plant Physiology, Second. ed. D. Van Nostrand Company.

Mordue(Luntz), A.J., Nisbet, A.J., 2000. Azadirachtin from the neem tree Azadirachta indica: its action against insects. An. da Soc. Entomológica do Bras. 29 (4), 615–632.

Mota-Sanchez, D., Cregg, B.M., McCullough, D.G., Poland, T.M., Hollingworth, R.M., 2009. Distribution of trunk-injected 14C-imidacloprid in ash trees and effects on emerald ash borer (Coleoptera: Buprestidae) adults. Crop Prot. 28 (8), 655–661. https://doi.org/10.1016/j.cropro.2009.03.012.

O'Brien, E.M., Herms, D.A., Gardiner, M.M., Goebel, P.C., Michel, A., 2017. Conserving Ash (*Fraxinus*) Populations and Genetic Variation in Forests Invaded. The Ohio State University.

Olofinboba, M.O., Kozlowski, T.T., 1982. Effects of three systemic insecticides on seed germination and growth of *Pinus halepensis* seedlings. Plant Soil 64 (2), 255–258. https://doi.org/10.1007/BF02184257.

Parween, T., Jan, S., Mahmooduzzafar, S., Fatma, T., Zahid, &, Siddiqui, H., Siddiqui, Z. H., 2016. Selective Effect of Pesticides on Plant-A Review. Crit. Rev. Food Sci. Nutr. 56, 160–179. https://doi.org/10.1080/10408398.2013.787969.

Poiras, A.A., 1998. Catalogue of the Weevils (Coleoptera, Curculionoidea) and their host plants in the Republic of Moldova. Pensoft Pub. https://doi.org/10.1649/0010-065x(2000)054[0548:cotwat]2.0.co;2.

Pritchard, H.W., Moat, J.F., Ferraz, J.B.S., Marks, T.R., Camargo, J.L.C., Nadarajan, J., Ferraz, I.D.K., 2014. Innovative approaches to the preservation of forest trees. For. Ecol. Manage. 333, 88–98. https://doi.org/10.1016/j.foreco.2014.08.012.

R Core Team, 2018. R: A Language and Environment for Statistical Computing.

Rajora, O.P., Mosseler, A., 2001. Challenges and opportunities for conservation of forest genetic resources. Euphytica 118, 197–212. https://doi.org/10.1023/A:1004150525384.

Ramsfield, T.D., Bentz, B.J., Faccoli, M., Jactel, H., Brockerhoff, E.G., 2016. Forest health in a changing world: Effects of globalization and climate change on forest insect and pathogen impacts. Forestry 89 (3), 245–252. https://doi.org/10.1093/forestry/cpw018.

Shawir, M.S., Abdel-Latif Abbassy, M., Salem, Y.M., 2014. Laboratory Evaluation of some Insecticides against Larval and Adult Stages of Red Palm Weevil's Rhynchophorus ferrugineus (Olivier). Alexandria Sci. Exch. J. An Int Q. J. Sci. Agric. Environ. 35, 75–79. https://doi.org/10.21608/asejaiqjsae.2014.2579.

Šijačić-Nikolić, M., Milovanović, J., Nonić, M., 2014. Conservation of Forest Genetic Resources. In: Ahuja, M.R., Ramawat, K.G. (Eds.), Biotechnology and Biodiversity. Springer International Publishing, Cham, pp. 103–128. https://doi.org/10.1007/978-3-319-09381-9_7.

Singh, V.K., Mathur, M., Mathur, S.N., 1982. Phyto-toxicity of the insecticide, phorate on germination of vigna mungo. Agric. Biol. Chem. 46 (6), 1681–1682. https://doi.org/10.1080/00021369.1982.10865307.

Solomon, J.D., Leininger, T.D., Wilson, A.D., Andernon, R.L., Thompson, L.C., McCracken, F.I., 1993. Ash pests: Guide to Major Insects, Diseases, Air Pollution Injury, and Chemical Injury.

St.Clair, J.B., Howe, G.T., 2011. Strategies for conserving forest genetic resources in the face of climate change. Turk. J. Botany 35, 403–409. https://doi.org/10.3906/bot-1012-98.

Subramanyam, C.K., 1926. A note on the life history of *Cryptorhynchus mangiferae* Fabricius. Madras Agric. Dep. Yearb. 1925, 29–36.

Sutherland, J.R., Woods, T.A.D., Miller, G.E., 1984. Effect of selected insecticides and fungicides on germination of Douglas-fir and white spruce pollen. Tree Plant. Notes 35, 22–24.

Tanis, S.R., Cregg, B.M., Mota-Sanchez, D., McCullough, D.G., Poland, T.M., 2012. Spatial and temporal distribution of trunk-injected 14C-imidacloprid in Fraxinus trees. Pest Manag. Sci. 68 (4), 529–536. https://doi.org/10.1002/ps.2281.

USDA Forest Service and Michigan State University, 2022. emeraldashborer.info [WWW Document]. URL http://www.emeraldashborer.info (Accessed February 22, 2022).

Wanat, M., Mocarski, Z., 2008. Current range of the ash seed weevil *Lignyodes bischoffi* Blatchley, 1916 (Coleoptera: Curiculionidae) in Poland. Polish J. Entomol. 77, 177–182.

Werner, R.A., 1974. Penetration and Persistence of Systemic Insecticides in Seeds and Seedlings of Southern Pines. J. Econ. Entomol. 67 (1), 81–84. https://doi.org/10.1093/jee/67.1.81.

Widrlechner, M.P., 2010. Building a comprehensive collection of Ash germplasm, in: Proceedings of the 4th Global Botanic Gardens Congress. pp. 1–10.

Woodcock, P., Marzano, M., Quine, C.P., 2019. Key lessons from resistant tree breeding programmes in the Northern Hemisphere. Ann. For. Sci. 76, 51. https://doi.org/10.1007/s13595-019-0826-y.