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Semiochemicals Affecting Attraction of Ambrosia Beetle *Euwallacea fornicatus* (Coleoptera: Curculionidae: Scolytinae) to Quercivorol: Developing Push-Pull Control

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Abstract

Euwallacea fornicatus (Eichhoff), the polyphagous shot hole borer (PSHB), is an ambrosia beetle infesting avocado *Persea americana* Mill. limbs in North America and Israel. We conducted field experiments with sticky traps in avocado orchards to develop push–pull semiochemical methods of managing PSHB. Traps baited with 10-fold increasing doses (0.01 to 100× or 1.26 µg to 12.6 mg/d) of attractant quercivorol were previously shown to increasingly capture female PSHB (males flightless). We converted trap catch of this relationship to a standardized effective attraction radius (EAR) that predicts capture power of baited-traps regardless of insect flight density. Earlier, piperitone and verbenone were shown to strongly inhibit attraction of PSHB to quercivorol-traps. We tested increasing numbers of 1× piperitone dispensers at 0.75-m distance surrounding a quercivorol-trap and found PSHB catch to decline exponentially. Increasing decadic doses (0.01 to 10×) of either verbenone or piperitone released at 1× quercivorol-traps caused a sigmoidal first-order kinetic-decay in catch. Verbenone (1×) placed at increasing distances (0, 0.25, 0.5, 1, and 2 m) from a 1× quercivorol-trap became increasingly ineffective in reducing catch of PSHB. We found no evidence that ethanol released from 7.5 to 480 mg/d affected attraction of PSHB, but *Scobicia chevrieri* (Villa and Villa) (Coleoptera: Bostrichidae) was increasingly attracted. Due to their relatively short-range (<0.5 m) inhibition of attractive sources, piperitone, and verbenone dispensers should be placed on avocado trunks where PSHB aggregations occur before the flight season.

Key words: quercivorol, verbenone, piperitone, inhibitors and repellents, chemical ecology

An ambrosia beetle from southeast Asia that invaded California in 2003, and shortly thereafter, Israel was designated the polyphagous shot hole borer (PSHB) because of its pest status attacking avocado *Persea americana* Mill. and many species of woody plants (Eskalen et al. 2012; Freeman et al. 2012; Mendel et al. 2012, 2017). PSHB appears to have similar behavior and ecology to the tea shot hole borer (TSHB) that invaded Florida (Carrillo et al. 2015, 2016; Cooperband et al. 2016). These two species are nearly identical morphologically but differ in DNA (Eskalen et al. 2013, O'Donnell et al. 2015, Stouthamer et al. 2017, Smith et al. 2019). A type specimen of *Euwallacea fornicatus* (Eichhoff) was recently located by Smith et al. (2019) who renamed TSHB as *Euwallacea perbrevis* (Schedl) and PSHB as *E. fornicatus* in agreement with DNA analyses. In Israel, avocado limbs are infested and killed by PSHB directly or by the vectoring of *Fusarium* dieback disease the beetles carry which reduces tree growth (Eskalen et al. 2012, Freeman et al. 2012, Mendel et al. 2012, Eskalen et al. 2013, Lynch et al. 2016, Mendel et al. 2017). Like other ambrosia beetles, PSHB carries symbiotic fungi that grow in beetle tunnels in the sapwood and serve as food (Wood 1982, Freeman et al. 2012, Hulcr and Stelinski 2017). Only the females of the species leave the brood tree limbs after mating since the males are flightless (Calnaido 1965, Carrillo et al. 2015).

Scolytine beetles including many bark beetles and some 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, was identified by Tokoro et al. (2007) as the aggregation pheromone of the oak-killing ambrosia beetle *Platypus quercivorus* (Murayama) of Japan. Quercivorol is commercially available and is attractive to TSHB in Florida (Carrillo et al. 2015, Kendra et al. 2017) and to PSHB in Israel (Byers et al. 2017, 2018). However, despite initial work on PSHB volatiles the reason for this attraction is unclear.

Quercivorol is highly attractive to PSHB females in the field as shown by a standardized comparative method termed the *effective attraction radius* (EAR) (Byers 2012a,b; Byers et al. 2017). The EAR of a baited-trap is the radius of a theoretical sticky sphere that would be required to catch the same number of insects as was actually caught by the attractive trap during the trapping period. The calculation of EAR uses a ratio of catches of the attractive trap to a passive (unattractive) trap of known interception size, the latter trap compensating for the particular insect flight density during trapping. This gives a consistent EAR for a specific bait and trap regardless of the insect density during measurement. A small radius EAR relative to other larger EAR of insect pheromones indicates a weak lure, whereas a relatively large radius EAR indicates a potent lure. Using sticky traps at six heights, the mean flight height of PSHB was calculated as 1.24 m with a SD of the vertical flight distribution of 0.88 m (Byers 2011, Byers et al. 2017). The EAR can be converted using the SD above to EARc, a circular radius in two dimensions, for use in simulation models (Byers et al. 2017, 2018). Our previous experiments in Israel showed that flying PSHB females are attracted to their aggregations on limbs of avocado trees, and we calculated that a typical aggregation has an EAR of 1.17 m and EARc of 0.98 m, which is nearly the same as 1× quercivorol-trap (Byers et al. 2017, 2018). A higher release rate of quercivorol (10×) gave an EAR of 1.98 m which is similar to EAR of aggregation pheromones of bark beetles (Byers 2012a, Byers et al. 2018). The EAR of a specific baited-trap is important to know when determining the practical number of baited traps needed to reduce a population of pest insects by mass trapping (Byers 2009, 2011; Byers et al. 2017).

Ethanol is commonly reported to be attractive to ambrosia beetles and some bark beetles (Byers 1989, 1992). Carrillo et al. (2015) showed the mean catch of TSHB in Florida on traps releasing a high dose of ethanol (~400 mg/d) was about three times more than on control traps, but this difference was not significant. In addition, they showed that ethanol plus quercivorol were significantly more attractive to TSHB than blank traps, but not significantly different from ethanol alone. Following this report, Byers et al. (2018) released ethanol over a 64-fold range (7.5–480 mg/d) together with a standard bait of quercivorol (0.126 mg/d). They found that ethanol at all release rates was moderately inhibitory of PSHB attraction to quercivorol. There was a weak trend of increasing inhibition with increasing release of ethanol; however, ethanol was not released alone.

Many conifer-infesting bark beetle species are inhibited in attraction by verbenone, a monoterpene ketone, that may indicate a degrading and unsuitable host tree (Byers and Wood 1980; Byers et al. 1984, 1989; Byers 1989; Hughes et al. 2017). Another inhibitor reducing attraction of the scolytine Douglas-fir beetle *Dendroctonus pseudotsugae* (Hopkins) is 3-methyl-2-cyclohexen-1-one (MCH) (Furniss et al. 1972). Earlier we tested several monoterpene ketones on PSHB response to quercivorol and found no evidence that either MCH, (±)-cryptone (4-isopropyl-2-cyclohexen-1-one), or (*S*)-carvone had an effect. However, piperitone and either enantiomer of verbenone each strongly reduced the attraction of PSHB to quercivorol, and the catch on quercivorol-baited sticky traps was increasingly reduced as the inhibitors verbenone plus piperitone were moved closer than 1 m to the trap (Byers et al. 2018).

Our first objective was to convert the dose–response curve of mean weekly catch per trap as a function of quercivorol dose to curves of EAR and EARc as functions of quercivorol release rate (mg/d). The curves of EAR/EARc in relation to dose/release of quercivorol are standardized functions ideal for comparing the potency of PSHB lures in traps to other pheromone traps of insects as well as in the design of mass trapping and push–pull programs (Cook et al. 2007, Byers et al. 2018). Our second objective was inspired by simulation models of push–pull where we envisioned placing one to three repellent dispensers inside the tree to cause reduction in attraction to aggregations of PSHB on limbs (Byers et al. 2018). Therefore, we wanted to test 0, 1, 2, or 3 dispensers of piperitone spaced 0.75 m from a quercivorol bait in a sticky trap. Since the effectiveness of nearby dispensers releasing inhibitors depends on the dose or release rate of these volatiles, a third objective was to determine the effect of increasing release rates of either verbenone or piperitone on attraction of PSHB to a standard 1× quercivorol-baited trap. A fourth objective was to quantify the effect of distance between the inhibitor verbenone and a quercivorol-baited trap. Finally, we wanted to test the same 64-fold range of release rates of ethanol (without quercivorol) in an attempt to observe either a higher capture rate (attraction) or a lowered capture rate (inhibition) when compared with control traps without chemicals.

Materials and Methods

Traps, Attractant Dispensers, and Study Area

Studies of PSHB were carried out in Hass avocado orchards (cultivated by Kibbutz Ma'agan Michael) 2.5 km east of Nahsholim, Israel (32° 36′ 25″ N; 34° 56′ 44″ E). Sticky screen cylinders (25-cm long × 25.5-cm diam.) of 6-mm mesh wire screen covered with sticky adhesive (80% polyisobutene, Rimifoot, Rimi, Petah Tikva, Israel) were used in all experiments. The 1× attractant dispenser consisted of 20 µl of quercivorol (racemic 85% *cis*, 15% *trans*; Synergy Semiochemicals, Burnaby, Canada) placed at the bottom of a glass flat-bottom 250-µl tube (3.29-mm i.d. × 30.6-mm long, J.G. Finneran Associates, Inc., Vineland, NJ) that gives an almost constant release rate at a specified temperature (Byers 1988). The release at 25°C for this lure was 0.126 mg/d according to previous weight loss measurements (Byers et al. 2017).

EAR and EARc of Sticky Trap Releasing Attractant Quercivorol

Catches from the dose–response curve for PSHB attraction to quercivorol in the field (Figure 1A in Byers et al. 2018) were converted to respective EAR (effective attraction radius). The three-dimensional EAR was calculated from the catches according to $EAR = [Ca \times S / (\pi \times Cb)]^{0.5}$, where Ca is the mean catch of the attractive traps, Cb is the mean catch of the unbaited traps (0.508), and S is the silhouette area (0.0924 m^2) of the sticky cylinder trap. A two-dimensional EARc for simulation models was calculated from the respective EAR using the SD of the vertical flight distribution of PSHB (0.88 m) as measured earlier (Byers et al. 2017, 2018) according to $EARc = \pi \times EAR^2/[2 \times SD \times (2 \times \pi)^{0.5}]$ (Byers 2011, 2012a,b). Nonlinear regression software (TableCurve 2D version 5.01, Systat Software Inc., Chicago, IL) was used to find a kinetic formation function that fit the dose–response data well (Byers 2013). Smaller and larger doses (0, 0.01, 0.1, 1, 10, and 108) of our standard quercivorol lure described above were converted to release rates (dose 1 = 0.126 mg/d) and analyzed by the same methods.

Catch on Quercivorol Trap as a Function of Number of Nearby Piperitone Dispensers

A 1× quercivorol lure was scotch taped inside an inverted plastic cup covered with aluminum foil to protect from sun and rain. The cup was attached centrally within a vertically aligned tubular sticky-screen trap described above placed at 1.2 m height. The piperitone inhibitors were also fixed inside foil-covered plastic cups at 1.2-m height but without sticky traps; and either one, two, or three inhibitors were placed 0.75-m away from the quercivorol-baited trap (two piperitone dispenser cups were on opposite sides, whereas three such cups were at corners of an equilateral triangle surrounding the attractant trap). A quercivorol-baited trap alone served as a control. The 1× piperitone inhibitor was released at 0.52 mg/d at 25°C (Byers et al. 2018) from a 2-ml glass vial (5.2-mm top opening, 32-mm long; Chrom4 GmbH, Thüringen, Germany) containing 60-µl neat piperitone (95% pure, 75% *R*-enantiomer, Synergy Semiochemicals). Each of the four treatment arrangements was replicated three times for a total of 12 groups randomized by position in a line with treatments separated by 15 m in the avocado orchard mentioned above. PSHB were picked from the traps every 7–10 d and replicate positions re-randomized by position in the line (12 September to 5 November 2018). The catch on traps of each collection was adjusted to catch per week for a total of 15 replicates for each treatment and analyzed by analysis of variance (ANOVA) with significant differences between pairs of treatments indicated by Tukey's HSD at $\alpha = 0.05$ (JMP 5.0.1a, SAS Institute Inc.). Nonlinear regression software (TableCurve 2D) was used to find a decay function that fit the dose–response data well (Byers 2013).

Catch on Quercivorol Trap as a Function of Release Rate of Piperitone or Verbenone Inhibitors

Earlier work showed that 1× piperitone together with 1× quercivorol caused the attraction of PSHB to decrease to only 10% compared with catch on 1× quercivorol traps (Byers et al. 2018). To investigate how the dose of piperitone affects the attraction, we released piperitone at 0, 0.01×, 0.1×, 1×, and 10× doses together with a 1× dose of quercivorol within a plastic cup inside a sticky trap as above. The 0.01 and 0.1× doses were prepared by dilution of piperitone with decanol based on a diffusion–dilution method (Byers 1988). This method blends the semiochemical and a solvent of similar volatility to obtain a mole proportion corresponding to the desired proportion of the release rate of the neat semiochemical. Thus, to obtain a 0.1 mole proportion dose requires 1.053 ml of decanol (MW 158.28, density 0.829 g/ml) and 100 µl piperitone (MW 152.23, density 0.933 g/ml). The lower 0.01 dose was made by 1:10 dilution (100 + 900 µl decanol). The 1× (standard experimental dose) and 10× doses were made from neat piperitone using one or ten 2-ml vials as described above. Each solution had 60 µl placed in 2-ml glass vials, whereas the control had only 60 µl of decanol in a vial. The sticky traps and foil cups described above were used with the different doses each replicated three times for a total of 15 traps. The traps were placed at random in a line with 15 m separating each trap (16 September to 21 November 2018) in the same avocado orchard but 150 m away from any other tests. PSHB were collected from sticky traps every week to 10 d, replicate positions re-randomized by position, and catch adjusted per week for a total of 21 replicates per treatment. Catch was analyzed by ANOVA as above. Nonlinear regression software (TableCurve 2D) was again used to find a decay function that fit the dose–response data well and the chosen curve plotted on a log scale according to previous methods (equation 2 in Byers 2013).

Earlier we showed that the attraction of PSHB to 1× quercivorol could be decreased to only 22 or 15% by adding 1× (*S*)-verbenone or 1× (*R*)-verbenone, respectively (Byers et al. 2018). A new source of verbenone (Sigma–Aldrich) was analyzed chemically by GC-MS (Levi-Zada et al. 2018) and found to be >95% pure and a mixture of 74% (*S*)- and 26% (*R*)-enantiomers. This verbenone was used in a similar dose–response tests as that done above for piperitone. The verbenone also was released at 0, 0.01×, 0.1×, 1×, and 10× doses each together with a 1x dose of quercivorol within an inverted plastic cup covered with aluminum foil inside a sticky trap. The doses of verbenone were prepared similarly by the diffusion–dilution method. Thus, 102.25-µl verbenone (MW 150.21, density 0.978 g/ml) was mixed with 1.128-ml decanol to give the 0.1 mole proportion. Dilution was used for the 0.01 dose and one or ten 2-ml vials for 1 or 10× doses as described above for piperitone. The 2-ml vial with 60 µl of verbenone (1×) released verbenone at 0.8 mg/d (Byers et al. 2018). The sticky traps and foil cups described above were used with the different doses each replicated two times for a total of 10 traps. The traps were placed in a line with 15 m separating each trap (3 October to 24 November 2019) in the same Hass avocado orchard near Nahsholim, Israel. PSHB were collected from sticky traps every 1–2 wk, replicate positions re-randomized by position, and catch transformed per week as above for a total of eight replicates per treatment. Catches on verbenone traps were analyzed graphically and statistically as described above for piperitone.

Catch on Quercivorol Trap as a Function of Distance from Verbenone Inhibitor

We tested the 1× dose of verbenone (same as above) together with 1× quercivorol in the inverted cups inside standard sticky traps (0 m distance of separation), or at separation distances between verbenone and quercivorol of either 0.25, 0.5, 1, or 2 m. In these latter four cases, the 1× quercivorol was alone in a cup at 1.2-m height inside a sticky trap, whereas verbenone was inside a cup on a pole at the same height but without a trap at each of the separation distances. The five distances were replicated three times at random in two lines with separation of 15 m between treatments and 20 m between lines. PSHB were collected from sticky traps every 1–2 wk, randomized as above, for a total of 12 replicates per treatment (19 August to 3 October 2019) and analyzed statistically as above.

Effect of Ethanol Release Rate

In previous tests, as ethanol was increasingly released over a 64-fold range, there was a small but significant inhibitory effect on attraction of PSHB to quercivorol (Byers et al. 2018). We wanted to test whether ethanol alone has an effect on attraction of PSHB using the same 64-fold dose range of ethanol using 10-ml vials filled initially half full with 95% ethanol. The vials had caps with drilled holes of either 0.5, 1, 2, or 4 mm giving release rates proportional to each hole's area or 7.5, 30, 120, and 480 mg/d, respectively.

Results

EAR and EARc of Sticky Trap Releasing Attractant Quercivorol

The mean catch per trap per week was converted to EAR (Y) and plotted as a function of quercivorol dose (X) (Fig. 1). The EAR increased with increasing dose (X) and fit perfectly a second-order hyperbolic kinetic function with three terms ($R^2 = 1$; Fig. 1). The quercivorol dose of 1 released 0.126 mg/d (Byers et al. 2018) and thus converting dosage to release rate (X) gave the same type curve and R^2 with identical coefficients $a = 0.20577$ and $b = 1.96894$ (Fig. 1) but different $c = 0.12615$. The relationship of EARc, obtained by converting the EAR using SD of vertical flight distribution, with quercivorol dose was fit well by a second-order hyperbolic equation with only two terms ($R^2 = 1.0$, Fig. 1). When release rate is substituted for dose, then the same curve is obtained with identical R^2 and coefficient $a = 3.44387$ but different $b = 0.30459$ (compare with Fig. 1). All these curves of EAR or EARc have a sigmoid shape when dose or release rate is plotted on the *x*-axis with a logarithmic scale (Fig. 1). The equations above can be used to calculate EAR or EARc at any release rate, for example, a release rate of 2 mg/d of quercivorol ($X = 2$) in the sticky trap is expected to give an EAR = 0.20577 + 1.96894 × 2/(0.12615 + 2) = 2.06 m, and an EARc = 3.44387 × 2/(0.30459 + 2) = 2.99 m.

Catch on Quercivorol Trap as a Function of Number of Nearby Piperitone Dispensers

The mean catch of PSHB per trap per week on the control quercivorol bait without a nearby piperitone repellent was highest, whereas the presence of one repellent 0.75 m away caused a decline in mean catch—but this was not significant statistically (Fig. 2). However, two or three piperitone repellents did significantly decrease the mean catch (Fig. 2). A first-order exponential decay function fit the data ($R^2 = 0.847$, adjusted $R^2 = 0.54$), but several other decay curves (not shown) gave similar curves and also fit the data.

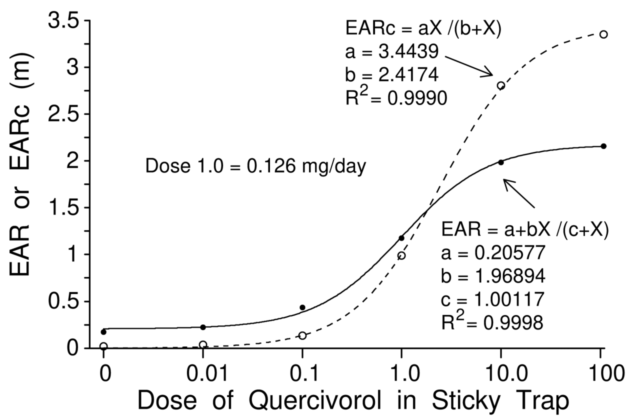

Fig. 1. Relationships of spherical EAR and circular EARc of PSHB with 10-fold increasing doses of quercivorol released from sticky traps (conversion from catch data in Byers et al. 2018, Figure 3, and plotted on logarithmic scale).

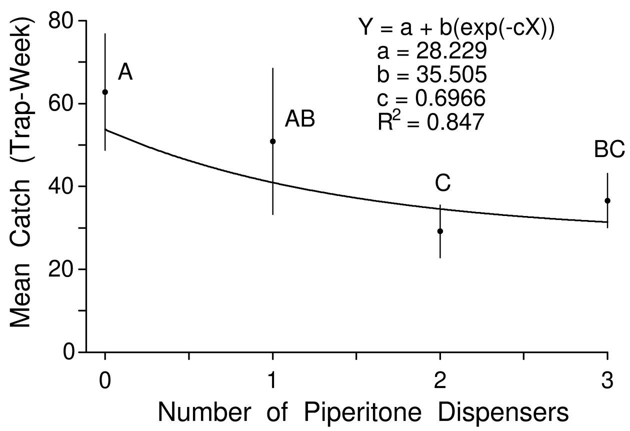

Fig. 2. Reduction in attraction of PSHB to quercivorol in relation to the number of piperitone repellent dispensers at 0.75-m distance (14 August to 5 November 2018, Nahsholim, Israel). Error bars are ±95% CL of means ($n = 15$). Means with the same letters were not significantly different ($\alpha = 0.05$, ANOVA and Tukey's HSD).

Catch on Quercivorol Trap as a Function of Release Rate of Piperitone or Verbenone Inhibitors

The mean catch per trap per week on the control quercivorol bait was highest and appears to decline with even a small amount of piperitone (at 0.01 dose, but not significant). Increasing the dose of piperitone to 0.1× released with quercivorol caused the attraction to decline significantly (Fig. 3). The release of 1 and 10× piperitone caused the lowest mean catches although these doses do not appear to be different in repellency (Fig. 3). It appears that 1× piperitone is sufficient to repel PSHB if placed at the source of attraction. The reduction in attraction to 1× quercivorol by 1× piperitone was 79% compared with quercivorol alone. A second-order decay function fit the data well ($R^2 = 0.997$, adjusted $R^2 = 0.987$). The dose–response curve can be expressed in terms of piperitone release rate (0.52 mg/d), which gives the same curve and coefficients (Fig. 3), but $c = 2.1399$.

The dose–response curve of mean catch per trap per week as a function of verbenone dose (Fig. 4) has a sigmoidal decline when plotted on a log scale. The reduction by 1× verbenone was 80%, similar to that of 1× piperitone above. A second-order decay function fit the data well ($R^2 = 0.990$, adjusted $R^2 = 0.959$). The dose–response curve expressed in verbenone release rate (0.8 mg/d) gives the same curve and coefficients (Fig. 4), but $c = 0.0438$.

Catch on Quercivorol Trap as a Function of Distance from Verbenone Inhibitor

Verbenone appears effective in inhibiting PSHB attraction to quercivorol beginning within about a meter distance but the effect is not significant until within 0.25–0.5 m (Fig. 5). This close-range effect is similar to previous results with a combination of verbenone and piperitone where the relationship of mean catch (Y) as a function of inhibitor distance (X) was given by the same exponential equation (Figure 1 in Byers et al. 2018). This equation means that as the distance X between the inhibitor and attractant source is increased, the mean catch Y more slowly increases at a rate proportional to X. This equation was solved for distance (equation 1 in Byers et al. 2018): $X = -\ln((Y - a)/b)$ in which the coefficient a represents the maximum mean catch so that 50% of maximum catch occurs at a distance $X = 0.6$ m. The same procedure can be done here using the equation (Fig. 5) at $0.5a = 132.22$ giving $X = 0.47$ m. These results reinforce our conclusion that any inhibitors such as

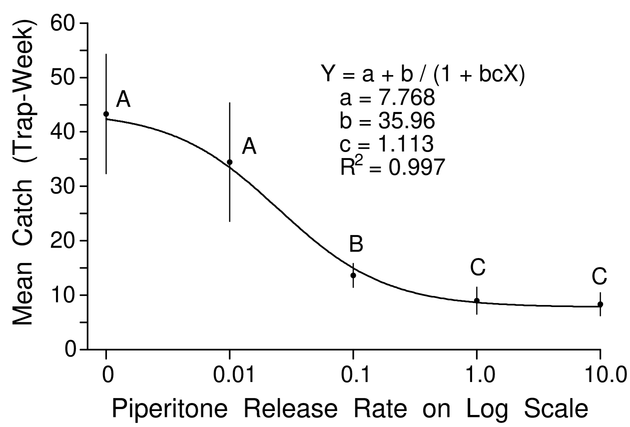

Fig. 3. Reduction in attraction of PSHB to 1× quercivorol in relation to 10-fold increasing release rates of repellent piperitone on a logarithmic scale (16 September to 21 November 2018, Nahsholim, Israel). Error bars are ±95% CL of means ($n = 21$). Means with the same letters were not significantly different ($\alpha = 0.05$, ANOVA and Tukey's HSD).

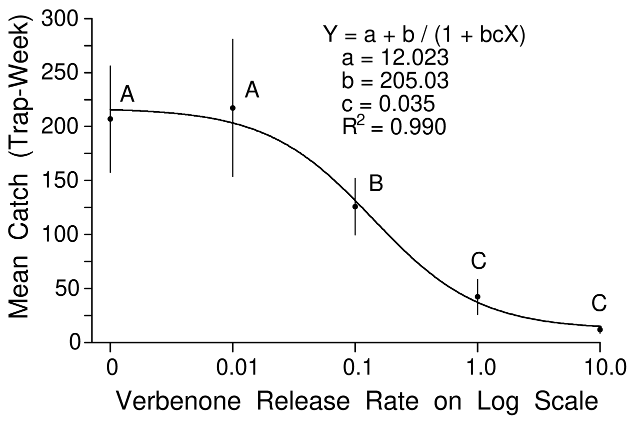

Fig. 4. Reduction in attraction of PSHB to 1× quercivorol in relation to 10-fold increasing release rates of repellent verbenone on a logarithmic scale (3 October to 24 November 2019, Nahsholim, Israel). Error bars are ±95% CL of means (n = 8). Means with the same letters were not significantly different (α = 0.05, ANOVA and Tukey's HSD).

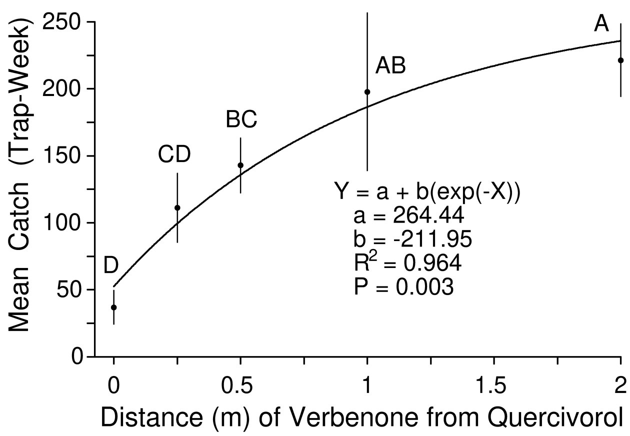

Fig. 5. Effect of increasing distance of inhibitor verbenone on attraction of PSHB to 1× attractant quercivorol in sticky traps (19 August to 3 October, Nahsholim, Israel). Error bars are 95% CL of means (n = 12). Means with the same letters were not significantly different (α = 0.05, ANOVA and Tukey's HSD).

verbenone or piperitone must be placed on the trunks of avocado in order to repel PSHB from landing in infestations.

Effect of Ethanol Release Rate

The relationship between ethanol release rate and attraction of a bostrichid beetle *Scobicia chevrieri* (Villa and Villa) (Buchelos 1991, Kollár 2014, Liu and Beaver 2017) shows a strong increase in attraction as the release rate of ethanol is increased (Fig. 6). For comparison, PSHB mean catch remains nearly constant regardless of release rate of ethanol (the R^2-value of 0.22 means that only 22% of the variation of catch is explained by the ethanol release rate). Such a low value for R^2 indicates that there likely is no relationship and no effect of ethanol on catch. Furthermore, mean catches at release rates from 7.5 to 480 mg/d of ethanol were not statistically different from each other or from that of a blank trap (Fig. 6).

Discussion

The dose–response (catch) curve reported in Byers et al. (2018) that fit a second-order hyperbolic kinetic function with two terms was

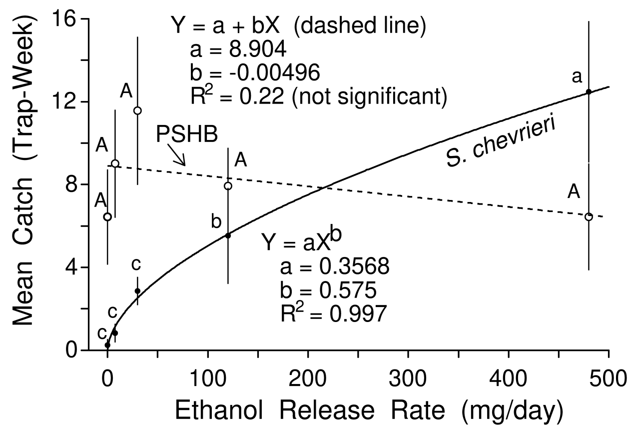

Fig. 6. Effect of increasing release rate of ethanol on attraction of PSHB compared with attraction of bostrichid beetle *Scobicia chevrieri* (19 August to 3 October, Nahsholim, Israel). Error bars are 95% CL of means (n = 8). Means with the same letters within a curve were not significantly different (α = 0.05, ANOVA and Tukey's HSD).

converted to dose–response (EAR or EARc) curves (Fig. 1). The dose–EAR or dose–EARc curves are more relevant to design of monitoring, mass-trapping, and push–pull methods in pest management since EAR and EARc determine the strength of the baited-trap that can be compared directly with those for other insect species. The EAR and EARc comparisons are based on a ratio of catches and thus theoretically do not depend on flight population density or length of trapping periods. The dose–EARc curve fit the same type of function as that for dose–catch, but the dose–EAR curve fit better the same function with three terms. Even more useful curves are obtained by replacing dose with release rate, in which case the curves are the same for EAR and EARc but the last coefficient (b for EARc and c for EAR, see Results) are different. With these latter curves one can use the measured release rate of quercivorol to predict the EAR and EARc of the trap with this lure. Unfortunately, very few studies have expressed their baited-traps for a particular insect pest in terms of a dose (release rate)–response (EAR or EARc) curve that predicts the trapping power of the baited-trap. Without this information the effectiveness of a particular baited-trap (consisting of chemicals, release rates, and specific trap) for capturing PSHB, or any pest insect, is uncertain. In addition, the release rate–EAR or release rate–EARc curves of PSHB can be compared with other insect species and trap-lures using these methods.

From our previous results (Byers et al. 2018) we found that attraction of PSHB was reduced to about 50% that of a 1× quercivorol baited-trap when 1× inhibitors of piperitone and verbenone were placed about 0.6 m away according to a curve of separation distance versus catch. Our simulation of 'push–pull' with up to three inhibitors per tree suggested that more dispensers would increase the PSHB avoidance of treated avocado trees (Byers et al. 2018). Thus, we tested from 0 to three inhibitors at 0.75 m distance from a 1× quercivorol baited-trap and found a gradual reduction in catch with increasing numbers of inhibitors (Fig. 2). The use of even more piperitone dispensers surrounding the 1× quercivorol attractant would likely further decrease catch but probably in diminishing amounts. The curve (Fig. 2) predicts that three piperitone dispensers would give a mean catch per week of 32 under the experimental trapping conditions, but even with eight such dispensers the mean catch is predicted to decline to 22/trap/wk. This indicates that multiple repellent dispensers at a distance of 0.75 m have diminishing efficiency in the 'push' of PSHB away from an attractive source. It would seem more effective to place the repellent on branches right next to where any attraction might begin.

Development of a push-repellent system for protecting avocado from PSHB attack would depend on the release rate (dose) of the inhibitors piperitone and verbenone. We wanted to release each inhibitor alone as we expected that it would be less expensive to use one inhibitor on a large scale. The piperitone dose–response curve (Fig. 3) is similar to the dose–response curve for verbenone (Fig. 4) but the effects of verbenone may inhibit attraction of PSHB at slightly higher doses, and the steepest decline occurs around 0.05 dose for piperitone, while the steepest decline occurs around 0.1 dose for verbenone. However, these minor differences may be due to variation in trap positions and dates in the field. Earlier we found that 1× dose piperitone [75% (*R*)-enantiomer] released at 0.52 mg/d, as well as (*S*)-verbenone and (*R*)-verbenone each released at 0.8 mg/d are among the most potent of inhibitors tested on any species of scolytine beetle, reducing response of PSHB to quercivorol by 90, 78, and 85%, respectively (Byers et al. 2018). In the present study, the same source of piperitone released at 1 and 10× rates (0.52 and 5.2 mg/d) reduced response of PSHB to quercivorol by 79 and 81%, respectively. The reduction at 1x piperitone dose in the present tests was slightly less than in tests the previous year, but similar to the repellency rates of verbenone above (Byers et al. 2018).

A reduction of 92% in attraction of the California five spined ips *Ips paraconfusus* Lanier to a 50-male infested log was shown for (*S*)-verbenone released at 4 mg/d (Byers and Wood 1980). Bedard et al. (1980a) also released (*S*)-verbenone at 4 mg/d and this reduced response of western pine beetle *Dendroctonus brevicomis* LeConte by 56% to its synthetic pheromone components. Males of this bark beetle release verbenone when joining a female in her gallery which causes other females and males to avoid landing and boring there and would reduce competition for the phloem food layer where they breed (Byers et al. 1984). The response of a European pine shoot beetle *Tomicus piniperda* L. to host monoterpenes was reduced by 75 or 80% by 0.25 mg/d of (*R*)-verbenone or (*S*)-verbenone, respectively (Byers et al. 1989). They also found that verbenone was increasingly released from infested logs as they aged and suggested this was probably due to microorganisms because the beetles contained little verbenone. The repellency of piperitone and verbenone to PSHB that we observed can be due naturally to microorganisms in decaying tissues, or may represent or mimic nonhost volatiles to avoid.

Earlier both piperitone and verbenone were placed together at increasing distances from an attractive quercivorol-baited trap whose catch increased as the distance was increased (Byers et al. 2018). We wanted to test only verbenone similarly to gain information for the development of verbenone treatments (push) on avocado limbs. Solving for the distance at 50% attraction rates for verbenone gave results of 0.47 m which is similar to 0.6 m for piperitone (Byers et al. 2018). The equation (Fig. 5) predicts a mean catch of 52.5 when verbenone and quercivorol are not separated (19.8%) compared with a maximum catch of 264.4 without verbenone (100%). The test of separation distance between quercivorol and verbenone clearly shows that verbenone would need to be very close to a PSHB aggregation on a limb/trunk of avocado in order to inhibit females from joining the aggregation over the flight season.

Carrillo et al. (2015) caught about three times more TSHB on ethanol (~400 mg/d, Synergy Semiochemicals) baited traps than on blank traps in Florida. They also showed that ethanol released with quercivorol attracted the most TSHB with a mean catch significantly more than for blank traps. However, quercivorol was not presented alone. In a following study, Kendra et al. (2017) showed that quercivorol plus α-copaene was significantly more attractive to TSHB than either compound alone and that either alone were more attractive than blank controls. We tested a 64-fold range of ethanol release (7.5–480 mg/d) with 1× quercivorol on PSHB in Israel and found that all ethanol doses reduced mean catch compared with blank controls, but we did not test ethanol alone (Byers et al. 2018). Thus, in the present study, we tested ethanol over the same dosage range on PSHB and did not find mean catches for any release rate to be significantly different from controls (Fig. 6). However, we did attract the bostrichid beetle *Scobicia chevrieri* in increasing numbers in relation to increasing release rate of ethanol (Fig. 6). This powder-post beetle is common throughout the Mediterranean region and infests wood of dead and decaying branches of angiosperm dicots (Buchelos 1991). The difference in behavior between the two species with regard to ethanol suggests that PSHB does not use ethanol to find suitable host tissues but rather to avoid degraded host tissues releasing ethanol.

In Israel, avocado trees are usually planted in rows with spacing of 3–4 m between trees in a row and 5–6 m between rows. Mature trees range in height from 4 to 6 m. Avocado trees are not treated with systemic insecticides in Israel, because of the possibility of absorption of chemicals into the fruits. Topical treatments of avocado wood with six insecticides only prevented attack by an ambrosia beetle *Xyleborus glabratus* Eichoff for about 2 wk, and once inside the limb the beetles are protected from pesticide applications (Carrillo et al. 2013). Therefore, use of attractant quercivorol traps to monitor PSHB populations is important in pest management to determine whether sanitation pruning of infested limbs is needed. Mass trapping is another management method that uses a relatively high density of monitoring traps in an area to lower mating frequency of the pest insect (Byers 2009, 2012b; Levi-Zada et al. 2018). Push-pull (Cook et al. 2007; Byers et al. 2018) combines semiochemical inhibitors or repellent plants next to the plant to be protected along with mass trapping or trap crop. In all these methods, the relative strength of the attractive trap should have its EAR (effective attraction radius) measured in order to understand whether the known attractive chemicals and release rates are effective in relation to the economic costs. The mean flight height of a pest insect needs to be determined to place traps at the optimal height, and the standard deviation of the mean height is used to convert EAR to EARc (circular effective attraction radius) for use in simulation models of mass trapping (Byers et al. 2017) and push–pull (Byers et al. 2018). The EAR for a 1× release of quercivorol is similar in strength to that of a PSHB aggregation on an avocado limb (Byers et al. 2017).

We have demonstrated that the inhibitors piperitone and verbenone are most effective when they are released very close to the attractive sources that are the aggregations found on the main trunks and limbs of avocado trees. The push–pull method should be initiated before PSHB fly in the spring in order to out-compete the newly forming aggregations that begin with just a female and are less attractive than quercivorol-baited traps. Finally, the push–pull method should be carried out for several consecutive years in the same areas to allow the treatments and natural mortality agents to have a chance to reduce populations of PSHB to low levels. Semiochemicals used in push–pull are released as volatiles in low quantities that are not expected to affect most insect natural enemies other than perhaps those of the target pest (Bedard et al. 1980b, Bakke 1981). Mass trapping and push–pull have an advantage in the case of PSHB because the egg-laying females are targeted as compared with moth systems, for example, where only male populations are lowered. Further work is warranted on push–pull methods targeting PSHB.

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