Evaluation of trunk-injected emamectin benzoate as a potential management strategy for Kuroshio shot hole borer in avocado trees

Frank J. Byrne [a,*], Janine Almanzor [b], Ivan Tellez [a], Akif Eskalen [c], Donald M. Grosman [d], Joseph G. Morse [a]

[a] Department of Entomology, University of California Riverside, USA
[b] Botanic Gardens, University of California Riverside, USA
[c] Department of Plant Pathology, University of California Davis, USA
[d] Arborjet Inc., 99 Blueberry Hill Rd, Woburn, MA, USA

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ABSTRACT

The polyphagous shot hole borer (PSHB) and the Kuroshio shot hole borer (KSHB) are newly invasive ambrosia beetles in California. They are vectors of the plant pathogen *Fusarium euwallaceae* (S. Freeman, Z. Mendel, T. Aoki, K. O' Donnell), the causal agent of Fusarium dieback in a broad host range that includes commercial avocados, landscape trees, and native tree species in urban and wildland environments. Management of these beetles using contact insecticides is challenging because the beetles spend little time outside their hosts. Trunk injection of systemic insecticides has been proposed as an alternative to contact treatments because insecticides can more effectively target the vascular tissues where the beetles establish their colonies. In this study, several field trials were conducted to evaluate the efficacy of trunk injections of the systemic insecticide emamectin benzoate in avocado trees. The uptake and persistence of emamectin benzoate were determined by quantifying residues in wood cores sampled at various heights within the trees where beetles would likely target. In conjunction with the field trials, a series of bioassays was conducted with a KSHB colony using an avocado-based artificial diet infused with the insecticide. The bioassays showed a dose-dependent effect of emamectin benzoate on the survival and development of the beetle in diet. We derived a tentative working threshold of 300 ng/g insecticide from the bioassay data that we subsequently used as a guide in evaluating the efficacy of the trunk injections. Emamectin benzoate established quickly within trees at the threshold concentration in the areas most vulnerable to attack and colonization by KSHB. Injection of the insecticide in a more dilute form promoted both faster uptake and more rapid establishment of effective concentrations than the undiluted form, thereby providing potential options in how the material is injected based on the levels of infestation of groves.

1. Introduction

Shot hole borer (SHB)-Fusarium dieback (FD) is a newly invasive pest-disease complex in California, that is comprised of two closely related ambrosia beetle vectors and their associated fungal symbionts (Freeman et al., 2013; Na et al., 2018). The ambrosia beetles have been identified and called the polyphagous shot hole borer (PSHB; *Euwallaceae whitfordiodendrus* Schedl) (Coleoptera: Curculionidae: Scolytinae) and the Kuroshio shot hole borer (KSHB; *Euwallaceae kuroshio* Gomez and Hulcr) ((Coleoptera: Curculionidae: Scolytinae) Eskalen et al., 2013; O'Donnell et al., 2015; Stouthamer et al., 2017; Gomez et al., 2018; Smith et al., 2019). Both species vector distinctive phytopathogenic

fungal symbionts that cause branch dieback and tree mortality in a broad host range that includes commercial avocados, landscape trees, and native tree species in urban and wildland environments (Eskalen et al., 2013). In reproductive host species, the beetles tunnel into trees and inoculate newly formed galleries with ectosymbiotic fungi (Fernando, 1959). The adult beetles and developing larvae feed on the fungi. Damage related to fusarium dieback is caused by the growth of the symbiotic fungus, *Fusarium euwallaceae* (S. Freeman, Z. Mendel, T. Aoki, K. O'Donnell) (Freeman et al., 2013), in the vascular tissues of the plant, which disrupts nutrient and water transport, eventually leading to branch dieback (Eskalen et al., 2012; Freeman et al., 2012, 2013; Mendel et al., 2012; Lynch et al., 2016). In landscape trees, the network

of tunnels compromises the integrity of branches, thereby posing a hazard to humans in public parks when trees become heavily infested. In addition, tunneling could potentially decrease the weight-bearing capacity of fruit-bearing trees, such as avocados.

Practical control options for shot hole borers are limited. Branch pruning and tree removal are recommended for heavily infested trees, combined with chipping and solarization of infested material (Eatough-Jones and Paine, 2015). The use of chemical insecticides is especially challenging given that the beetles spend most of their lives within their hosts. Contact insecticides applied to the bark can be used to target newly-emerging adults when they exit tunnels to establish new colonies. However, in California where the beetle reproduces year-round (Eatough-Jones and Paine, 2015), no clear windows of beetle emergence can be accurately predicted in a given area (Mayorquin et al., 2018). Therefore, multiple applications would likely be required throughout the year in order to provide effective control. Combination treatments with systemic insecticides and fungicides have been proposed as a strategy to better target both the insect and its symbiotic fungi within the vascular system (Mayorquin et al., 2018; Grosman et al., 2019). However, implementation of such a strategy has not yet been optimized. One of the major challenges appears to be the effective introduction of the chemicals into the vascular system, particularly in infested trees where the distribution of the chemical may be disrupted by tunneling and fungal colonization (Eatough-Jones et al., 2017; Mayorquin et al., 2018). In healthy mature avocados, trunk injection has been shown experimentally to be an effective delivery method for introducing systemic neonicotinoid (dinotefuran and imidacloprid) and organophosphate (acephate) insecticides to the tree canopy at doses that are effective against avocado thrips (Byrne et al., 2012, 2014). In the case of the neonicotinoids, the quantification of insecticide residues in leaves used for bioassays permitted the establishment of threshold levels of insecticide needed for effective control. In contrast, bioassays alone were used to determine the efficacy of the organophosphate. Assessments of the efficacy of trunk injection chemicals for the management of shot hole borers and other ambrosia beetles have relied upon documenting the incidence of new beetle attacks on trees (Eatough-Jones et al., 2017; Grosman et al., 2019), and through the *in vitro* exposure of fungal pathogens to wood cores sampled from treated trees (Mayorquin et al., 2018). Documenting the number of beetle attacks provides a direct measure of the efficacy of the systemic treatments. However, this approach provides little information on the movement and distribution of compounds within the tree. Evaluations of the efficacy of trunk-injected systemic insecticides in the management of ambrosia beetles would greatly benefit from the possibility of correlating beetle attack rates and fungal inhibition with residue levels in the trees.

Several studies have evaluated the efficacy of trunk-injected insecticides against nematodes, bark and ambrosia beetles, and other pests, in commodity and landscape trees, including avocado (Peña et al., 2011), apple (Coslor et al., 2019), oak (Svihra et al., 2004), elm (Pajares and Lanier, 1989), ash (McCullough et al., 2019) and several pine species (Grosman et al., 2010; Fettig et al., 2013). Trunk injections of emamectin benzoate were highly effective at preventing the establishment of emerald ash borer (*Agrilus planipennis* Fairmaire) in ash trees (*Fraxinus pennsylvanica* Marsh) for up to 3 years following injections (McCullough et al., 2019). But, injections of emamectin benzoate in ponderosa pine (*Pinus ponderosa* Laws) were only effective against the western pine beetle (*Dendroctonus brevicomis* LeConte) in the third year following trunk injections (Grosman et al., 2010), and were ineffective against spruce beetle on Engelmann spruce (*Picea engelmannii* Parry) or mountain pine beetle (*Dendroctonus ponderosae* Hopkins) on lodgepole pine (*Pinus contorta* Douglas) (Grosman et al., 2010). In Japan, trunk injections were effective at controlling the nematode-vectored pine wilt disease in two species of pine trees (Takai et al., 2003). It appears from several of these studies that emamectin benzoate is effective as a trunk injection insecticide, although it may require a lengthy period of time to

establish within trees before becoming fully effective (Fettig et al., 2013). Such delays may not be acceptable in avocado groves for protection against KSHB, where delays in responding to an incipient attack could allow the beetle time to establish colonies, thereby making subsequent control more difficult (Grosman et al., 2019).

Research evaluating the efficacy of trunk injection of emamectin benzoate in avocados is limited, but recent data are encouraging for the control of ambrosia beetles. Of four systemic insecticides evaluated, two of which were trunk-injected (imidacloprid and emamectin benzoate), emamectin benzoate was the most effective at controlling the redbay ambrosia beetle *Xyleborus glabratus* Eichhoff, the vector of laurel wilt disease, and a serious threat to the Florida avocado industry (Peña et al., 2011). Injections with emamectin benzoate resulted in higher beetle mortality, a reduction in the number of live beetles found inside the beetle galleries, and no brood production. Currently, the redbay ambrosia beetle does not occur in California. Looking forward, however, if this pest is introduced into the state, then trunk injections of emamectin benzoate may be one of the few chemical control options available to growers. Having data on the uptake and retention of emamectin benzoate in avocado trees will be important if this method of control is considered by the avocado industry to be a viable option for the management of several potentially debilitating ambrosia beetle pests. In apple pest management, a similar approach was taken for the evaluation of trunk-injected imidacloprid as an alternative to topical pesticide applications. Both temporal and spatial distribution of the insecticide in tree canopies showed considerable promise for the use of this technology in commercial orchards (Aćimović et al., 2013).

In this study, we report on our evaluations of trunk injections of emamectin benzoate as a potential management option for ambrosia beetles in avocados. The movement of insecticide within trees was documented by quantifying residues over time in wood cores taken at different distances from the injection points. Such information is useful for quantifying the uptake and retention of currently available and experimental systemic insecticides, and establishing threshold levels of insecticides required to effectively control the pest. In conjunction with the trunk injection evaluations, we conducted a series of bioassays on a strain of KSHB using a bioassay system in which emamectin benzoate was incorporated into an avocado sawdust-based artificial diet (Peer and Taborsky, 2004). The same artificial diet was used to study the biology of the PSHB and tea shot hole borer (TSHB) (Cooperband et al., 2016), two species closely related to the KSHB (Stouthamer et al., 2017).

2. Materials and methods

2.1. Efficacy of emamectin benzoate against KSHB in artificial diet

2.1.1. Artificial medium and insect rearing

A colony of the recently described KSHB, *E. kuroshio* Gomez and Hulcr (Gomez et al., 2018), was established in 2014 on artificial diet in the UCR Insectary and Quarantine Facility. The insects originated from a commercial avocado grove in Escondido, San Diego County, and were confirmed by genetic analysis to be KSHB (Cooperband et al., 2016). The beetles were reared in a sawdust-based diet prepared according to the method of Peer and Taborsky(2004), but with the exclusion of antibiotics (to preserve the fungal symbionts) and the substitution of avocado as the source of sawdust (Cooperband et al., 2016). The final diet preparation consisted of a mixture of 20 g agar, 10 g sucrose, 5 g casein, 5 g yeast extract, 1 g Wessons salts, 5 g corn starch, 75 g avocado sawdust, 5 ml 95% ethanol, 2.5 ml wheat germ oil, and 500 mls water. After autoclaving, 25 ml aliquots of the freshly prepared diet were added to 50 ml sterile Falcon® conical polypropylene centrifuge tubes (Corning Life Sciences, 33210 Central Ave, Union City, CA94587; cat. # 352070), and allowed to cool and solidify in a laminar flow hood before use.

Each diet tube was inoculated with a single mated female beetle. Beetles were surface sterilized with 75% EtOH, and then placed on filter

paper to dry. Before introducing a beetle to the diet tube, a hole approximately 1.5 cm deep was made in the diet using a sterilized 0.1-cm diameter plastic rod. A beetle was then placed into each tube using soft forceps and directed head first into the hole using a fine paintbrush. A layer (0.5 cm) of dry autoclaved sawdust was added to the tube to cover the surface. The caps were then loosely placed on the tubes, and the tubes left upright for 1–2 days. After that time, the tubes were oriented horizontally by placing them in sections of PVC tubing (5 cm diameter x 15 cm long) that were arranged in a grid and glued to a plastic backing board. The colonies were maintained in a rearing room at 25 °C, 50% RH, and a photoperiod of 16:8 h.

2.1.2. Diet bioassay

In a preliminary assessment, we found no difference in toxicity between technical grade emamectin benzoate and a commercially-available 4% emamectin benzoate formulation (TREE-äge®; Arborjet, Inc., Woburn, MA). Therefore, the formulated insecticide was used as the source of insecticide for all bioassays because it was more cost-effective than purchasing technical grade emamectin benzoate from online vendors. Final concentrations of emamectin benzoate in diet bioassays ranged from 1 to 1000 ng/g. Diet was prepared according to the standard protocol (section 2.1.1), and divided into equal volumes in 2 l beakers to accommodate different concentrations of insecticide. When the diet had cooled to 60 °C, the chemical was added to each beaker, and then vigorously stirred to distribute the insecticide evenly throughout the medium. The diet was then poured into 50-ml Falcon® tubes (25 mls per tube), and allowed to cool. Fifty tubes were prepared for each concentration, and seeded with a single mated female. At weekly intervals following the initiation of the bioassay, 10 tubes from each concentration were destructively sampled to determine the progress of development. Bioassays were terminated after 4 weeks, when the F1 adults were detected in the control diet. The survivorship of the parental beetles, oviposition, and the number of all instars and F1 adults were documented throughout the assessment period.

2.2. Field evaluation of trunk injections of emamectin benzoate

2.2.1. Field sites

In 2015, three field trials were conducted to evaluate the uptake and retention of emamectin benzoate administered to avocado trees by trunk injection. Two of those field trials (January and October timings) were conducted at Pine Tree Ranch, a 53-acre citrus and avocado ranch located near Santa Paula in Ventura County. The California Avocado Commission (CAC) (12 Mauchly #L, Irvine, CA 92618) leases land at Pine Tree Ranch for research and demonstration purposes. The CAC provided us with a block of 15-year old Hass avocado trees to conduct trunk injection experiments. The tree spacing was 4.57 m × 6.1 m. A second October trial was conducted at Hidden Valley Ranch, a commercial avocado ranch located 140 miles south of Pine Tree Ranch. At that site, we were allocated an avocado block consisting of 1 acre of 15-years old Hass avocados by the property owner. The tree spacing at Hidden Valley Ranch was 6.1 m × 6.1 m.

2.2.2. Insecticide application technique

The insecticide and trunk injection equipment for the trials were provided by Arborjet, Inc. (99 Blueberry Hill Rd, Woburn, MA 01801, USA). The source of formulated emamectin benzoate was TREE-äge®, a commercially-available product that is labeled for use as a trunk injection treatment for the management of a wide range of wood-boring insects in forest and landscape trees. TREE-äge is not registered for use on avocados, but was chosen for this study because of our prior experience with both this product and the injection equipment. Registration of a product suitable for use on food crops would require a formulation that did not contain certain constituents (other than the active ingredient) that are currently prohibited by EPA. Insecticide was injected into freshly drilled ports that were evenly spaced around the circumference

of each tree at a height of 15 cm above the soil surface, and beneath the area of the trunk where the scaffold branches emerge. Four holes were drilled per tree using a 9.525 mm titanium nitride coated wood drill bit to a depth of 2.54 cm. A #4 Arborplug® was set into each hole according to manufacturer's recommendations (Arborjet, 2020), using an Arbor-plug setter and rubber mallet. The drill bit was cleaned with a 10% bleach solution between trees to avoid any possible introduction of fungal and Oomycete pathogen contamination into the drill wounds. Table 1 summarizes details of the three trunk injection trials.

2.2.3. Trial 1, January 2015 – injections at Pine Tree Ranch

The objective of this trial was to measure the effect of dilution on the uptake and retention of formulated emamectin benzoate in trees injected with a single rate of insecticide over a 1-year period. Twelve trees were randomly selected within the grove that were of similar height (*ca.* 4.5 m) and trunk diameter. The trunk diameters of the trees were determined at 15 cm above soil level, and had a mean of 22.5 cm (±2 cm). Prior to all treatments, trees were numbered and treatments assigned to individual trees using a random number generator. Two sets of 4 trees each were chosen to receive the 2 pesticide treatments (undiluted and diluted), and a third set of trees was used as the untreated control treatment. The QUIK-jet Air® microinjection system was used to inject undiluted (neat) formulated insecticide, giving a final dose of 0.95 g emamectin benzoate per tree (n = 4). A second set of trees (n = 4) was injected with a 10% aqueous dilution of the formulated product. However, with a limit of 4 injection ports per tree, and a maximum injection volume capacity of 1.81 mls per injection port, it was necessary to inject the diluted insecticide using a TREE I.V. micro infusion® system. This system used the same injection ports as the QUIK-jet Air, but could accommodate the higher injection volume required to deliver the desired concentration of 0.95 g emamectin benzoate per tree.

At 3, 6, 9, and 12 months post-injection, wood cores were sampled from scaffold branches at heights ranging from 30 to 150 cm from the injection ports. Our decision to focus most of the sampling at 150 cm and above in our study trees was predicated on results of research that indicated scaffold branches and, in particular, branch collars as prime targets for colonization by the beetles (Mendel et al., 2012). Cores were extracted using a 15-cm, 3-thread Haglöf increment borer (Forestry Suppliers, Inc., 205 West Rankin St, Jackson, MS 39201; cat. # 63256) that removed a 5.15 mm diameter core to a depth of 2.5 cm. We were initially concerned about the potential variability in residue concentrations associated with different core sampling positions around the circumference of the branches. Therefore, at 3 and 6 months post-injection, 4 cores were sampled at all four cardinal directions around the circumference of the branches at each of the sampling heights. Following extraction, the wood cores were inserted into plastic drinking straws to prevent breakage of the core during handling and storage. The cores were stored in a lab freezer (−20 °C) pending residue analysis. To minimize damage to the trees from the increment borer, sampling was alternated between scaffold branches on successive

Table 1

Summary of trunk injection trials conducted at Pine Tree Ranch (Trials 1 and 2) and Hidden Valley Ranch (Trial 3) in January and October 2015. All trees were injected with TREE-äge in undiluted (neat) or diluted form in order to deliver 0.95 g active ingredient per tree.

Trial	Injection Timing	Injection Rate (product/inch trunk diam.)	Grams a. i./tree	Method of Application
1	Jan 12, 2015	2.5 ml/inch; neat solution	0.95 g	QUIK-jet Air
		2.5 ml/inch; 10% aq. dilution	0.95 g	TREE I.V.
2	Oct 6, 2015	2.5 ml/inch; neat solution	0.95 g	QUIK-jet Air
3	Oct 5, 2015	2.5 ml/inch; neat solution	0.95 g	QUIK-jet Air

sampling dates. In addition, the holes left after cores were removed were plugged using cork stoppers that were pressed firmly into the holes using a rubber mallet.

We had planned to continue sampling trees up to 2 years after the trees were injected. However, in Jan 2016, several trees throughout the grove were exhibiting signs of significant decline, including trees used for our trial. The problem with the trees arose from issues that were later attributed to the quality of a new source of irrigation water that was used within the grove. As a consequence, the trial was terminated, with no samples available for analysis after the 12 month samples were collected in Jan 2016.

2.2.4. Trials 2 and 3, October 2015 – injections at Pine Tree Ranch and Hidden Valley Ranch

The objectives of these trials were to compare seasonal injection timings (Pine Tree Ranch) and site location on the uptake and retention of emamectin benzoate. The October trial at Pine Tree Ranch was conducted in the same block as the January trial (section 2.2.3), with 6 replicate trees per treatment. At Hidden Valley Ranch, 6 replicate trees were chosen for each treatment (control and injected), and the mean trunk diameter of the trees at 15 cm above the soil line was 24 cm ($\pm$2 cm). A single rate of 2.5 mls of undiluted formulated emamectin benzoate was injected into the trees (n = 6) at both field sites using the Quikjet Air®, giving a final concentration of 0.95 g emamectin benzoate per tree (Section 2.2.2). Samples were taken initially at 1 and 3 months after injections at 150 and 300 cm above the injection ports, and then at a 3-month frequency thereafter. Based on our assessments of the distribution of insecticide around the circumference of the branches during the trial at Pine Tree Ranch (section 2.2.3), a single core was taken at each height. Due to the decline and death of several of the trees at Pine Tree Ranch (Section 2.2.3), the October trial at that location was terminated at 3 months after the trees were injected. However, at Hidden Valley Ranch, sampling continued up to 2 years after the injections.

2.2.5. Chemical quantification of emamectin benzoate in wood cores

A commercially available ELISA kit (FujiFilm Wako Chemicals, Richmond, VA; cat #309–33991) was used to quantify emamectin benzoate residues in the outer 1.25 cm of each wood core sample using a protocol optimized specifically for avocado wood cores during this project. Briefly, the individual wood cores were weighed, chopped into small pieces using a razor blade, and soaked in 100% methanol overnight (0.5 g core material per 5 mls methanol). An aliquot of each extract was dried completely in a TurboVap® LV evaporator (Caliper Life Sciences, Hopkinton, MA, USA) and then reconstituted in water prior to analysis by ELISA, following the steps outlined in the kit. Wood cores taken from untreated avocado trees at both sites were used as controls, and for estimating the level of dilution required to eliminate matrix effects from the assays (Byrne et al., 2005).

2.3. Statistical analysis

All statistical analyses of field data for emamectin benzoate were performed using JMP® Pro Statistical Discovery Software, v.13.0. When necessary to satisfy model assumptions, data were $\log_{10}$ (ng/g + 1) transformed prior to analysis, but were back transformed to original units on all graphical representations of data. The effects of emamectin benzoate on the development of KSHB in diet bioassays were analyzed using a repeated-measures multivariate analysis of variance (MANOVA), and contrasts were used to further assess significant effects. We also tested the significance of sample coordinate on the detection of emamectin benzoate using the repeated measures MANOVA. We analyzed the effect of dilution on the uptake of emamectin benzoate at Pine Tree Ranch using a linear mixed-effects model that included fixed effects of time, treatment (diluted or undiluted) and sample height, and a random effect of tree ID to account for autocorrelation associated with repeated measurements of the same trees over time. We used a similar model to

compare the uptake of emamectin benzoate at Pine Tree and Hidden Valley ranches following the October injections. Multiple comparisons were performed using the Tukey HSD test, with Bonferroni correction.

3. Results

3.1. Effect of emamectin benzoate on the development of KSHB in diet bioassays

A typical profile showing the temporal development of the KSHB in the avocado sawdust diet is shown in Fig. 1. At 1 week after a single mated female was introduced to each diet tube, eggs were detected, and oviposition continued throughout the 4-week assessment period. All four instars (including pupae) were present at week 3. The life cycle was completed within 4 weeks, with the emergence of the first F1 adults.

The incorporation of emamectin benzoate into the diet had a concentration-dependent effect on development (Figs. 2 and 3). An initial rate-finding bioassay that was conducted over a one week period showed the effects of emamectin benzoate at 1 ng/g and 1000 ng/g on adult mortality and oviposition (Fig. 2). In that bioassay, each tube was seeded with 2 adult female beetles. While there was no statistically significant impact on adult mortality at 1000 ng/g (Fig. 2a), there was clearly an effect on surviving adults at this concentration, with a significant decline in oviposition ($F_{1,18}$ = 6.99, P = 0.02) (Fig. 2b).

Assuming that the dead adults did not contribute to oviposition before they died, then egg production at 0, 1, and 1000 ng/g emamectin benzoate was 1.8, 1.3, and 0.07 eggs per surviving adult, respectively, in that bioassay. A clearer picture of the concentration-dependent effects of emamectin benzoate on the development of the KSHB is shown in Fig. 3. Although oviposition was not affected within the first 2 weeks of exposure, as demonstrated by a similar increase in the number of eggs at all concentrations in week 2 relative to week 1, the insecticide appeared to exhibit a triple effect on population development thereafter that resulted in a reduction in oviposition, inhibition of egg development that reduced the eclosion of 1st instars, and mortality of emerging 1st instars. These effects were especially evident at 300 ng/g, and to a lesser extent at 30 ng/g. The high number of eggs at 300 ng/g remained relatively constant after two weeks. However, in the control and lower doses, the egg numbers also remained high, while the number of immatures continued to increase. This indicates that the eggs were continually
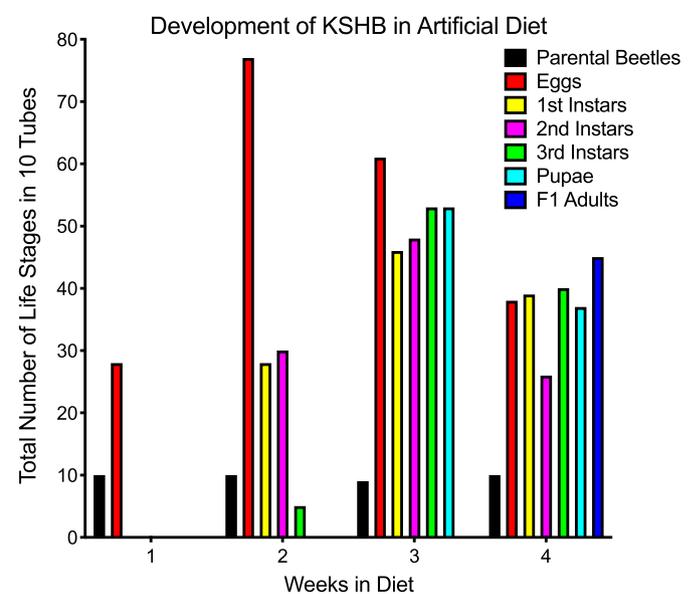
Fig. 1. Development of KSHB in avocado sawdust diet in the absence of emamectin benzoate. Ten diet tubes were opened weekly and the total numbers of each life stage determined.
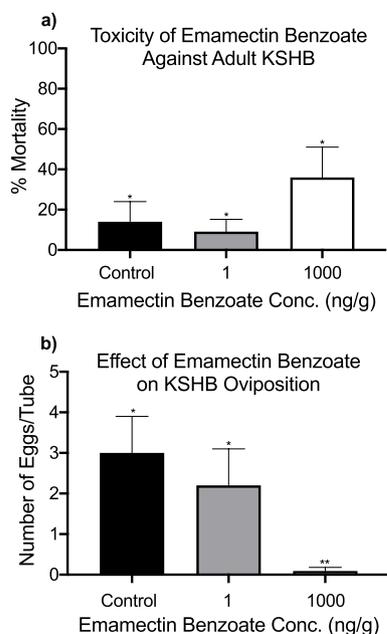
Fig. 2. Effect of emamectin benzoate concentration on (a) survival and (b) oviposition of the KSHB. Mortality bars (2a) represent mean percentages for 10 tubes (2 beetles per tube). Oviposition bars (2b) are means (±SEM) for 10 tubes, and significant differences between doses are indicated by asterisks (P < 0.05).

hatching at the lower doses, while new eggs were being added by surviving adults. At 300 ng/g, very few of the eggs that were oviposited during the first weeks hatched, and the lack of additional eggs indicates that the adults had stopped ovipositing. A repeated-measures MANOVA showed that there was an overall significant effect of emamectin benzoate concentration on the numbers of emerging 1st instars ($F_{3,36} = 18.64$, P < 0.0001). Pairwise contrasts showed that the general treatment effect was due to a significant reduction in 1st instar numbers at 300 ng/g relative to the control ($F_{1,18} = 38.55$, P < 0.0001), and both the 3 ng/g ($F_{1,18} = 30.73$, P < 0.0001) and 30 ng/g ($F_{1,18} = 86.46$, P < 0.0001) concentrations. All other pair-wise contrasts were non-significant (P ≥ 0.17). The significant reduction in 1st instars at 300 ng/g resulted in extremely low numbers of subsequent instars, and no pupae or F1 adults.

3.2. Uptake and retention of trunk-injected emamectin benzoate

3.2.1. Cardinal distribution of emamectin benzoate at different core-sampling heights

In the January 2015 trial conducted at Pine Tree Ranch, a repeated measures MANOVA showed that, at any given height, the cardinal position around the circumference of the branch from which the wood core was extracted did not have a significant effect on the detectability of emamectin benzoate at either 3 months ($F_{3,12} = 0.35$; P = 0.79) or 6 months ($F_{3,12} = 0.28$; P = 0.84) after injections (Fig. 4). Therefore, the 4 core residue concentrations at each sampling height were combined and averaged to simplify further analyses and presentation. And, for the two subsequent trials conducted in October at Pine Tree Ranch and Hidden Valley Ranch, a single core was taken at each sampling height on each sampling date, based on the assumption that the insecticide would be evenly distributed within the branches at any given distance from the injection port.

3.2.2. Effect of dilution on emamectin benzoate uptake and retention

At Pine Tree Ranch, concentrations of emamectin benzoate were consistently higher at all sample heights in trees that were injected with the diluted formulation (Fig. 5). At 3-months post-injection, the mean
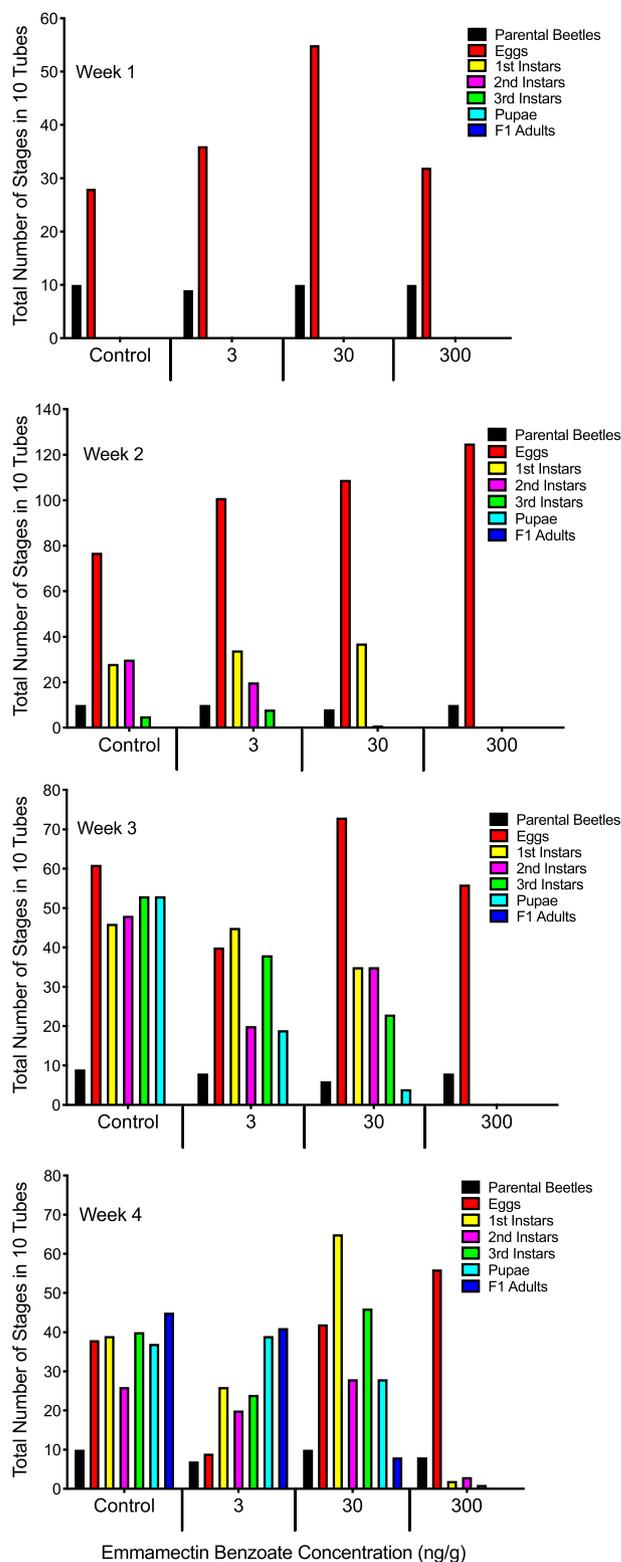
Fig. 3. Effect of emamectin benzoate concentration on the development of KSHB in avocado sawdust diet medium. Each bar represents the total number of each stage in 10 tubes opened on a weekly basis.

levels of emamectin benzoate in wood cores were 6-fold and 14-fold higher at 30 cm and 90 cm, respectively, in trees treated with the diluted formulation (Fig. 5), indicating more rapid vertical movement within the tree. At 6-months, the concentrations of emamectin benzoate were still consistently higher in the core samples from the trees treated
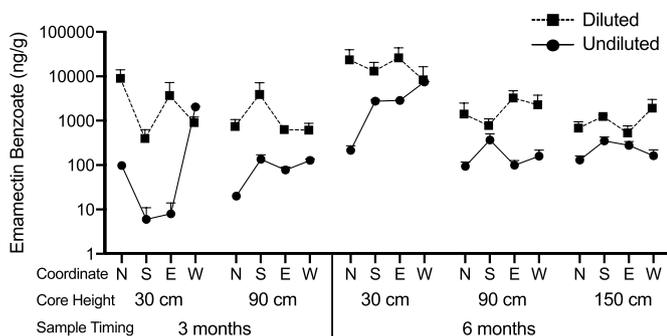
Fig. 4. Concentrations of emamectin benzoate in wood cores sampled from each of 4 cardinal positions around the circumference of avocado branches at 3 and 6 months after treatment with either undiluted or diluted formulation. Points are means (±SEM) for each coordinate sampled from 4 trees. Core height represents the distance from the injection port to the sample location.

with the diluted formulation, including those sampled at the additional height of 150 cm (Fig. 5). For the final two sampling dates, cores were only sampled at 150 cm, with higher mean residues in the diluted treatments.

In the analysis of emamectin benzoate concentrations at 30 and 90 cm, that were measured at 3 and 6 months after injections, there was a significant treatment effect ($F_{1,6} = 23.0$; $P = 0.003$) detected across the two sampling dates, but non-significant effects of time ($F_{1,18} = 1.84$, $P = 0.19$) and core height ($F_{1,18} = 2.42$, $P = 0.14$). A separate analysis was conducted on the 6-month residue data to incorporate the additional residues measured at 150 cm. With three sample heights in the analysis, we were able to detect a significant effect of height in the dilution treatments ($F_{2,6} = 46.23$; $P = 0.0002$), but not in the undiluted treatments ($F_{2,6} = 0.06$; $P = 0.94$). This result highlights the more rapid movement of the insecticide when the formulation was injected in a more dilute solution. At 9 and 12 months, residues at 150 cm remained consistent with those that were measured at 6-months(Fig. 5), indicating good persistence of insecticide at that height. Interestingly, the difference between the undiluted and diluted treatments was not significant at 12 months ($t = -1.244$, $P = 0.13$, $df = 6$), suggesting that the residues in the diluted treatment had stabilized by 12 months, whilst those in the undiluted treatment were still increasing.

3.2.3. Comparison of location

A comparison of the temporal changes in emamectin benzoate residues at Hidden Valley and Pine Tree ranches at 1- and 3-months

following the October injections identified significant effects for time ($F_{1,30} = 8.01$, $P = 0.01$), height ($F_{1,30} = 9.16$, $P = 0.005$), and the interaction between time and height ($F_{1,30} = 8.03$, $P = 0.008$), but not location ($F_{1,10} = 1.30$, $P = 0.28$), or the interactions between location and time ($F_{1,30} = 3.73$, $P = 0.63$), or location and height ($F_{1,30} = 3.43$, $P = 0.07$) (Fig. 6). At Hidden Valley Ranch, we continued to monitor emamectin benzoate levels for up to 2 years. After peaking at 1 month, residues at 150 cm dipped dramatically, before increasing again at 6 months. At 300 cm, mean concentrations peaked at 3 months after injection. Residues at both heights declined gradually after the 6-month samples. However, at 9 and 12 months, the mean concentrations were still close to 300 ng/g, the concentration of insecticide that showed good activity in diet bioassays (Fig. 3). Before the study at Pine Tree Ranch was terminated, data for 1 and 3 months after injections showed mean residues well above the 300 ng/g threshold at both 150 and 300 cm (Fig. 6). Upon examination of residue measurements for individual trees, the proportion of trees in which the concentrations of emamectin benzoate were above 300 ng/g never reached 100% at either study site; however, 80% of trees at Hidden Valley Ranch achieved that threshold level within 1 month of injection, and maintained that level for 6 months. In contrast, during the January trial at Pine Tree Ranch, in trees treated with the diluted formulation, residues exceeded 300 ng/g in 100% of trees up to 9 months, and in 75% of trees at 12 months.
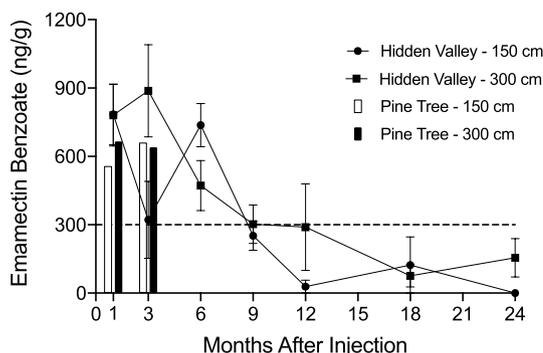
Fig. 6. Temporal profiles of mean (±SEM) concentrations of emamectin benzoate in wood cores sampled at 150 and 300 cm from avocado trees treated by trunk injection. The line graphs represent data sampled over 2 years from trees at Hidden Valley Ranch. The bars represent data sampled at 1 month (clear bars) and 3 months (black bars) from trees at Pine Tree Ranch. Bars are offset on each sampling date for easier presentation.

Fig. 5. Line graph (with SEM) showing a comparison of emamectin benzoate residues in wood cores sampled from avocado trees treated with dilute and undiluted (labeled as 'neat') trunk injections of formulated emamectin benzoate. Residues were determined for each treatment in wood cores sampled at 3, 6, 9 and 12 months after injection of insecticide. The sample heights represent the distance from the injection port to the sample location. Treatment means for neat vs dilute injections at each height and on each sampling date were compared separately using Student t-tests. Means followed by different lower-case letters, upper-case letters, or numbers, are significantly different (P < 0.05).

4. Discussion

Recent studies have shown that control of the polyphagous shot hole borer with contact insecticides alone is untenable (Eatough Jones et al., 2017; Mayorquin et al., 2018), and the greater the level of infestation prior to the initiation of treatments, the less effective contact insecticides become. The need for repeated applications is especially problematic given the year-round activity of this pest in Southern California. The use of systemic pesticides with extended residual activities has more appeal (Mayorquin et al., 2018; Grosman et al., 2019), although they are also limited in efficacy in trees that are already infested (Grosman et al., 2019). In this study, we have shown that emamectin benzoate establishes quickly in woody tissues of avocado trees following injection, and that the rate of uptake could be manipulated by diluting the insecticide prior to injection. Injections with undiluted insecticide moved more slowly, and at any given height within the tree, concentrations were always lower than when the insecticide was diluted prior to injection. This flexibility in injection protocol could allow pest managers to tailor their management strategies depending on whether the injections are targeting an existing population, thereby requiring faster uptake, or are being used prophylactically to prevent an incipient attack. Targeted trunk injections using undiluted insecticide could be used on border trees, for example, which are most vulnerable to attack during the initial invasive phase, or to protect trees adjacent to infested trees within a grove that is at the early phase of beetle invasion.

With an understandable reluctance on the part of growers to allow us to conduct *in vivo* bioassays in commercial groves, the use of the avocado sawdust rearing diet as a bioassay medium worked very well for our assessments of the effects of emamectin benzoate on the development of the KSHB. The bioassays permitted us to determine a working threshold of 300 ng/g for the insecticide that could be used as a guide for evaluating the efficacy of trunk injections in field trials. Clearly, it is important to be cautious when directly relating insecticide residue data from wood cores with bioassay data conducted using artificial diets, although the comparison may be justified in this case. The artificial diet is saturated with the insecticide, and the beetle has little option but to tunnel through the medium. Following trunk injection, emamectin benzoate is distributed throughout the trees within the xylem tissue, essentially saturating that medium also. The wood cores were designed to focus the residue testing on the vascular region of the trees, thereby maximizing the quantification of insecticide in the area of the tree where the beetles are most likely to construct their tunnels.

The effect of emamectin benzoate on the KSHB in diet bioassays was three-fold. First, at sufficiently high concentrations, adult mortality of the founding females occurred. However, even at concentrations as high as 5000 ng/g emamectin benzoate, we have never observed 100% mortality (F. Byrne, unpublished data). Second, despite the survival of a substantial number of founding insects, and the excavation of tunnels within the diet, emamectin benzoate clearly exhibited an effect on surviving adults that resulted in a dramatic reduction in oviposition after 2 weeks of exposure within the diet. And third, there was a concentration-dependent mortality of the immature stages. These combined effects mirror those described under field conditions for the management of active populations of redbay ambrosia beetle in avocado trees treated with emamectin benzoate (Peña et al., 2011), and show the potential benefits of using this insecticide as a chemical control agent for the management of KSHB. Emamectin benzoate will only be successful, however, if threshold concentrations of the insecticide can be established at the colonization sites of the beetles (150 cm and above in our study trees). In our field studies, the proportion of trees in which the concentrations of emamectin benzoate measured at 150 cm were at least 300 ng/g never exceeded 50% (January injection timing) and 60% (October injection timing) at Pine Tree ranch over a 6-month period when the neat formulation was injected. The proportion reaching the threshold increased to 100% when the diluted formulation was injected, and persisted for up to 9 months, again highlighting the advantage of being able to manipulate the injection volume to enhance delivery of the insecticide. In fact, when trees were injected with the diluted formulation, residues above 1000 ng/g were recorded, thus improving the effectiveness of the insecticide against the colony as a whole. At Hidden Valley Ranch, threshold concentrations at 150 and 300 cm were reached within 1 month following the injection of the neat formulation, and mean residues at 300 cm persisted at this concentration for up to 12 months. Although the diluted formulation was not tested at Hidden Valley Ranch, we would expect even more rapid uptake and distribution in these trees, based on the data generated from the trials at Pine Tree Ranch.

5. Conclusions

In California, insecticides formulated for trunk injection are currently labeled solely for the control of pests on ornamental, landscape, and forest trees, with no approved applications by trunk injection to trees that may be harvested for food consumption by humans or used in animal feed. Given the success of emamectin benzoate in the management of many key pests on non-food crops, our goal with this research was to determine whether emamectin benzoate could be used in avocado trees for shot hole borer management. We showed that avocado trees can be successfully injected with the insecticide, and effective concentrations established within trees in the areas most vulnerable to attack and colonization by both PSHB and KSHB. In particular, trunk injection of emamectin benzoate may be an effective treatment for early infestations of ambrosia beetles, given that infested trees could be targeted specifically, thereby eliminating the need to treat an entire grove. We understand that adding a food crop usage to a registration would be a costly and time-consuming process, requiring multiple trials to evaluate residues of active ingredient and other formulation constituents in fruit. However, should a potential registration of emamectin benzoate for trunk injection in avocados come under consideration, data from this study would be highly supportive of such an effort. Given the current lack of effective chemical control options, the availability of a trunk-injectable formulation for ambrosia beetle control would be an important much-needed tool for the management of this pest complex.

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.

CRediT authorship contribution statement

Frank J. Byrne: Conceptualization, Methodology, Formal analysis, Investigation, Writing - original draft, Visualization, Funding acquisition. **Janine Almanzor:** Methodology, Investigation. **Ivan Tellez:** Investigation. **Akif Eskalen:** Conceptualization, Methodology, Investigation. **Donald M. Grosman:** Methodology, Investigation. **Joseph G. Morse:** Conceptualization, Methodology, Writing - review & editing, Funding acquisition.

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