

Evaluations of Insecticides and Fungicides for Reducing Attack Rates of a new invasive ambrosia beetle (*Euwallacea* Sp., Coleoptera: Curculionidae: Scolytinae) in Infested Landscape Trees in California

Michele Eatough Jones,^{1,2} John Kabashima,³ Akif Eskalen,⁴ Monica Dimson,³ Joey S. Mayorquin,⁴ Joseph D. Carrillo,⁴ Christopher C. Hanlon,¹ and Timothy D. Paine¹

¹Department of Entomology, University of California Riverside, Riverside, CA 92521 (michele.eatough@ucr.edu; christopher.hanlon@ucr.edu; timothy.paine@ucr.edu), ²Corresponding author, e-mail: michele.eatough@ucr.edu, ³University of California Cooperative Extension, 7601 Irvine Boulevard, Irvine, CA 92618 (jnkabashima@ucanr.edu; mjdimson@ucanr.edu), and ⁴Department of Plant Pathology and Microbiology, University of California Riverside, Riverside, CA 92521 (akif.eskalen@ucr.edu; jmayo001@ucr.edu; jcarr022@ucr.edu)

Subject Editor: Timothy Schowalter

Received 21 March 2017; Editorial decision 22 May 2017

Abstract

A recently discovered ambrosia beetle with the proposed common name of polyphagous shot hole borer (*Euwallacea* sp., Coleoptera: Curculionidae: Scolytinae), is reported to attack >200 host tree species in southern California, including many important native and urban landscape trees. This invasive beetle, along with its associated fungi, causes branch dieback and tree mortality in a large variety of tree species including sycamore (*Platanus racemosa* Nutt.). Due to the severity of the impact of this *Euwallacea* sp., short-term management tools must include chemical control options for the arboriculture industry and private landowners to protect trees. We examined the effectiveness of insecticides, fungicides, and insecticide–fungicide combinations for controlling continued *Euwallacea* sp. attacks on previously infested sycamore trees which were monitored for 6 mo after treatment. Pesticide combinations were generally more effective than single pesticide treatments. The combination of a systemic insecticide (emamectin benzoate), a contact insecticide (bifenthrin), and a fungicide (metconazole) provided some level of control when applied on moderate and heavily infested trees. The biological fungicide *Bacillus subtilis* provided short-term control. There was no difference in the performance of the three triazole fungicides (propiconazole, tebuconazole, and metconazole) included in this study. Although no pesticide combination provided substantial control over time, pesticide treatments may be more effective when trees are treated during early stages of attack by this ambrosia beetle.

Key words: ambrosia beetle, bifenthrin, emamectin benzoate, triazole fungicide

The as yet undescribed beetle with the proposed common name of polyphagous shot hole borer (Coleoptera: Curculionidae: Scolytinae) vectors fungal pathogens that cause the disease known as Fusarium Dieback. The beetle was first identified in southern California in 2003 (Rabaglia et al. 2006; Eskalen et al. 2012, 2013). It has now been reported in Los Angeles, Orange, Ventura, Riverside, and San Bernardino Counties (Eskalen 2016). The beetle, hereafter referred to as *Euwallacea* sp., is an ambrosia beetle closely related to and morphologically indistinguishable from the Southeast Asian species, *Euwallacea formicatus*. Females are black (1.8–2.5 mm) and the much less abundant males are wingless and smaller in size (1.5–1.67 mm). Newly enclosed adults mate with siblings while still in the maternal gallery.

Female beetles carry fungal spores in mandibular mycangia and transmit them to host trees while boring into the tree (Fernando 1959). Fungal species carried by *Euwallacea* sp. include *Fusarium euwallaceae*, *Graphium euwallaceae*, and *Paracremonium pembeum* (Freeman et al. 2012, Eskalen et al. 2013, Lynch et al. 2016). Females initiate galleries, inoculate galleries with fungi, and continue to expand that gallery over time, laying clusters of eggs (Umeda et al. 2016). The initial entry hole to the gallery is used as both an entrance and an exit. Both adult beetles and developing larvae feed on the fungi. Fusarium Dieback is a vascular disease in plants caused by fungi associated with the beetle (Eskalen et al. 2012, Freeman et al. 2013, Lynch et al. 2016). The disease is a result of fungal colonization in active xylem tissue, leading to interruption

Table 1. Information for each chemical used

Active ingredient	Class	Rate (amount a.i.)	Method	Trade name	Manufacturer
Bifenthrin	Pyrethroid insecticide	240 g/liter	Trunk spray	Onyx	FMC Corporation, Philadelphia, PA
Emamectin benzoate	Avermectin insecticide	2.4 ml/cm DBH	Injection	TREE-äge	Arborjet, Woburn, MA
Tebuconazole	Triazole fungicide	2.4 ml/cm DBH	Injection	Tebuject 16	Mauget, Arcadia, CA
Propiconazole	Triazole fungicide	2.4 ml/cm DBH	Injection	Propizol	Arborjet, Woburn, MA
Metconazole	Triazole fungicide	18.14 g/cm DBH	Trunk spray	Tourney + surfactant	Valent, Walnut Creek, CA
Surfactant for metconazole	Penetrating agent	2.9 ml/cm DBH	Trunk spray	Pentra-Bark	AgBio Inc., Westminster, CO
<i>Bacillus subtilis</i> QST 713 strain	Microbial fungicide	1% solution	Trunk spray	Cease + surfactant	Bayer Environmental Science, Research Triangle Park, NC
Surfactant for <i>B. subtilis</i>	Penetrating agent	2.9 ml/cm DBH	Trunk spray	Pentra-Bark	AgBio Inc., Westminster, CO

of nutrient and water transport, causing branch dieback, and in severe cases, tree death.

In California, *Euwallacea* sp. has been recorded attacking >200 species of trees, with 49 currently known suitable reproductive hosts from >20 plant families (Eskalen 2016). Reproductive hosts include many California natives (e.g., California sycamore (*Platanus racemosa* Nutt.), coast live oak (*Quercus agrifolia* Née), and arroyo and black willows (*Salix lasiolepis* Benth. and *S. gooddingii* C.R. Ball)), common urban species (e.g., Japanese maple (*Acer palmatum* Thunb.) and *Liquidambar styraciflua* L.), and important commercial crops (avocado, *Persea americana* Mill) (Eskalen et al. 2013). Due to its broad host range, *Euwallacea* sp. causes dieback and tree mortality in trees in parks, residential neighborhoods, other public landscapes, and riparian areas. It also has the potential for ecological impacts in natural forests and ecosystems, as well as economic impacts in avocado-growing regions in southern California. Management tools are needed by home owners, park managers, and arborists to help protect landscape trees and minimize the aesthetic and economic impacts of *Euwallacea* sp. The range of options for the immediate term may include direct control using contact insecticides, systemic insecticides, and fungicides to prevent infestation or manage infesting beetles and their associated fungi to limit spread of this ambrosia beetle (Cranham 1966, Paine et al. 2011). The efficacy of sanitation options, such as chipping or solarization of infested wood to prevent beetle emergence, has previously been reported (Eatough Jones and Paine 2015).

Trunk sprays have conventionally been used to protect trees from bark beetle attack (Fettig et al. 2013a). Laboratory tests with cut logs showed bifenthrin was most effective for deterring attack by *Euwallacea* sp. (Eatough Jones and Paine 2017). Additionally, fungicides have been used to protect trees from a variety of beetle-vectored fungal diseases (e.g., Appel and Kurdyla 1992, Haugen and Stennes 1999, Mayfield et al. 2008). Systemic insecticides that are injected into the lower bole of the tree have been receiving increased attention as alternatives to bole sprays for preventing bark beetle attacks (Fettig et al. 2013a).

The objective of this study was to test the effectiveness of insecticides, fungicides, and insecticide–fungicide combinations for controlling *Euwallacea* sp. We chose one systemic insecticide, one contact insecticide, three triazole fungicides, and one bacterial fungicide applied individually and in combinations. To test the effectiveness of the pesticide treatments, we treated mature, infested sycamore trees, and monitored for increasing attacks over time.

Materials and Methods

Naturally infested, mature California sycamore (*Platanus racemosa* Nutt.) trees were selected on the campus of University of California, Irvine (Orange Co.). We initially selected 100 trees and nine

treatments. The nine treatments were as follows: 1) bifenthrin, 2) emamectin benzoate, 3) tebuconazole, 4) propiconazole, 5) metconazole, 6) *Bacillus subtilis*, 7) bifenthrin + tebuconazole, 8) emamectin benzoate + tebuconazole, and 9) untreated controls. Table 1 shows application rates and manufacturer information for all pesticides used. Metconazole was applied using a backpack sprayer (Chapin Tree/TurfPro model 62000, Batavia, NY) to 2.4 m trunk height. Bifenthrin and *B. subtilis* were applied by spraying to run-off up to 2.4 m trunk height with a 200-gallon truck-mounted sprayer. Tebuconazole was injected with a ChemJet tree injector (Mauget, Arcadia, CA). Emamectin benzoate and propiconazole were injected using a Tree I.V. system (Arborjet, Woburn, MA).

We measured tree diameter at 1.5 m above ground (diameter at breast height, DBH) for all selected trees and assessed infestation levels as light, moderate, or heavy (categories based on a quick visual assessment as approximately: light <50 attacks, moderate 50–200 attacks, or heavy >200 attacks in ~1 m of trunk length centered at breast height). All selected trees were GPS mapped and randomly assigned to one of the nine treatments using a random treatment assignment tool (Urbaniak and Plous 2015), resulting in 11 trees per treatment (with the extra tree going to the control group). Group assignments were checked to confirm that there were no significant differences in DBH and estimated infestation level. During the initial period of evaluation, a 10th treatment was added: emamectin benzoate + propiconazole. These trees were scattered around the circumference of the area where the initial nine treatments were located. In July 2015, one month after other treatments were initiated, an 11th treatment was added: bifenthrin + emamectin benzoate + metconazole. Mean DBH for selected trees was 41.1 ± 1.3 cm (mean and S.E.).

In June 2015, prior to beginning treatments, we quantified the number of attacks on each tree. Pesticide treatments were applied in late June 2015, after the initial attack data were collected, except for treatment 11 (bifenthrin + emamectin benzoate + metconazole), where initial counts and pesticide application were performed in July 2015. The number of attacks were assessed again 1 mo after pesticide application (July 2015), and then at ~3 mo (September 2015) and 6 mo (December 2015) after pesticide application. During the period of pesticide application, several trees that had been selected were excluded from the study due to tree removal or other unforeseen situations. Tree removals (outside the control of the investigators) continued sporadically throughout the course of monitoring due to hazard conditions as tree health declined. This resulted in an unbalanced number of trees assigned to each treatment.

Data Collection

We counted all *Euwallacea* sp. entrance holes around the entire circumference of the main trunk within an area 0.9 m in length,

Table 2. Number of trees included at each count period for each treatment

Treatment	No. of trees in each treatment			
	June	July	Sept.	Dec.
Untreated control	10	9	10	11
Bifenthrin	10	11	9	9
Emamectin benzoate	8	9	10	10
Tebuconazole	8	10	10	10
Propiconazole	8	9	9	8
Metconazole	8	8	7	8
<i>Bacillus subtilis</i>	7	7	7	7
Emamectin benzoate + Propiconazole	8	7	8	9
Emamectin benzoate + Tebuconazole	9	8	9	9
Bifenthrin + Tebuconazole	11	11	10	10
Emamectin benzoate + Bifenthrin + Metconazole	NA	10	10	10

Data audits resulted in counts from a few trees being excluded during some months. Additionally, some trees were removed, as branch dieback lead to hazard conditions in public spaces.

starting at a trunk height of ~0.9 m from the ground. Each hole was marked with a small dot to the side of the hole using a paint marker, and counts were tallied with a hand-held tally counter. Entrance holes were determined by their shape and size, a round hole approximately the size of the tip of a ball point pen (0.85 mm diameter). If there was an accumulation of sap or boring dust, but it was unclear if there was a hole underneath, the area was cleared with a plastic putty knife or pen cap to verify the presence of a hole. At the end of the count, the length of the count area was measured in two arbitrary locations around the trunk and recorded. Length of the count area and DBH were used to calculate the total surface area counted for each tree. Attack density for each tree and each sampling period was calculated as total entrance holes/m².

During subsequent monitoring periods in July 2015, September 2015, and December 2015, all holes in the same area on the main trunk were counted again. A different color of paint marker was used each period, and both new holes and all previously marked holes were marked with the new color and tallied. Marking and counting all existing holes was necessary because bark regularly peeled away from the trunk.

Additionally, in October 2015, due to the large number of people participating in the counts, recorded data were audited against paint marks visible on the trees. The audit was conducted by a smaller group of graduate students and researchers that had been working extensively with this ambrosia beetle, with three to four people checking each tree. Some discrepancies, particularly a lower number of paint marks visible than had been recorded, were expected due to shedding of bark, weathering, and public access to the trees. In these cases, the originally recorded data were used. For other unaccounted discrepancies between recorded data and paint marks visible on the tree, data for that tree from that count period were excluded from the dataset. This audit resulted in 25 of the 310 recorded tree counts between June and September being excluded from the dataset due to probable counting errors. An additional 10 data points were missing due to tree removal after treatments were begun. For the monitoring period in December, two or more people checked each tree, each checking over the entire count area two or three times. A list of all treatments, and the number of trees included in each treatment at each count period, is given in Table 2.

Statistical Analysis

We analyzed the effectiveness of each pesticide treatment in two ways. First, we compared the number of attacks within each

individual treatment over time using a paired comparison *t*-test (SAS Institute Inc. 2012). We compared the attacks/m² for each tree for pretreatment counts in June 2015 to subsequent counts at 1, 3, and 6 mo posttreatment. Since trees treated with bifenthrin + emamectin benzoate + metconazole were added in July 2015, paired comparison *t*-tests for this treatment were evaluated at 2 and 5 mo posttreatment.

Second, we contrasted the increase in attacks for each pesticide treatment to the increase in attacks for untreated control trees. We have observed that newly emerging beetles are more likely to initiate attacks on their natal host tree, rather than migrating to a new host. Because of this, the number of new attacks recorded for each tree was significantly correlated with the total number of attacks already present on the tree ($r=0.66$, $P<0.001$, $n=303$). We used % increase in attacks for each counting interval to compare among treatments, to weight the new attacks by the total number of attacks on the tree. This limited variation due to differences in the initial number of attacks among trees both within and between treatments. The % increase in attacks for each tree at each sampling period was calculated using attacks/m² for each count period as:

$$\% \text{ new attacks} = \frac{\text{current period} - \text{previous period}}{\text{current period}}$$

Significant differences in the % new attacks for each pesticide contrasted with untreated controls for each count period were assessed by a nonparametric ANOVA and significant differences were evaluated using the Kruskal–Wallis χ^2 at $\alpha=0.10$ (SAS Institute Inc. 2012).

We also compared the effectiveness of the three triazole fungicides when each was applied individually. The fungicides compared were tebuconazole, propiconazole, and metconazole. We used % increase in attacks, as calculated above, for each counting interval to compare among fungicide treatments. Significant differences in % new attacks for the fungicides were assessed by nonparametric ANOVA and significant differences were evaluated using the Kruskal–Wallis χ^2 at $\alpha=0.10$ (SAS Institute Inc. 2012).

Results

For treatments assessed individually over time, only three treatments had not had a significant increase in attacks after 1 mo (Table 3; Figs. 1 and 2). These treatments included *Bacillus subtilis*,

Table 3. Statistical results for each treatment over time

Treatment	1 mo		3 mo		6 mo	
	<i>t</i>	<i>P</i>	<i>t</i>	<i>P</i>	<i>t</i>	<i>P</i>
Untreated control	-2.22	0.06	-2.59	0.03	-3.05	0.01
Bifenthrin	-2.47	0.04	-2.19	0.07	-3.14	0.02
Emamectin benzoate	-2.97	0.03	-3.63	0.01	-4.13	0.004
Tebuconazole	-2.51	0.07	-2.54	0.04	-2.25	0.06
Propiconazole	-2.89	0.02	-2.22	0.08	-2.56	0.06
Metconazole	-3.57	0.01	-3.20	0.02	-2.95	0.03
<i>Bacillus subtilis</i>	-1.08	0.32	-4.19	0.01	-3.14	0.02
Emamectin benzoate + Propiconazole	-2.37	0.06	-2.71	0.03	-2.25	0.06
Emamectin benzoate + Tebuconazole	-1.75	0.12	-2.59	0.04	-2.20	0.06
Bifenthrin + Tebuconazole	-1.81	0.10	-2.30	0.05	-3.13	0.01
Emamectin benzoate + Bifenthrin + Metconazole	NA	NA	-4.57	0.001	-2.16	0.06

Paired comparison *t*-tests were performed comparing the initial pretreatment number of attacks/m² on each tree to attacks at 1, 3, and 6 mo posttreatment. Treatments with significant differences indicated that the number of attacks increased significantly compared to initial attacks.

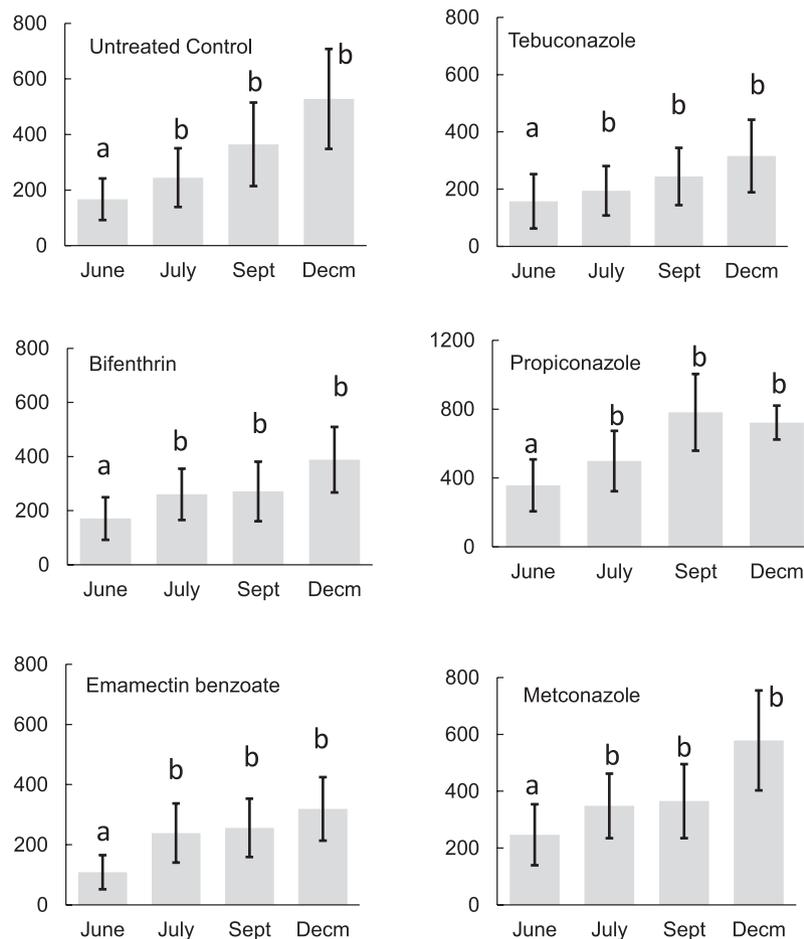


Fig. 1. Total attacks/m² for each treatment at each sampling period (mean and S.E.) for untreated controls and individual insecticides. For each treatment, bars with different letters indicate that there was a significant increase in attacks compared to pretreatment counts in June for paired *t*-tests at $\alpha=0.10$. A significant difference indicated that there was a significant increase in the number of attacks over time, which was associated with poorer performance for that treatment.

emamectin benzoate + tebuconazole, and bifenthrin + tebuconazole. Paired comparisons for total attacks comparing pretreatment counts with 3 mo posttreatment and with 6 mo posttreatment counts showed a significant increase in attacks for all treatments at $\alpha=0.10$, indicating all treatments had a significant increase in

attacks by *Euwallacea* sp. during later months in the monitoring period (Table 3).

For each treatment compared to untreated control trees, trees treated with the three pesticide combination of bifenthrin + emamectin benzoate + metconazole had a significantly lower

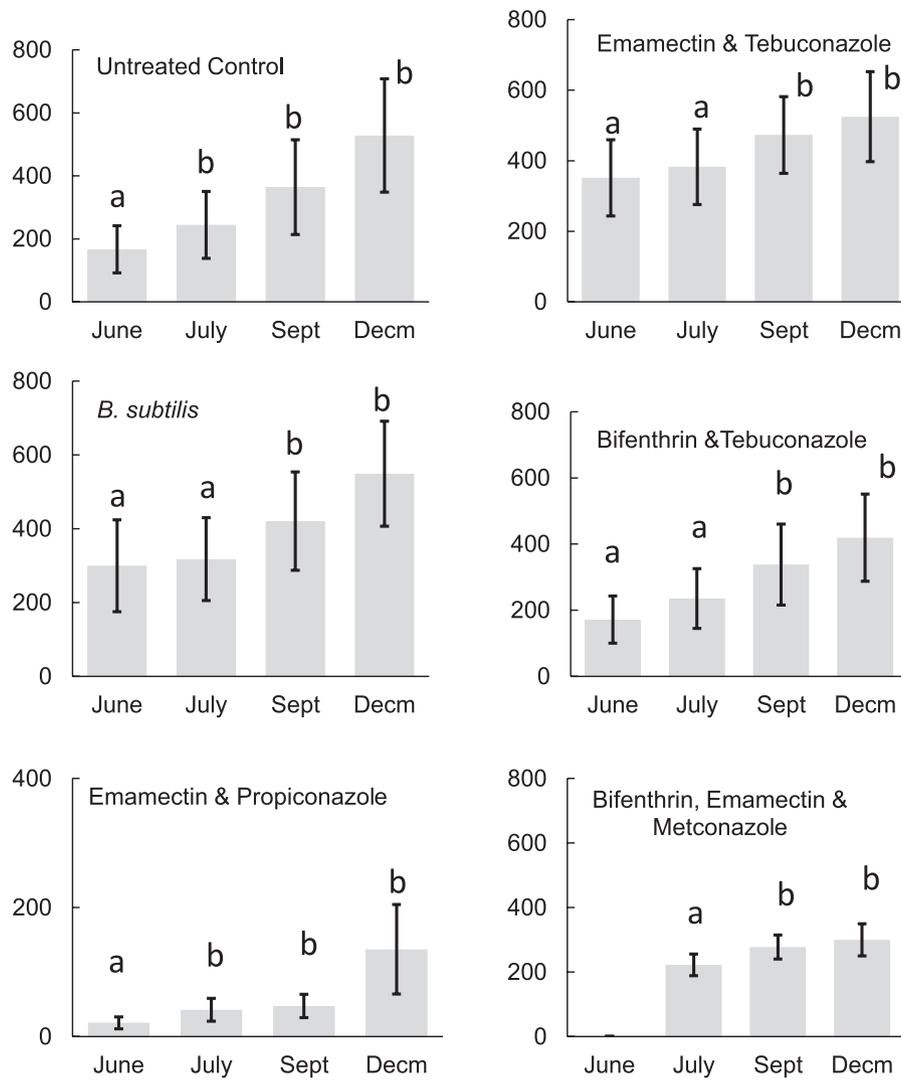


Fig. 2. Total attacks/m² for each treatment at each sampling period (mean and S.E.) for untreated controls, *Bacillus subtilis*, and insecticide combinations. For each treatment, bars with different letters indicate that there was a significant increase in attacks compared to pretreatment counts in June for paired *t*-tests at $\alpha = 0.10$. A significant difference indicated that there was a significant increase in the number of attacks over time, which was associated with poorer performance for that treatment.

Table 4. Statistical results for each pesticide treatment compared to untreated control

Treatment	July		Sept.		Dec.	
	χ^2	<i>P</i>	χ^2	<i>P</i>	χ^2	<i>P</i>
Bifenthrin	0.11	0.74	0.78	0.38	0.01	0.93
Emamectin benzoate	0.14	0.71	3.78	0.05	0.09	0.76
Tebuconazole	0.39	0.53	0.24	0.62	2.06	0.15
Propiconazole	1.45	0.23	0.47	0.49	0.09	0.77
Metconazole	0.23	0.63	0.47	0.49	0.46	0.49
<i>Bacillus subtilis</i>	2.69	0.10	0.47	0.49	0.09	0.77
Emamectin benzoate + Propiconazole	0.81	0.37	0.63	0.43	0.96	0.33
Emamectin benzoate + Tebuconazole	1.56	0.21	0.75	0.39	3.08	0.08
Bifenthrin + Tebuconazole	0.09	0.76	0.14	0.71	0.97	0.33
Emamectin benzoate + Bifenthrin + Metconazole	NA	NA	2.67	0.10	3.82	0.05

A nonparametric ANOVA compared the % new attacks for one treatment to untreated control trees for the sampling interval ending in the month indicated.

% new attacks compared to untreated control trees for the count intervals ending in both September and December (Table 4; Fig. 3). This treatment was not assessed for increased attacks in July, as the

treatment was applied in July. Trees treated with *Bacillus subtilis* had a significantly lower % new attacks compared to untreated controls in July, but not in September or December (Fig. 3).

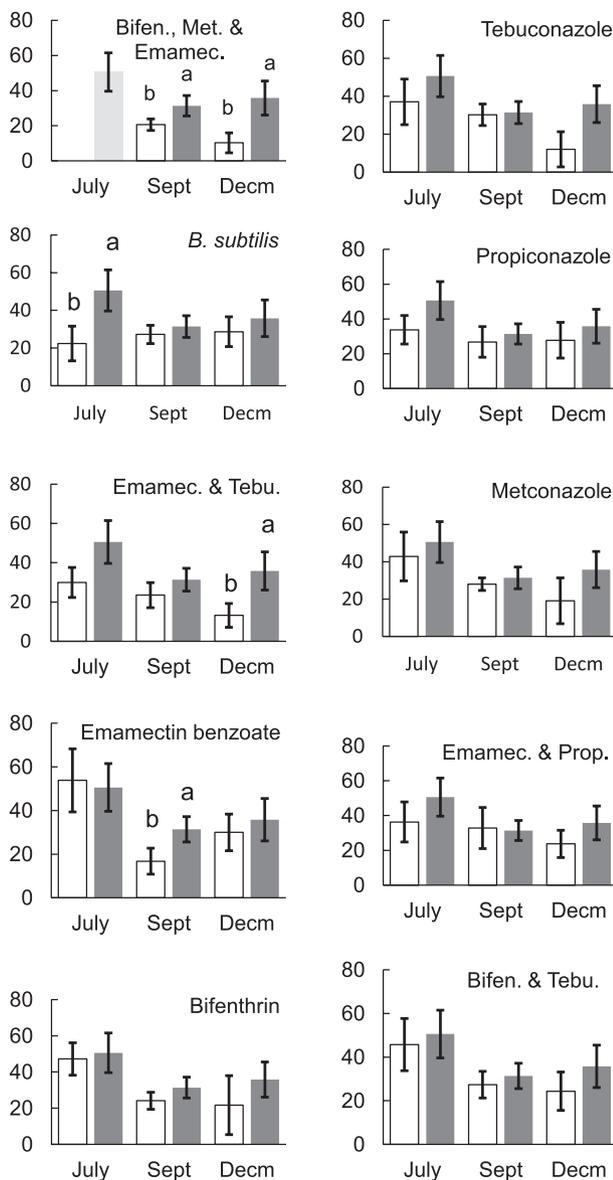


Fig. 3. Each individual treatment (white bars) compared to the untreated control (gray bars) contrasting the % new attacks for each sampling interval (mean and S.E.). The month listed on the graph is the end of the interval, for the periods June to July, July to September, and September to December. Within each treatment and time period, bars with different letters were significantly different at $\alpha = 0.10$. Abbreviations are Bifen, bifenthrin; Met, metconazole; Emamec, emamectin benzoate; Tebu, tebuconazole; and Prop, propiconazole.

Trees treated with the emamectin benzoate + tebuconazole combination had a significantly lower % new attacks compared to untreated control trees in December, but not in earlier months (Fig. 3). Trees treated with emamectin benzoate had a significantly lower % new attacks compared to untreated control trees in September only. No other treatments were significantly different from untreated controls.

When comparing among the three triazole fungicides, there were no significant differences in the % new attacks for trees treated with the three triazole fungicides for any sampling interval (period ending July, $\chi^2 = 0.16$, $P = 0.92$; period ending September, $\chi^2 = 0.46$, $P = 0.80$; period ending in December, $\chi^2 = 1.09$, $P = 0.58$).

Discussion

Pesticide Treatments Over Time

When comparing the total number of attacks over time within each treatment, none of the individual pesticides or pesticide combinations examined were effective at curbing the number of new attacks for the entire 6 mo that treatments were tracked. Newly emerged *Euwallacea* sp. tend to re-infest the maternal tree more often than taking flight and finding new host material (personal observation). It is likely that the majority of new attacks on each tree arose from newly emerged beetles re-attacking the maternal tree, rather than from new beetles colonizing the tree from other host material. Therefore, the increase in attacks over time most likely indicates that the pesticide treatments were unable to inhibit beetle larvae from completing development and establishing new galleries.

However, three treatments did curtail new attacks on trees during the first month posttreatment. Trees treated with the biological fungicide *B. subtilis* and trees treated with the triazole fungicide tebuconazole in combination with either bifenthrin or emamectin benzoate did not have increased attacks during the first month after treatment. Although *B. subtilis* has not previously been studied as a management option for ambrosia beetle management, it has been successfully used to control root and foliar fungal diseases for a wide variety of plants (Cawoy et al. 2011). Although none of the pesticides tested provide complete control for *Euwallacea* sp. attacks on infested trees, there are several options that may mitigate attack rates compared to untreated trees.

Pesticide Treatments Compared to Untreated Control

Trees treated with *B. subtilis* had significantly fewer attacks than untreated controls during the first month after treatment, but not at later time periods. Although *B. subtilis* only provided short-term control on previously infested trees, having a biopesticide option available for home owners and land managers may provide an important tool for managing this invasive ambrosia beetle in areas where chemical sprays are undesirable.

Only the three-agent pesticide combination with a combined trunk spray, systemic insecticide, and a fungicide (bifenthrin, emamectin benzoate, and metconazole) provided significant control compared to untreated trees for the entire monitoring period. Some two-agent combinations showed more limited periods of control. These treatments all included the systemic insecticide emamectin benzoate. Trees treated with emamectin benzoate in combination with tebuconazole had accumulated fewer attacks than control trees 6 mo after treatment, but not during earlier time periods. Trees treated with emamectin benzoate showed transitory control, with fewer attacks than control trees at 3 mo, but not earlier or later. Previous trials for *Euwallacea* sp. control indicated the systemic insecticide imidacloprid may also mitigate new attacks on previously infested trees (Eatough Jones and Paine 2017). Although both have shown some efficacy in separate trials, the systemic insecticides emamectin benzoate and imidacloprid have not yet been directly compared for control of this beetle.

Systemic insecticides that are injected into the lower bole of the tree have been receiving increased attention as alternatives to bole sprays for preventing bark beetle attacks (Fettig et al. 2013a). Studies focusing on bark beetles have examined the efficacy of preventative sprays applied to uninfested trees that were subsequently baited to attract bark beetles. Fettig et al. (2014) found that emamectin benzoate in combination with propiconazole was more effective than emamectin benzoate alone for protecting pine trees from *Dendroctonus ponderosae* Hopkins. Additionally, timing of the

preventative treatment was important, with treatments applied in the fall, 6 mo before beetle attack, being more efficacious than treatments applied in the late spring, 1 mo before beetle attack (Fettig et al. 2014). Similarly, abamectin and tebuconazole applied ~9 mo before beetle attack were effective for protecting trees from *D. ponderosae* (Fettig et al. 2013b). Factors that influence chemical transportation through the plant's vascular system, such as low temperatures and soil moisture, may affect the time it takes for adequate distribution of the insecticides, and impact the effectiveness of injected insecticides (Grosman et al. 2010; Fettig et al. 2013a,b, 2014).

However, unlike many *Dendroctonus* and *Ips* species, which have a limited period of tree attack, *Euwallacea* sp. is actively laying eggs year-round, and both adults and larvae may be found in an infested tree at any time of year. Unlike bark beetles, which attack phloem and cambium tissues, ambrosia beetles such as *Euwallacea* sp. are active throughout the sapwood. These differences in behavior may make timing and efficacy of systemic insecticides more difficult to predict for this beetle. In the early stages of attack, trees are not likely to exhibit symptoms associated with Fusarium Dieback, and beetle entry holes are difficult to find, often making it unfeasible to treat trees before beetles are present. Trees in this study were all infested with *Euwallacea* sp. prior to treatment. However, emamectin benzoate may still be effective when applied after wood-boring beetles are present. Ash trees infested with emerald ash borer that were injected with emamectin benzoate had significantly lower symptoms of canopy decline than untreated trees (Flower et al. 2015). Although emamectin benzoate alone did not reduce ambrosia beetle attacks in this study, it may increase tree protection when used in combination with fungicides and contact insecticides. Additionally, long-term monitoring over several years, rather than for 6 mo, could provide better information on the effectiveness of emamectin benzoate alone, or in combination with other pesticides, for maintaining or improving symptoms associated with *Euwallacea* sp. and Fusarium Dieback.

We did not see any significant differences among the three triazole fungicides tested in this study. None of the triazole fungicides provided significant control when used alone. Our findings are similar to Fettig et al. (2013b), indicating that insecticides in combination with fungicides will likely be more effective for controlling wood-boring beetles. However, further research is needed to determine if a particular fungicide is more effective. Although fungicides were tested in combination with insecticides, the limited number of available infested trees did not allow us to test all combinations.

Pyrethroids such as bifenthrin are typically effective against bark beetles for one or two field seasons (DeGomez et al. 2006; Fettig et al. 2006, 2013a). In this study, bifenthrin by itself did not provide any significant control, but trees treated with bifenthrin in combination with emamectin benzoate and a fungicide had significantly fewer attacks compared to control trees. Previous laboratory trials with cut logs have shown bifenthrin was more effective for preventing *Euwallacea* sp. attack and reducing gallery formation than other insecticides used as trunk sprays, including clothianidin, dinotefuran, and fenprothrin (Eatough Jones and Paine 2017).

In conclusion, ambrosia beetles can be difficult to control with pesticides because of their cryptic habits. Ingestion of wood by polyphagous shot hole borer is limited, and beetles spend little time on the tree surface. This may limit the beetle's interaction with pesticides. However, due to the severity of the impact of this ambrosia beetle on a wide variety of tree species in southern California, immediate management options, including pesticides, are needed. We found that *B. subtilis* provided short-term control (<3 mo) for *Euwallacea* sp. Pesticide combinations were generally more effective

than single pesticides. We used one contact insecticide applied as a trunk spray (bifenthrin) and one systemic insecticide applied through trunk injections (emamectin benzoate). Three triazole fungicides were included in this study, propiconazole, tebuconazole, and metconazole. There was no difference in performance among the three fungicides, but limitations in available infested trees did not allow for a complete comparison of fungicide and insecticide combinations. The combination of a systemic insecticide, a contact insecticide, and a fungicide provided the best control; we used emamectin benzoate, bifenthrin, and metconazole for this combination. Testing of other three-agent combinations may also prove to be efficacious. Ongoing research will continue to investigate the efficacy of insecticide–fungicide combinations for controlling this ambrosia beetle. Additionally, many of the trees included in this study were heavily infested, with 51% of the trees having >100 attacks/m² and 13% having >500 attacks/m² at the beginning of the trail. Several of the trees exhibited symptoms of branch dieback, and some had to be removed because heavy infestation created hazard conditions in public spaces. Pesticide treatments may be more efficacious if trees can be treated at the beginning of the infestation before symptoms of Fusarium Dieback are evident while trees xylem vessels are still active for the transportation of the systemic pesticides. Note that this experiment was only done on previously infested sycamore trees. Further studies need to be done on other hosts tree species for better management options of this pest disease complex.

Acknowledgments

We wish to thank Robin Veasey, Colin Umeda, Gabby Martinez, Francis Na, Kameron Y. Sugino, and Beth B. Peacock for assistance with lab and field work. We would like to thank the University of California Irvine Office of Environmental Planning & Sustainability for providing resources and support. We would especially like to thank Matthew Deines and Richard Demerjian at University of California Irvine. We thank Target Specialty Products and Valent for providing resources. We would like to thank RPW Services Inc., Great Scott Tree Service, Inc., Arborjet, and Mauget for valuable cooperative support. We would particularly like to acknowledge Donald Grossman at Arborjet, Ann Hope at Mauget, Paul Webb at RPW, and Scott Griffiths at Great Scott Tree Service, Inc. for their assistance. This research was funded by grants from the California Association of Nurseries and Garden Centers, the Nursery Growers Association, and a California Department of Food and Agriculture Specialty Crop Block Grant. This material is based upon work that is supported by the National Institute of Food and Agriculture, U.S. Department of Agriculture, Hatch project under CA-R-ENT-7607-MS.

References Cited

- Appel, D. N., and T. Kurdyla. 1992. Intravascular injection with propiconazole in live oak for oak wilt control. *Plant Dis.* 76: 1120–1124.
- Cawoy, H., W. Bettiol, P. Fickers, and M. Ongena. 2011. *Bacillus*-based biological control of plant diseases, pp. 273–302. In M. Stoytcheva (ed.), *Pesticides in the modern world – pesticides use and management*. InTech, Rijeka, Croatia.
- Cranham, J. E. 1966. Shot-hole borer (*Xyloborus fornicatus* Eichh) of tea in Ceylon. 1. chemical control and population dynamics. *B. Entomol. Res.* 56: 481.
- DeGomez, T. E., C. J. Hayes, J. A. Anhold, J. D. McMillin, K. M. Clancy, and P. P. Bosu. 2006. Evaluation of insecticides for protecting southwestern ponderosa pines from attack by engraver beetles (Coleoptera: Curculionidae: Scolytinae). *J. Econ. Entomol.* 99: 393–400.
- Eatough Jones, M., and T. D. Paine. 2015. Effect of chipping and solarization on emergence and boring activity of a recently introduced ambrosia beetle (*Euwallacea* sp., Coleoptera: Curculionidae: Scolytinae) in southern California. *J. Econ. Entomol.* 108: 1852–1559.

- Eatough Jones, M., and T. D. Paine. 2017. Potential pesticides for control of a recently introduced ambrosia beetle (*Euwallacea* sp.) in southern California. *Journal of Pest Science*. In Press. DOI 10.1007/s10340-017-0866-8
- Eskalen, A. 2016. Fusarium dieback/polyphagous shot hole borer. (<http://eskalenlab.ucr.edu>) (accessed 10 March 2017).
- Eskalen, A., D. H. Wang, and M. Twizeyimana. 2012. A new pest: *Fusarium* sp. and its vector tea shot-hole borer (*Euwallacea fornicatus*) causing Fusarium dieback on avocado in California. *Phytopathology* 102: 35.
- Eskalen, A., R. Stouthamer, S. C. Lynch, P. F. Rugman-Jones, M. Twizeyimana, A. Gonzalez, and T. Thibault. 2013. Host range of Fusarium dieback and its ambrosia beetle (Coleoptera: Scolytinae) vector in southern California. *Plant Dis.* 97: 938–951.
- Fernando, E.F.W. 1959. Storage and transmission of ambrosia fungus in the adult *Xyleborus fornicatus* Eich. (Coleoptera: Scolytidae). *Ann. and Mag. Nat. Hist.* 2: 475–480.
- Fettig, C. J., K. K. Allen, R. R. Borys, J. Christopherson, C. P. Dabney, T. J. Eager, K. E. Gibson, E. G. Hebertson, D. F. Long, A. S. Munson, et al. 2006. Effectiveness of bifenthrin (Onyx) and carbaryl (Sevin SL) for protecting individual, high-value conifers from bark beetle attack (Coleoptera: Curculionidae: Scolytinae) in the western United States. *J. Econ. Entomol.* 99: 1691–1698.
- Fettig, C. J., D. M. Grosman, and A. S. Munson. 2013a. Advances in insecticide tools and tactics for protecting conifers from bark beetle attack in the western United States, pp. 472–492. In S. Trdan (ed.), *Insecticides - development of safer and more effective technologies*. InTech, Rijeka, Croatia, 2013.
- Fettig, C. J., D. M. Grosman, and A. S. Munson. 2013b. Efficacy of abamectin and tebuconazole injections to protect of lodgepole pine from mortality attributed to mountain pine beetle attack and progression of blue stain fungi. *J. Entomol. Sci.* 48: 270–278.
- Fettig, C. J., A. S. Munson, D. M. Grosman, and P. B. Bush. 2014. Evaluations of emamectin benzoate and propiconazole for protecting individual *Pinus contorta* from mortality attributed to colonization by *Dendroctonus ponderosae* and associated fungi. *Pest Manag. Sci.* 70: 771–778.
- Flower, C. E., J. E. Dalton, K. S. Knight, M. Brikha, and M. A. Gonzalez-Meler. 2015. To treat or not to treat: diminishing effectiveness of emamectin benzoate tree injections in ash trees heavily infested by emerald ash borer. *Urban for. Urban Gree* 14: 790–795.
- Freeman, S., A. Protasov, M. Sharon, K. Mohotti, M. Eliyahu, N. Okon-Levy, M. Maymon, and Z. Mendel. 2012. Obligate feed requirement of *Fusarium* sp. nov., an avocado wilting agent, by the ambrosia beetle *Euwallacea aff. fornicatus*. *Symbiosis* 58: 245–251.
- Freeman, S., M. Sharon, M. Maymon, Z. Mendel, A. Protasov, T. Aoki, A. Eskalen, and K. O'Donnell. 2013. *Fusarium euwallaceae* sp. nov. - a symbiotic fungus of *Euwallacea* sp., an invasive ambrosia beetle in Israel and California. *Mycologia* 105: 1595–1606.
- Grosman, D. M., C. J. Fettig, C. L. Jorgensen, and A. S. Munson. 2010. Effectiveness of two systemic insecticides for protecting western conifers from mortality due to bark beetle attack. *West. J. Appl. For.* 25: 181–185.
- Haugen, L., and M. Stennes. 1999. Fungicide injection to control Dutch elm disease: Understanding the options. *Plant Diagnostics Qrtly.* 20: 29–38.
- Lynch, S. C., M. Twizeyimana, J. S. Mayorquin, D. H. Wang, F. Na, M. Kayim, M. T. Kasson, Q. T. Pham, C. Bateman, P. Rugman-Jones, et al. 2016. Identification, pathogenicity and abundance of *Paracremonium pembedum* sp nov and *Graphium euwallaceae* sp nov.-two newly discovered mycangial associates of the polyphagous shot hole borer (*Euwallacea* sp.) in California. *Mycologia* 108: 313–329.
- Mayfield, A. E., E. L. III, Barnard, J. A. Smith, S. C. Bernick, J. M. Eickwort, and T. J. Dreaden. 2008. Effect of propiconazole on laurel wilt disease development in redbay trees and on the pathogen in vitro. *Arboriculture Urban For.* 34: 317–324.
- Paine, T. D., C. C. Hanlon, and F. J. Byrne. 2011. Potential risks of systemic imidacloprid to parasitoid natural enemies of a cerambycid attacking *Eucalyptus*. *Biol. Control* 56: 175–178.
- Rabaglia, R. J., S. A. Dole, and A. I. Cognat. 2006. Review of American *Xyloborina* (Coleoptera: Curculionidae: Scolytinae) occurring north of Mexico, with an illustrated key. *Ann. Entomol. Soc. Am.* 99: 1034–1056.
- SAS Institute Inc. 2012. SAS, Version 9.4. SAS Institute, Cary, NC.
- Urbaniak, G. C., and S. Plous. 2015. Research Randomizer (Version 4.0). (<http://www.randomizer.org>) (accessed June 2015).
- Umeda, C., A. Eskalen, and T. D. Paine. 2016. Polyphagous shot hole borer and fusarium dieback in California, pp. 757–767. In T. D. Paine and F. Lieutier (eds.), *Insects and diseases of Mediterranean forest systems*. Springer, New York.