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Aggregation volatiles and behavior of the polyphagous shot hole borer attacking avocado in Israel

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Introduction

The polyphagous shot hole borer (PSHB), *Euwallacea fornicatus*, is an ambrosia beetle in the family Curculionidae and subfamily Scolytinae. The bark and ambrosia beetles (Scolytinae) that feed or kill living trees usually are attracted from tens of meters to aggregation pheromones consisting of one to three chemicals (Byers 1989, 1995) that are produced by either one or both sexes. Often host tree odors enhance the attraction to aggregation pheromone. Some ambrosia beetles are known to use aggregation pheromones, such as *Trypodendron lineatum, Gnathotricus sulcatus*, and *Platypus quercivorus* (Byers 1989; Tokoro et al. 2007). A closely related species of PSHB found in Florida has been reported to be attracted to quercivorol (Carrillo et al. 2015), an aggregation pheromone of the oak ambrosia beetle *P. quercivorus* colonizing oaks. However, Carrillo et al. and other unpublished studies have not determined the strength of this attraction compared to other semiochemicals of bark beetles and ambrosia beetles.

The PSHB ambrosia beetle appears to have arrived in Israel from Southeast Asia or Africa and has also invaded parts of California as early as 2003, and Israel shortly thereafter (Eskalen et al. 2012; Mendel et al. 2012; Freeman et al. 2012). The beetle has also been classified earlier as *Xyleborus fornicatus.* Like many scolytid beetles, this ambrosia beetle carries symbiotic fungi (species) that grow in the tunnels of adults and their larvae in the host tree sapwood and serve as primary food or supplemental nutrition. In the case of PSHB, only the females appear to leave the brood tree after mating since the males are not capable of flying (Carrillo et al. 2015). The females of PSHB are supposed to fly up to 24 minutes in the laboratory and thus were calculated to fly from 400 to 800 m without aid of wind (http://caforestpestcouncil.org/wp-content/uploads/2008/07/Polyphagous-Shot-Hole-Borer.pdf). Of course, females could rest and possibly feed between flights, and wind would increase their potential dispersal range. Our observations and that of others suggest that females generally do not bore alone into their host avocado tree but are commonly found together in an aggregation of female attacks in a relatively concentrated area of a branch or nearby branches.

We hypothesized that either females land at random on host trees or some weak host chemicals attract the first few females over a short range, then after she bores into the tree's bark/wood a stronger attraction is created that may consist of fungal and/or beetle-produced odors. Because female PSHB mate with males before leaving the brood tree and males are incapable of flight, one could ask why the PSHB needs a long-range 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 sexes of the progeny of non-related females that were attracted to the aggregation in order to reduce harmful genetic inbreeding. Similarly, females that responded 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 mass attack. At the minimum, the odors of infesting beetles would indicate a food and habitat resource to exploit. A few reports have suggested that ethanol and quercivorol = (1S,4R)-p-menth-2-en-1-ol are weakly attractive to PSHB (Carrillo et al. 2015). However, ethanol was released in all formulations so it is not clear if quercivorol is attractive or even enhances attraction to ethanol. Also, there were no methods used to determine the relative strengths of the baits.

Objectives

We proposed to determine the attractive strength of various odor sources of avocado wood infested with PSHB or not infested in the field. If attractive sources are indicated, or other attractants, then these would be tested in the field for their relative attractive strengths to determine if they can be practical for monitoring and mass trapping.

Methods and Results:

Experiments testing infested PSHB avocado branches:

Avocado logs infested with PSHB ambrosia beetles were cut from trees and placed inside cardboard boxes with clear plastic window to collect emerged adults as they walked to the light and slipped in a funnel where the beetles fell into a cup with damp paper. The adults were collected and stored at 4° C until use.

Fig. 1. Cardboard boxes containing PSHB-infested avocado logs in which beetles attracted to light are collected by funnels and cup.

Experiment I. In the first experiment, avocado logs (6 cm diameter x 28 cm long) were cut from a tree and after one week were drilled with 1-mm drill through the bark and phloem and a female was introduced to each of 30 holes per log. Two infested logs were then wrapped with nylon mesh to prevent beetles from leaving and these placed into a sticky trap in an avocado orchard in the field (1 km from Binyamina, Israel). A set of two control logs were wrapped similarly and placed into a second sticky trap. Traps were made of 6-mm mesh wire screen covered with

Rimifoot sticky adhesive (Rimi, Petah Tikva, Israel) formed into a cylinder 28 cm diameter × 33 cm high and placed at 1.2 m height on poles spaced 15 m apart (see Fig. 2 below). Traps were picked of PSHB every week for three weeks (21 June to 7 July 2016). The catches (0 to 1 per week per trap) were not significantly different from seven blank traps. Dissection of the infested logs at the end of the experiment showed that the beetles died shortly after introduction (probably because beetles had been stored too long and were unhealthy).

Fig. 2. Sticky cylinder traps containing infested logs or quercivorol baits.

Experiment II. In order to obtain healthier beetles in infested avocado logs, we placed a quercivorol bait on several cut logs in a pile for a week (18-24 Aug 2016) in the field and then placed the naturally-infested logs in the trap compared to control logs. There is some weak evidence that the infested logs were more attractive because this trap over three weeks caught 8, 6, and 2 beetles each week for a total of 16 compared to the control logs with 0, 3, and 1, respectively, for a total of 4. The logs probably dried out too fast in the summer heat.

Experiment III. In order to use naturally-attacking beetles in living branches, the closest to nature, we wrapped sticky screen into a cylinder (33 cm long x 28 cm diam.) surrounding naturally infested branches (N = 7) within avocado trees and compared these to control branches (N = 7), however, the latter became infested to a lesser extent during the trapping period (26 Sept. to 15 Nov. 2016). The average catch on the infested limbs was 140.4 ± 55.8 (±SE) and 35.1 ± 5.8 which were significantly different (Mann Whitney test, U = 2, P = 0.005). This indicates that the naturally infested limbs were significantly attractive compared to control limbs (although some infestation). During this period a blank sticky trap caught only 8 while two uninfested logs caught a mean of 2 and the older attacked logs a mean of 3. This indicates that beetle attacks in living trees are more attractive than cut logs with or without beetles (the cut logs probably dried out by the time the branch test was started and beetles had likely died).

In another experiment conducted at the same time nearby, the 1x quercivorol baits (N=2) caught 100 and 234 for a mean of 167 (not much more than 140.4 caught on the infested limbs). This experiment suggests there may be a potent attractant released by living avocado limbs infested by PSHB. This experiment needs to be repeated with nylon wrap on the control limbs and a comparison to blank control traps inside and outside the monitored avocado trees.

Experiments testing attractive strength of quercivorol

Previous reports and preliminary tests in Israel indicated that quercivorol was attractive but the strength of this attraction as related to the release rate (dose) was not known.

Experiment IV. A small glass test-tube dispenser (3.29 mm i.d. x 30.6 mm long) was developed that gives an almost constant release rate at a specified temperature (Byers 1988) when 20 µl of quercivorol is placed at the bottom of the tube (1x dose). This dispenser in the laboratory (average of three dispensers) lasted about 4 months at 25º C and gave a constant release of 0.126 mg/day as measured by weight loss with a microbalance. The 1x dose of quercivorol (1 glass tube) was placed inside an inverted aluminum foil covered cup to prevent sun and rain damage and then surrounded by a sticky screen trap described above. Three of these traps were compared with seven control traps in order to obtain a ratio of catch that is used to calculated the ***effective attraction radius*** (EAR) according to the equation in Byers (2007, 2008, 2009). A total of 35 PSHB were caught on seven blank traps and 709 beetles on three treatment traps from 21 June – 18 Aug. The per trap total of 5 on control trap compared to 236.33 on 1x dose treatment trap gives an EAR = 1.179 m due to the sticky trap silhouette interception area of 0.0924 square meters. In order for the EAR to be used in simulation models of monitoring and mass trapping, the EAR needs to be converted to two-dimensions and to the EARc (c for circular). This conversion requires knowledge of the standard deviation (SD) of the vertical flight distribution which is obtained in the next experiment.

Experiment V. A pole (Fig. 3 to left) was constructed with six sticky traps (25.5 cm diam. x 25 cm high) each baited with 1x quercivorol. The pole's trap centers were at 0.25, 1.35, 2.45, 3.55, 4.65, and 5.75 m height above ground in an avocado orchard (26 June – 18 Aug.). PSHB caught on the traps were picked weekly for three weeks and once more after 3 more weeks. The catches at each height were used to calculate mean flight height and the SD (standard deviation) of the vertical flight distribution according to an iterative equation (Byers 2011). This gave means heights of 1.12, 1.37, 1.15, and 1.11 for a grand mean flight height of 1.24 m; the SD was 0.85, 0.85, 1.38, and 0.86 for a grand SD of 0.88 m. Using the SD and the EAR calculated above, then the EARc = 0.99 m (Byers 2008, 2009).

Experiment VI. A dose-response experiment was done to determine whether higher or lower dosages than 1x would be more attractive. Also the EAR and EARc can be calculated for each dose (release rate of quercivorol). Two replicates of each dosage at 10-fold increasing magnitude: 0 (control), 0.01x, 0.1x, 1x, and 10x (x=0.126 mg/day) were placed in the same avocado orchard and picked eight times approximately every one to two weeks (18 Aug – 15 Nov.). The small test-tube dispenser was used for the various release rates with the two lower rates made by diluting quercivorol with decanol because both compounds have similar vapor pressures and therefore would evaporate at similar rates (Byers 1988). The 10x dose was made by placing 10 dispensers of 1x in the cup. The mean catch per trap per week (Y) with increasing release rate (X) fit a first-order kinetic formation function perfectly (R^2 = 1), and indicates that the 10x dose catches the most (see Fig. 4). The 10x dose released 1.26 mg/day of quercivorol. The EAR was calculated for each of the doses 0.01, 0.1, 1, and 10x as 0.29, 0.54, 1.02, and 2.00 m, respectively. These doses had catches that calculated EARc of 0.06, 0.21, 0.73, and 2.86 m, respectively.

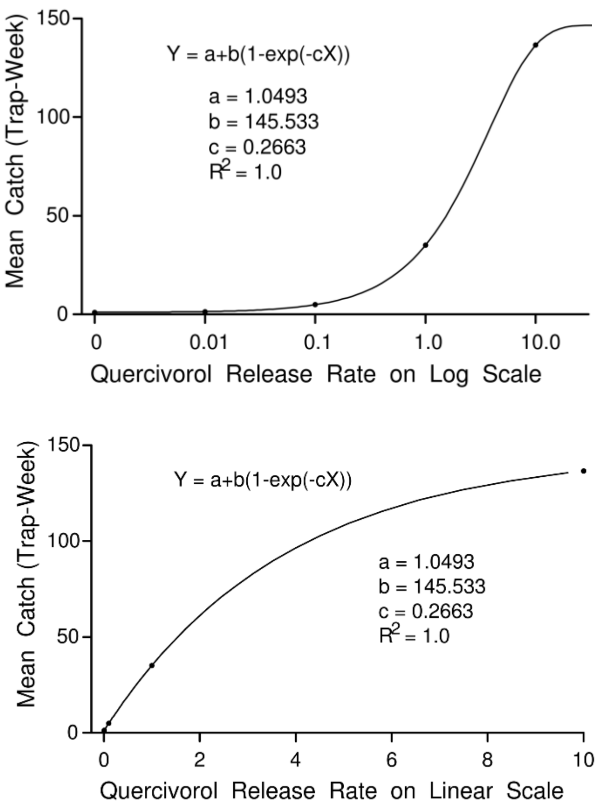

Fig. 4. Top graph shows the catch of PSHB attracted to 10-fold increasing doses of quercivorol on a logarithmic scale. Bottom graph shows the same catches on a linear scale; both graphs are fit with the same kinetic formation equation.

Experiment VII. Chemical analysis by GC and GCMS of gray rubber septa lures from Costa Rica (Chemtica) showed a total of 2 mg of quercivorol which would expire in only 2 days at the 10x rate. However, rubber septa lures are well known to exhibit an exponential decline in release rate (Byers 1988) such that initially the lure may release similar to the 1x rate and then after several days become close to the 0.1x rate that was hardly attractive. The commercial plastic-bubble lures from Canada contained 150 times more quercivorol (300 mg of 90% active isomer) and are expected to release at a constant rate at a constant temperature (Byers 1988). The release of these lures was measured by others at Tapazol (Israel) to be about 4 mg per day which would be comparable to 317% of the 10x rate. However, both rates would catch similarly according to theoretical expectation (for example, a doubling of release causes an insignificant increase in attraction for most pheromones). Accordingly, the Canadian bubble lure would provide 2.5 months of significant attraction, or somewhat less at summer temperatures. We suggest using the highest release rate lure for monitoring (Byers and Naranjo 2014) and mass trapping (Byers 2012b). It is possible to calculate the expected EARc from the Canadian lure and model this in a mass trapping scenario (Byers 2012a, b).

Discussion

An attractive lure could appear strongly attractive by catching high numbers of insects when population densities are high, but the same lure would appear weak if presented when population density was low. Thus, lure strength cannot be assessed by captures in the field without knowing the population flight density. 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 an attractive lure is presented to compensate for different possible densities. For example, if the mean catch per trap is 5 on blank traps and their silhouette area (area as seen from one side) is 0.09 m^2 while the mean catch on the traps with lures is 236, then the effective attraction radius (EAR) is 1.18 m (Fig. 5).

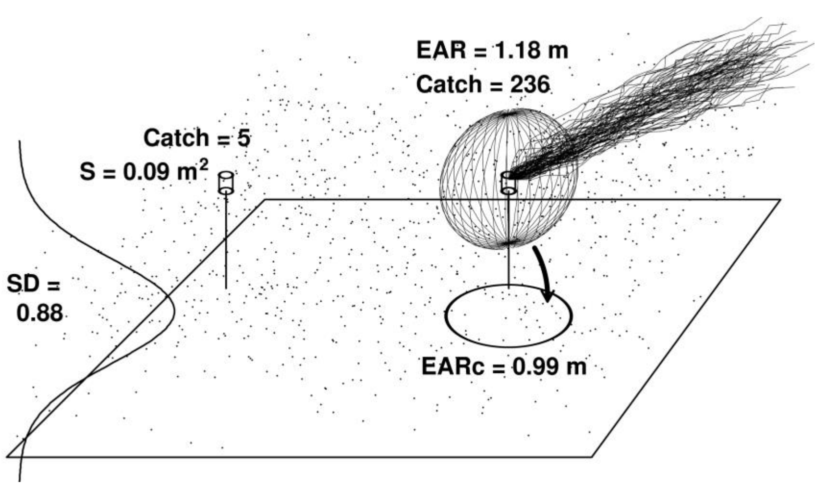

Fig. 5. Diagram depicting the ratio of catch on blank trap (at left) and pheromone trap (at right) giving an EAR and its conversion to EARc depending on SD of vertical flight distribution of PSHB.

The EAR is a spherical three-dimensional construct that represents the interception area of a large sticky trap without a lure. In effect the 1.18 m radius trap (no lure) would catch the same number of insects as a pheromone bait in a trap that only attracts some of the insects flying by (as represented by the "smoky plume" in Fig. 5). 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 PSHB by recording catches on baited traps at various heights above ground. The formulas for calculating SD are standard statistical ones but require each trap height to be entered as many times as the catch at that height 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 height for PSHB was 1.24 m (shoulder height or similar) and the SD was 0.88 m.

Theoretically, the EAR of 1.18 m and SD of 0.88 m could be used to model PSHB flight in three dimensions (Byers 2009) but the same catch on traps can be done in two dimensions in the same area with the EARc (which was 0.99 m) much faster by computer simulation. In the case of PSHB, the EAR and EARc are similar but if the insect was a moth flying just above the crop canopy (e.g. cotton pink bollworm) 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 large pines then the SD would be larger and the EARc would be much smaller than the EAR (Byers 2012a, Byers and Naranjo 2014). The EAR of 1.17 m for PSHB indicates that quercivorol (1x) is a potent attractant similar to some other bark beetle pheromones. For example, in bark beetles, the standard pheromone bait for the European spruce bark beetle, *Ips typographus*, has an EARc = 0.54 m, while a 50-male infested pine log of California 5-spined ips, *Ips paraconfusus*, has EARc = 1.23 m (Byers 2012a).

The EAR and EARc of quercivorol (10x) of 2.0 and 2.86 m, respectively, appears similar to some moth pheromones such as the pink bollworm moth, a major pest of cotton, with an EARc = 2.61 m (Byers and Naranjo 2014), while recently the pheromone monitoring traps for lesser date moth were estimated to have an EARc = 3.43 m (Levi-Zada et al. 2016 submitted). While quercivorol has so far not been found in PSHB, there may be other similar compounds (terpene alcohols) that comprise the aggregation pheromone. Until these compounds are found in the beetle, quercivorol should not classified as a pheromone but rather as a kairomone or parapheromone. In practical respects, a 1 to 3 mg/day release rate of quercivorol should attract significant numbers of PSHB and be suitable for mass trapping or monitoring. In determining the density of traps, a too high density can cause interference among traps due to confusion of the PSHB and thereby lower trap efficiency, while a too low density will not give good coverage and will take longer to trap out the population so that many females find suitable host branches/trees. Thus, the density of competing attractive sources is very important to the success of mass trapping. This means that it is imperative that mass trapping begin early in the spring season before females fly and attack new colonization sites. The great advantage with PSHB is that the females (the reproductive sex) are caught and not just males as with moths. Thus PSHB is a very good candidate for mass-trapping.

In mass-trapping it is an economically necessity to have an inexpensive trap that can be reused and with adequate capacity to hold killed insects. Also baits need to highly attractive as well as last for several weeks or more. In this respect, the Canadian bait is much better than the Costa Rican bait, both in terms of attraction (closer to 10x dose) and amounts of active ingredient that will last longer at a constant release rate (at a constant temperature). The rubber septa of Costa Rican lure should decline rapidly in release of quercivorol and then become a more constant release but at an unacceptably low level (probably closer to 0.1x or lower release) that catches too little.

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