Journal of Economic Entomology, 110(4), 2017, 1512–1517
doi: 10.1093/jee/tox151
Advance Access Publication Date: 24 May 2017
Research article

Ecology and Behavior

Attraction of the *Euwallacea* sp. near *fornicatus* (Coleoptera: Curculionidae) to Quercivorol and to Infestations in Avocado

John A. Byers,[1,2] Yonatan Maoz,[3] and Anat Levi–Zada[4]

[1]Department of Entomology, Robert H. Smith Faculty of Agriculture, Food and Environment, The Hebrew University of Jerusalem, Rehovot, 76100, Israel (john.a.byers@gmail.com), [2]Corresponding author, e-mail: john.a.byers@gmail.com, [3]The Plants Production and Marketing Board, Yehud, 56000, Israel (yoni369@gmail.com), and [4]Institute of Plant Protection, Agricultural Research Organization, Volcani Center, Rishon LeZion, 7505101, Israel (anatzada@volcani.agri.gov.il)

Subject Editor: Qiao Wang

Received 22 February 2017; Editorial decision 4 May 2017

Abstract

The *Euwallacea* sp. near *fornicatus* (*Euwallacea* sp. 1 hereafter) feeds on many woody shrubs and trees and is a pest of avocado, *Persea americana* Mill., in several countries including Israel and the United States. Quercivorol baits are commercially available for *Euwallacea* sp. 1 females (males do not fly), but their attractive strength compared to other pheromones and potential for mass trapping are unknown. We used sticky traps baited with quercivorol released at 0.126 mg/d (1×) and at 0.01×, 0.1×, and 10× relative rates to obtain a dose–response curve of *Euwallacea* sp. 1 attraction. The curve fitted well a kinetic formation function of first order. Naturally infested limbs of living avocado trees had attraction rates equivalent to 1× quercivorol. An effective attraction radius (EAR) was calculated according to previous equations for each of the various baits (1× EAR = 1.18 m; 10× EAR = 2.00 m). A pole with six sticky traps spaced from 0.25–5.75 m in height had captures of *Euwallacea* sp. 1 yielding a mean flight height of 1.24 m with vertical flight distribution SD of 0.88 m (0.82–0.96 m, 95% CI). The SD with specific EAR was used to calculate EARc, two-dimensional EAR (1× EARc = 0.99 m; 10× EARc = 2.86 m), for comparison with other insect pheromone traps and for use in simulations. The simulation methods described previously were performed with combinations of 1–16 traps with 1–50 aggregations per 9-ha plot. The simulations indicate mass trapping with quercivorol could be effective if begun in spring before *Euwallacea* sp. 1 establishes competing sources of attraction.

Key words: kairomone, vertical flight distribution, mean flight height, effective attraction radius, semiochemical

The *Euwallacea* sp. near *fornicatus* (*Euwallacea* sp. 1 hereafter) is an ambrosia beetle (Coleoptera: Curculionidae: Scolytinae) that invaded California as early as 2003, and shortly thereafter Israel (Eskalen et al. 2012, Mendel et al. 2012, Freeman et al. 2012). *Euwallacea* sp. 1 in both California and Israel appears to be the same species closely related to the *Euwallacea fornicatus* (Eichhoff) from Sri Lanka that has become a pest in Florida (Carrillo et al. 2015, 2016; Cooperband et al. 2016). These two species originated in Southeast Asia and are morphologically identical but differ enough in DNA to be considered distinct species (Eskalen et al. 2013, O'Donnell et al. 2015, Stouthamer et al. 2017). *Euwallacea* sp. 1 has a relatively broad host range of woody shrubs and trees and can be a serious pest because it may carry *Fusarium* dieback disease (Freeman et al. 2012, Eskalen et al. 2013, Cooperband et al. 2016, Lynch et al. 2016). In Israel, *Euwallacea* sp. 1 (O'Donnell et al. 2015) is potentially a serious problem in avocado because, although the tree does not usually die from the disease, the beetle does kill infested limbs and reduces tree growth over a period of years (Freeman et al. 2012, Mendel et al. 2012).

Like many scolytid beetles, this ambrosia beetle carries symbiotic fungal species that grow in the adult and larval tunnels in the sapwood and serve as food (Wood 1982, Freeman et al. 2012, Hulcr and Stelinski 2017). Only the females of *Euwallacea* sp. 1 and *E. fornicatus* appear to leave the brood tree after mating, as the males are not capable of flight (Calnaido 1965, Carrillo et al. 2015). The females of *E. fornicatus* were observed to fly for up to 24 min in the laboratory at 0.3 to 0.6 m/s and thus were calculated to fly for up to 864 m on the first dispersal flight without aid of wind (Calnaido 1965). It is probable that *Euwallacea* sp. 1 in the field has a similar fight capacity. Females of this species complex could rest and possibly feed between flights and wind would increase their potential dispersal range. Our observations of *Euwallacea* sp. 1 and that of others (Eskalen et al. 2013) suggest that females generally do not bore alone into their host avocado tree but are commonly found together in an aggregation of females in a relatively concentrated area of a branch or branches.

Scolytid beetles including bark beetles and ambrosia beetles usually are attracted from tens of meters to aggregation pheromones that consist of one to three chemicals produced by either one or both sexes depending on the species (Byers 1989). Quercivorol, (1*S*,4*R*)-*p*-menth-2-en-1-ol, is the aggregation pheromone of *Platypus quercivorus* (Murayama) that colonizes oaks in Japan (Tokoro et al. 2007), and is also attractive to *E. fornicatus* in Florida (Carrillo et al. 2015). Preliminary tests in Israel and United States indicate that *Euwallacea* sp. 1 is attracted to quercivorol. However, there is no evidence that quercivorol is produced by *E. fornicatus* or *Euwallacea* sp. 1. Perhaps females of *Euwallacea* sp. 1 are attracted to quercivorol because either 1) it is a kairomone released by the host or its fungal infection, 2) it elicits cross-attraction of *Euwallacea* sp. 1 in the ancestral range, 3) it is a mimic of a pheromone component of *Euwallacea* sp. 1, or 4) it is a pheromone component of *Euwallacea* sp. 1.

There have been no studies that have investigated the attraction of *Euwallacea* sp. 1 to infested host substrates in the field or determined the attractive strength of quercivorol relative to other bark beetle pheromones. Ethanol is commonly reported to be attractive to ambrosia beetles and some bark beetles (Byers 1989, 1992). Carrillo et al. (2015) based on catch data from Florida suggest that ethanol together with quercivorol are weakly attractive to *E. fornicatus* (and by inference to *Euwallacea* sp. 1). However, in the Carrillo et al.'s study ethanol is released in all formulations, so it is not clear whether quercivorol alone is attractive or enhances attraction to ethanol.

In the present study we aimed to 1) determine the attractive strength of various odor sources of avocado wood compared to such wood infested with *Euwallacea* sp. 1 in the field using the effective attraction radius method that does not depend on population levels (Byers et al. 1989; Byers 2008, 2009, 2012a, b), and 2) test quercivorol over a dose–response range of several orders of magnitude in release rate. Effective attraction radius (EAR) for attractive traps with quercivorol or infested avocado will be converted to a circular EAR (EARc) and used in simulations in two dimensions and in encounter rate equations. This should aid in pest detection and monitoring, and design of mass trapping programs (Byers 2012b, Byers and Naranjo 2014, Levi-Zada et al. 2017).

Materials and Methods

Insects and Emergence Cages

Avocado branches infested with *Euwallacea* sp. 1 were cut from trees (Hass variety) in an orchard 2.5 km east of Nahsholim, Israel (32° 36′31″ N, 34° 56′49″ E), hereafter called the study site. The logs ranged from 4 to 10 cm diameter × 30–40 cm long and were placed inside several closed cardboard boxes (45 by 30 by 50 cm) each fitted with a clear plastic window (20 cm wide by 25 cm high, made of acetate plastic sheeting) through which natural light attracted emerged adults. The emergence boxes were placed on a shelf at room temperature. As the beetles emerged from the logs, they either flew into or slipped near the plastic window and fell down a flat rectangular funnel made of the plastic sheet and scotch tape (1 cm wide by 20 cm width of window). The funnel was fitted and taped into the bottom edge of the cardboard box. Beetles that fell into the funnel continued down through a flexible plastic tube into a cup with damp paper. The adults were collected daily and stored at 4 °C until use within 2 wk.

Attractive Strength of 1× Quercivorol Dispenser in Sticky Trap

Quercivorol was obtained from commercial bubble-cap dispensers (Synergy Semiochemicals Corp., Burnaby, Canada) each containing 300 µl of the compound. Chemical analysis by gas chromatography (GC) and GC–mass spectrometry (GC–MS) of the quercivorol was conducted as described by Levi-Zada et al. (2017). We used a glass flat-bottom 250 µl dispenser (3.29 mm i.d. × 30.6 mm long, J.G. Finneran Associates, Inc., Vineland, NJ) with 20 µl of quercivorol placed at the bottom that gives an almost constant release rate at a specified temperature (Byers 1988). Weight loss of the dispenser ($N = 3$) on a 0.01 mg precision microbalance over 4 mo in the laboratory at 25 °C was used to determine the constancy of the release rate and coefficient of variation (CV = SD/mean). This release rate was arbitrarily designated 1× (1 glass dispenser). The dispenser tube was scotch taped upright inside an inverted aluminum foil-covered plastic cup (8 cm diameter by 10 cm) to prevent sun and rain damage and then surrounded by a sticky screen trap. The sticky traps (cylinder, 28 cm diameter by 33 cm high) were made of 6-mm mesh wire screen covered with sticky adhesive (80% polyisobutene, Rimifoot, Rimi, Petah Tikva, Israel) and placed at 1.2 m in height on poles spaced 15 m apart at the field site. Three of these 1×-baited traps were compared with seven blank control traps in order to obtain a ratio of catch that is used to calculate the EAR (Byers 2007, 2008, 2009) according to $\mathrm{EAR} = [Ca \times S/(\pi \times Cb)]^{0.5}$, where Ca is the mean catch of the pheromone traps, Cb is the mean catch of the unbaited traps, and S is the silhouette area (0.0924 m^2) of the cylinder trap.

Mean Flight Height and SD of Vertical Flight Distribution

In order that EAR could be used in simulation models of monitoring and mass trapping (Byers 2012b, Byers and Naranjo 2014), the EAR needs to be converted to two dimensions and to the EARc (c for circular). This is done with the following equation: $\mathrm{EARc} = \pi \times \mathrm{EAR}^2/[2 \times \mathrm{SD} \times (2 \times \pi)^{0.5}]$ that requires the EAR and the standard deviation (SD) of the vertical flight distribution. The SD was obtained in another experiment with a pole with six sticky traps (cylinder, 25.5 cm diameter by 25 cm high) with their centers at 0.25, 1.35, 2.45, 3.55, 4.65, and 5.75 m height above ground placed in an avocado orchard at the study site (26 June–18 Aug. 2016). Each sticky trap was baited with 1× quercivorol. *Euwallacea* sp. 1 caught on the traps were picked weekly for 3 wk and once more after 3 more weeks. The catches at each height were used to calculate mean flight height and the SD of the vertical flight distribution, in which trap height is entered repeatedly for each insect caught (Byers 2011). It is possible to calculate a 95% confidence interval for SD of specific N assuming a normal distribution from the following square root formulas:

$$\left[\frac{(N-1)\cdot \mathrm{SD}^2}{\chi^2_{\alpha/2,N-1}}\right]^{0.5} \le \mathrm{SD} \le \left[\frac{(N-1)\cdot \mathrm{SD}^2}{\chi^2_{1-\alpha/2,N-1}}\right]^{0.5} \qquad (1)$$

The denominator on the left is the lower critical value and the denominator on the right is the upper critical value of the chi-square distribution (Larson and Farber 2012).

Dose–Response Curve of Quercivorol

A dose–response experiment at the field site was done to determine whether higher or lower dosages than 1× would be more attractive. Two replicates of each dosage at 10-fold increasing magnitude: 0

(control), 0.01×, 0.1×, 1×, and 10× were placed in cylinder sticky traps (28 by 33 cm) in the same avocado orchard. *Euwallacea* sp. 1 were taken off the sticky traps every 1 to 2 wk for eight collections (18 Aug.–15 Nov. 2016). The glass dispenser was used for the various release rates, with the two lower rates made by diluting quercivorol (154.25 FW, 0.0457 mm Hg, 25 °C, density 0.922 g/ml) with appropriate amounts of decanol (158.28 FW, 0.048 mm Hg, 25 °C, density 0.83 g/ml) because both compounds have similar vapor pressures and therefore would evaporate at similar rates. According to equations in Byers (1988), the 0.1× solution was made by diluting 11 µl quercivorol in 111 µl decanol. The 0.01× solution was made by 1:10 dilution of the 0.1× solution. The 10× dose was made by placing 10 dispensers of 1× inside the cup. Nonlinear regression software (TableCurve 2D version 5.01, Systat Software Inc., Chicago, IL) was used to find a function that fit the catch data best (Byers 2013). The EAR and EARc were also calculated from catches at each dose (release rate of quercivorol).

Attraction of *Euwallacea* sp. 1 to Infested Avocado Logs or Branches

In the first experiment, avocado logs (6 cm diameter by 28 cm long) were cut from a tree and after 1 wk holes of 1 mm diameter were drilled through the bark and phloem (stopping at the sapwood) and a female *Euwallacea* sp. 1 from the laboratory emergence boxes was introduced to each of 30 holes per log. Two infested logs were then wrapped with nylon mesh to prevent beetles from leaving and placed into a 28- by 33-cm sticky trap in the avocado orchard as described above. A set of two control logs also were drilled (without females), wrapped, and placed into a second sticky trap. Traps were picked of *Euwallacea* sp. 1 every week for 3 wk (21 June–7 July 2016).

In experiment 2, we attempted to obtain naturally attacking beetles by placing three 1× quercivorol baits on several cut logs in a pile for a week (18–24 Aug. 2016) in the field and then placed two naturally infested logs in each of two sticky traps compared to two control logs in each of two other sticky traps as above. The traps subsequently were picked every week for 4 wk.

In experiment 3, we monitored naturally attacking beetles in branches on living avocado trees (26 Sept. to 15 Nov. 2016). We formed a sticky screen into a 28 cm diameter by 33 cm cylinder encircling an infested branch section (one per tree, $N = 7$) and compared these to an equal number of sticky screens around uninfested control branches (one per tree) from nearby trees. At the end of the experiment, traps were collected and *Euwallacea* sp. 1 counted in the laboratory and compared with a Mann–Whitney U test (R Foundation for Statistical Computing version 3.1.2).

Simulations Using Measured EARc

Computer simulations programmed in Java 1.6 as described in Byers (2012b) were performed with a trap EARc of 2.86 m for the 10× bait of quercivorol (from Results). One thousand initial beetles were simulated moving up to 7,200 m and caught by several densities of traps (1, 4, or 16) placed in a grid in a 300- by 300-m area with no competition from natural *Euwallacea* sp. 1. The same model was used with the same parameter sets but with additional infested avocado aggregation sources (1, 10, or 50) of EARc = 0.98 m (from Results) placed at random in the area. The numbers attracted to aggregations on trees and caught in traps were counted and converted to a percentage of the initial population. The simulations for both scenarios were repeated eight times for each parameter combination.

Results

Attractive Strength of 1× Quercivorol Dispenser in Sticky Trap

GC and GC–MS analysis of quercivorol from a commercial source revealed that it was 90% of the *1S,4R*-isomer that is the major aggregation pheromone component of *P. quercivorus* in Japan (Tokoro et al. 2007). The 1× dispenser in the laboratory (average of three dispensers) lasted over 4 mo at 25°C and gave a constant release as measured by weight loss. At the end of the period the three dispensers had a coefficient of variation (CV) of weight loss of only 1.2%, indicating consistent release among dispensers. The best fitting simple equation was linear, $Y = 0.6233 - 8.79\text{E-}08X$ ($N = 29$, $R^2 = 0.99$, $P < 0.0001$) with X in min and Y in ng. This converts to a release rate of 0.126 mg/d for the 1× glass tube. A total of 35 *Euwallacea* sp. 1 were caught on seven blank traps and 709 beetles on three 1× treatment traps from 21 June–18 Aug. The mean per trap total of 5 on control trap compared to 236.3 on treatment trap gives an EAR = 1.18 m due to the sticky trap silhouette interception area of 0.0924 square meters.

Mean Flight Height and SD of Vertical Flight Distribution

The catches on the 1× sticky traps on the pole analyzed by methods of Byers (2011) gave mean flight heights of 1.12 m ($N = 83$; 26 June–3 July), 1.37 m ($N = 147$; 7–17 July), 1.15 m ($N = 11$; 17–30 July), and 1.11 m ($N = 65$; 30 July–18 Aug.). The total catches over all four flight periods on traps from lowest to highest were 97, 156, 42, 8, 3, and 0, respectively, giving a grand mean flight height of 1.24 m. The SD of the vertical flight distribution was 0.85, 0.85, 1.38, and 0.86 m, respectively, for each flight period giving a grand SD of 0.88 m. The asymmetrical 95% confidence interval for the grand SD was 0.82 to 0.96 ($N = 306$, equation 1). Using the SD and the EAR of 1.18 m calculated above gave an EARc = 0.99 m (Byers 2008, 2009).

Dose–Response Curve of Quercivorol

The mean catch per trap per week (Y) with increasing release rate (X) on a logarithmic scale had a sigmoidal curve that fitted a first-order kinetic formation function perfectly (Fig. 1A), and indicated that the 10× dose caught the most. The same function when fitted on a linear scale of dosage had a convex shape (Fig. 1B). The 10× dose corresponds to 1.26 mg/d release of quercivorol. The EAR was calculated for each of the doses 0.01, 0.1, 1, and 10× as 0.21, 0.38, 1.02, and 2.00 m, respectively. The EAR conversion gave two-dimensional EARc of 0.02, 0.10, 0.73, and 2.86 m, respectively.

Attraction of *Euwallacea* sp. 1 to Infested Avocado Logs or Branches

In the first experiment, the catches of *Euwallacea* sp. 1 (0 to 1 per week per trap) did not differ significantly between artificially infested avocado logs and seven blank traps. Dissection of the infested logs at the end of the experiment showed that the beetles died shortly after introduction, possibly because beetles had been stored too long and were unhealthy or the logs may have dried out rapidly and killed the beetles.

In the second experiment in which *Euwallacea* sp. 1 were attracted to freshly cut avocado logs with quercivorol baits, there is some weak evidence that the infested logs were more attractive than control logs. The infested-log trap over 3 wk caught 8, 6, and 2 beetles each successive week for a total of 16 compared to the control logs with 0, 3, and 1, respectively, for a total of 4. It is possible that the logs dried out too fast in the summer heat.

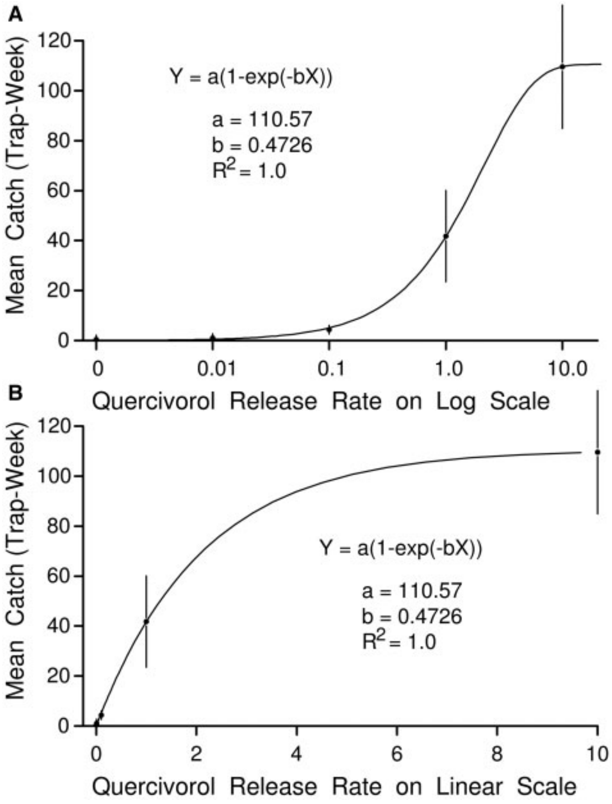

Fig. 1. Mean catch per trap-week of *Euwallacea* sp. 1 attracted to 10-fold increasing release rates of quercivorol on a logarithmic scale (**A**) or on a linear scale (**B**). Error bars are ±SE ($N = 16$).

In the third experiment, we monitored attraction of *Euwallacea* sp. 1 to limbs infested naturally with *Euwallacea* sp. 1 on living trees compared to uninfested branches on trees. Some of the latter, however, became infested to a lesser extent during the trapping period (our observations). The average catch on the infested limbs was 140.4 ± 55.8 (±SE) compared to 35.1 ± 5.8 on the less infested or uninfested limbs. These mean catches were significantly different (Mann–Whitney test, U = 2, N1 = N2 = 7, $P = 0.005$ two-tailed), indicating that the naturally infested limbs were significantly more attractive compared to control limbs. During this period a blank sticky trap caught only 8 while two uninfested logs in a sticky trap caught a mean of 2 and the older attacked logs from the second experiment a mean of 3. This indicates that beetle attacks in living trees are more attractive than cut logs with or without beetles (the logs appeared dried out by the time the branch test was started). The 1× quercivorol baits (N = 2) during this time caught 100 and 234 for a mean of 167 (not much more than 140.4 on the infested limbs). The EAR and EARc of the *Euwallacea* sp. 1-infested avocado limbs and quercivorol are summarized in Table 1. Although usually not considered, the EAR or EARc of a blank trap is simply its silhouette interception area $S = 0.0924\ \text{m}^2$ which gives an EAR = $(S/\pi)^{0.5} = 0.17$ m and this converts to an EARc = 0.02 m.

Simulations Using Measured EARc

Effective mass trapping of the female population occurred in simulations using EARc of 2.86 m for the 10× bait of quercivorol with 1,000 beetles and either 1, 4, or 16 sticky traps in a 9-ha area without attractive competition from natural *Euwallacea* sp. 1. For example, if females fly for up to 7,200 m, then 36.1 ± 1.8, 84.3 ± 0.8, or 99.9 ± 0.1 percent of the females (±95% C.L.) are captured, respectively. Addition of competing densities of beetle aggregations (EARc = 0.98 m, Table 1) at the three trap densities demonstrates how mass trapping can become less effective (Fig. 2). These results emphasize the need to set up mass trapping before *Euwallacea* sp. 1 start to fly in the spring in order to catch beetles before they can create aggregation odor sources on avocado limbs.

Discussion

A lure could appear strongly attractive when catching high numbers of insects at high population density, but the same lure would appear weak if presented when population density is low. Therefore, in order to measure the attractive strength of a lure that does not depend on the insect population density, several blank sticky traps of known dimensions are needed to estimate the density of flying insects at the same time when an attractive lure is presented. For example, if the mean catch per blank trap was 5, the trap silhouette area (seen from one side of trap) was $0.092\ \text{m}^2$, and the mean catch on the traps with lures was 236, then the EAR should be 1.18 m (Fig. 3).

The EAR is a spherical three-dimensional construct that represents the interception area of a large sticky trap without a lure. Such a trap with 1.18-m radius would be expected to catch the same number of insects as a smaller pheromone-baited trap that attracts only some of the insects flying by (as represented by the "smoky plume," Fig. 3). In order to model mass trapping of insects, two-dimensional models are less computationally intensive but do require that the EAR be converted to a circular EARc. This conversion is done by using the SD (standard deviation) of the vertical flight distribution of *Euwallacea* sp. 1 by recording catches on baited traps at various heights above ground. The formulas for calculating SD are standard but require each trap height be entered as many times as the catches into the formula (Byers 2011). The mean height of flight can also be calculated which gives the best height to place traps for monitoring or mass trapping. We found that the mean flight height for *Euwallacea* sp. 1 was 1.24 m (shoulder height or similar) and the SD was 0.88 m.

Theoretically, the EAR of 1.18 m and SD could be used to model *Euwallacea* sp. 1 flight in three-dimensions (Byers 2009, 2011), but the same catches on traps can be more easily done in two-dimensions in the same area with the EARc (which was 0.99 m). In the case of *Euwallacea* sp. 1, the EAR and EARc are similar but if the insect was a moth flying just above the crop canopy, then its SD would be shallow and its EARc would be several times larger than its EAR, while if the insect was a bark beetle of tall pines, then the SD would be larger and the EARc would be much smaller than the EAR (Byers 2012a, Byers and Naranjo 2014). The EARc of 1× quercivorol for *Euwallacea* sp. 1 of 0.99 m (earlier test) or 0.73 m (later test) indicates that the compound is a potent attractant similar to some other bark beetle pheromones. For example, the standard pheromone bait for *Ips typographus* (L.) has an EARc = 0.54 m, while a 50 male-infested pine log of California fivespined ips, *Ips paraconfusus* Lanier, has EARc = 1.23 m (Byers 2012a). The EAR and EARc of 10× quercivorol of 2.0 and 2.85 m, respectively, appears similar to some sex pheromones of moths. For example, the pink bollworm moth, *Pectinophora gossypiella* (Saunders), a pest of cotton, was calculated to have an EARc = 2.61 m (Byers and Naranjo 2014), while recently the monitoring traps for *Batrachedra amydraula* Meyrick were estimated to have an EARc = 3.43 m (Levi-Zada et al. 2017).

To date there is no evidence that *E. fornicatus* or *Euwallacea* sp. 1 females produce quercivorol that they are clearly attracted to.

Table 1. Mean catches of various treatments and controls used to calculate EAR and EARc (equations in Methods and Materials)

Treatment	Mean catch per trap	EAR (m)	EARc (m)
21 June–9 Aug.			
Blank ($N = 7$)	5	0.17	0.02
1 × ($N = 3$)	236.3	1.18	0.99
23 Aug.–19 Sept.			
Control ($N = 2$)	0 (if 0.5) (if 1)[a]		
Control logs ($N = 2$)	2.5	(0.38) (0.27)[a]	(0.10) (0.05)[a]
Hand-infested logs ($N = 2$)	8	(0.59) (0.49)[a]	(0.25) (0.17)[a]
18 Aug.–15 Nov.			
Blank ($N = 1$)	8	0.17	0.02
0.01 × ($N = 2$)	11.5	0.21	0.03
0.1 × ($N = 2$)	40	0.38	0.10
1 × ($N = 2$)	280.5	1.02	0.73
10 × ($N = 2$)	1091.5	2.00	2.86
19 Sept.–15 Nov.			
Blank ($N = 1$)	3	0.17	0.02
1 × ($N = 2$)	167	1.28	1.17
More infested *Euwallacea* sp. 1 limb ($N = 7$)	140.4	1.17	0.98
Less infested *Euwallacea* sp. 1 limb ($N = 7$)	35.1	0.59	0.25

[a] The control did not catch insects, but if a mean of 0.5 had been caught or 1 then the EAR and EARc in parentheses would result.

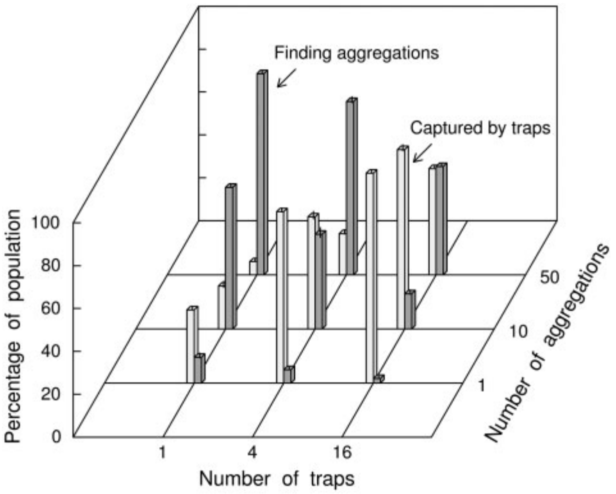

Fig. 2. Mean percentages of initial populations of 1,000 simulated beetles that were trapped by various numbers of traps (EAR = 2.86 m, lighter bars) or found aggregations of various numbers (EAR = 0.98 m, darker bars) in a 9-ha area. Each bar mean of eight simulations, and vertical error line on top of each bar represents 95% CI (bars are significantly different if error lines do not overlap).

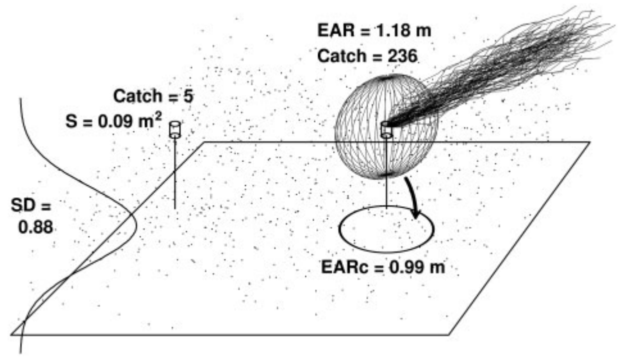

Fig. 3. Diagram depicting the ratio of catch on blank trap (at left) and pheromone trap (at right) giving a three-dimensional EAR and its conversion to two-dimensional EARc depending on SD of vertical flight distribution of *Euwallacea* sp. 1.

However, we did find some evidence that infested limbs of avocado trees were attractive with EARc equivalent to a 1× quercivorol bait. It is well known that males are incapable of flight and female *E. fornicatus* and *Euwallacea* sp. 1 mate with males before leaving the brood tree (Calnaido 1965, Carrillo et al. 2015). This raises the question why the *Euwallacea* sp. 1 female needs a long-range volatile attractant or aggregation pheromone. One argument for a female to release a long-range pheromone is that she would have an interest in her daughters and sons mating with the progeny of other females that arrived in order to reduce harmful genetic inbreeding. Similarly, females that were attracted to aggregation pheromone would benefit because their progeny could also outbreed and perhaps incur less mortality due to the host tissues having less resistance when succumbing to a cooperative attack. At the minimum, the odors of infesting beetles would indicate a food and habitat resource to exploit. Thus, we hypothesize that a pioneer female of *Euwallacea* sp. 1 randomly lands on a host tree (or is weakly attracted to host tree chemicals over a short range) and after she bores into the bark or wood, a stronger attraction results that may consist of fungal and beetle-produced odors. With time a stronger attraction may result from an aggregation of females, similar but different from bark beetle aggregations involving pheromones attractive to both sexes (Byers 1989). While quercivorol has not been found in *Euwallacea* sp. 1, there may be other similar compounds (terpene alcohols) that could comprise an aggregation pheromone. Until these compounds are found in the beetle, quercivorol should not be classified as a pheromone but rather as an attractant.

In practical respects, a 1 to 3 mg/d release rate of quercivorol should attract significant numbers of *Euwallacea* sp. 1 and be suitable for mass trapping or monitoring. The 10× rate of quercivorol (1.26 mg/d) caught about 2.6 times more *Euwallacea* sp. 1 than the 1× rate, and the latter rate attracted similar numbers of *Euwallacea* sp. 1 compared to infested limbs of avocado trees. In determining the optimal density of traps for mass trapping, it is important to consider that an overly high density of traps is expensive and can cause multiple odor plumes disrupting orientation of *Euwallacea* sp. 1 and thereby lower trap efficiency, while a very low density will not give good coverage and take longer to trap out the population, allowing many females to find suitable host branches and trees. In addition, the density of competing attractive sources (baited traps and natural sources) is very important to the success of mass trapping. In order to reduce natural sources of competition, it is imperative that mass trapping begin early in the spring season before females fly and attack new colonization sites. The great advantage with *Euwallacea* sp. 1 is that females, the reproductive sex, are caught and not males alone as with moths (Byers 2007, 2012b). Thus, *Euwallacea* sp. 1 is a good candidate for mass trapping.

In mass trapping, it is an economic necessity to have an inexpensive trap that can be reused and that is not saturated with insects. Moreover, baits need to be highly attractive as well as last for several weeks or more. Our dose–response curve joins a number of field dose–response curves of insects which fit well the kinetic formation functions, especially the first-order nonintercept type (Byers 2013). The commercial Canadian bait contains about 300 mg of quercivorol and releases about three times the rate of our 10× bait (unpublished data) and is expected from the curves to catch slightly more *Euwallacea* sp. 1, although this needs investigation. We conclude that the 10× bait or similar baits are suitable for mass trapping. Currently pilot mass trapping tests are being initiated by avocado growers in Israel.

Acknowledgments

We thank Mrs. Daniella Fefer (Chemistry-Entomology, Volcani Center) for technical assistance in analysis of the quercivorol isomers. We thank Kibbutz Ma'agan Michael avocado growers for assistance in setup and maintenance of field experiments. This research was supported by a grant from The Plants Production and Marketing Board, Fruit Division: Avocado table #0390320 of 2016.

References Cited

Byers, J. A. 1988. Novel diffusion-dilution method for release of semiochemicals: Testing pheromone component ratios on western pine beetle. J. Chem. Ecol. 14: 199–212.

Byers, J. A. 1989. Chemical ecology of bark beetles. Experientia 45: 271–283.

Byers, J. A. 1992. Attraction of bark beetles, *Tomicus piniperda, Hylurgops palliatus*, and *Trypodendron domesticum* and other insects to short-chain alcohols and monoterpenes. J. Chem. Ecol. 18: 2385–2402.

Byers, J. A. 2007. Simulation of mating disruption and mass trapping with competitive attraction and camouflage. Environ. Entomol. 36: 1328–1338.

Byers, J. A. 2008. Active space of pheromone plume and its relationship to effective attraction radius in applied models. J. Chem. Ecol. 34: 1134–1145.

Byers, J. A. 2009. Modeling distributions of flying insects: Effective attraction radius of pheromone in two and three dimensions. J. Theor. Biol. 256: 81–89.

Byers, J. A. 2011. Analysis of vertical distributions and effective flight layers of insects: Three-dimensional simulation of flying insects and catch at trap heights. Environ. Entomol. 40: 1210–1222.

Byers, J. A. 2012a. Estimating insect flight densities from attractive trap catches and flight height distributions. J. Chem. Ecol. 38: 592–601.

Byers, J. A. 2012b. Modelling female mating success during mass trapping and natural competitive attraction of searching males or females. Entomol. Exp. Appl. 145: 228–237.

Byers, J. A. 2013. Modeling and regression analysis of semiochemical dose−response curves of insect antennal reception and behavior. J. Chem. Ecol. 39: 1081–1089.

Byers, J. A., and S. E. Naranjo. 2014. Detection and monitoring of pink bollworm moths and invasive insects using pheromone traps and encounter rate models. J. Appl. Ecol. 51: 1041–1049.

Byers, J. A., O. Anderbrant, and J. Löfqvist. 1989. Effective attraction radius: A method for comparing species attractants and determining densities of flying insects. J. Chem. Ecol. 15: 749–765.

Calnaido, D. 1965. The flight and dispersal of shot-hole borer of tea (*Xyleborus fornicatus* Eichh., Coleoptera: Scolytidae). Entomol. Exp. Appl. 8: 249–262.

Carrillo, D., T. Narvaez, A. A. Cossé, R. Stouthamer, and M. Cooperband. 2015. Attraction of *Euwallacea* nr. *fornicatus* (Coleoptera: Curculionidae: Scolytinae) to lures containing quercivorol. Fla. Entomol. 98: 780–782.

Carrillo, D., L. F. Cruz, P. E. Kendra, T. I. Narvaez, W. S. Montgomery, A. Monterroso, C. De Grave, and M. F. Cooperband. 2016. Distribution, pest status and fungal associates of *Euwallacea* nr. *fornicatus* in Florida avocado groves. Insects 7: 55–11. p. doi: 10.3390/Insects7040055

Cooperband, M. F., R. Stouthamer, D. Carrillo, A. Eskalen, T. Thibault, A. A. Cossé, L. A. Castrillo, J. D. Vandenberg, and P. F. Rugman-Jones. 2016. Biology of two members of the *Euwallacea fornicatus* species complex (Coleoptera: Curculionidae: Scolytinae), recently invasive in the U.S.A., reared on an ambrosia beetle artificial diet. Agric. For. Entomol. 18: 223–237.

Eskalen, A., A. Gonzalez, D. H. Wang, M. Twizeyimana, and J. S. Mayorquin. 2012. First report of a *Fusarium* sp. and its vector tea shot hole borer (*Euwallacea* nr. *fornicatus*) causing *Fusarium* dieback on avocado in California. Plant Dis. 96: 1070.

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.

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. *fornicata*. Symbiosis 58: 245–251.

Hulcr, J., and L. L. Stelinski. 2017. The ambrosia symbiosis: From evolutionary ecology to practical management. Annu. Rev. Entomol. 62: 285–303.

Larson, R., and B. Farber. 2012. Elementary Statistics: Picturing the World. Prentice Hall, Boston.

Levi-Zada, A., A. Sadowsky, S. Dobrinin, T. Ticuchinski, M. David, D. Fefer, E. Dunkelblum, and J. A. Byers. 2017. Monitoring and mass-trapping methodologies using pheromones: the lesser date moth *Batrachedra amydraula*. Bull. Entomol. Res. doi:10.1017/S000748531700048.

Lynch, S. C., M. Twizeyimana, J. S. Mayorquin, D. H. Wang, F. Na, M. Kayim, M. T. Kasson, P. Q. Thu, C. Bateman, P. Rugman-Jones, et al. 2016. Identification, pathogenicity and abundance of *Paracremonium pembeum* 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.

Mendel, Z., A. Protasov, M. Sharon, A. Zveibil, S. B. Yehuda, K. O'Donnell, R. Rabaglia, M. Wysoki, and S. Freeman. 2012. An Asian ambrosia beetle *Euwallacea* nr. *fornicatus* and its novel symbiotic fungus *Fusarium* sp. pose a serious threat to the Israeli avocado industry. Phytoparasitica 40: 235–238.

O'Donnell, K., S. Sink, R. Libeskind-Hadas, J. Hulcr, M. R. Kasson, R. C. Ploetz, J. L. Konkol, J. N. Ploetz, D. Carrillo, A. Campbell, et al. 2015. Discordant phylogenies suggest repeated host shifts in the *Fusarium*–*Euwallacea* ambrosia beetle mutualism. Fungal Gen. Biol. 82: 277–290.

Stouthamer, R., P., Rugman-Jones, P. Q., Thu, A., Eskalen, T., Thibault, J., Hulcr, L. J., Wang, B. H., Jordal, C. Y., Chen, M., Cooperband, C. S., Lin, N., Kamata, S. S., Lu, H., Masuya, Z., Mendel, R., Rabaglia, S., Sanguansub, H. H., Shih, W., Sittichaya, and S. Zong. 2017. Tracing the origin of a cryptic invader: phylogeography of the Euwallacea fornicatus (Coleoptera: Curculionidae: Scolytinae) species complex. Agr. Forest Entomol. doi: 10.1111/aef.12215.

Tokoro, M., M. Kobayashi, S. Saito, H. Kinuura, T. Nakashima, E. Shoda-Kagaya, T. Kashiwagi, S. Tebayashi, C. Kim, and K. Mori. 2007. Novel aggregation pheromone, (1S,4R)-p-menth-2-en-1-ol, of the ambrosia beetle, *Platypus quercivorus* (Coleoptera: Platypodidae). Bull. FFPRI 6: 49–57.

Wood, S. L. 1982. The bark and ambrosia beetles of North and Central America (Coleoptera: Scolytidae), a taxonomic monograph. Great Basin Nat Memoirs 6: l–1359.