



Inhibitory Effects of Semiochemicals on the Attraction of an Ambrosia Beetle *Euwallacea nr. fornicatus* to Quercivorol

John A. Byers¹ · Yonatan Maoz² · David Wakarchuk³ · Daniela Fefer⁴ · Anat Levi–Zada⁴

Received: 8 February 2018 / Revised: 2 April 2018 / Accepted: 9 April 2018 / Published online: 17 April 2018
© Springer Science+Business Media, LLC, part of Springer Nature 2018

Abstract

The *Euwallacea fornicatus* (Eichhoff) species complex includes the polyphagous shot hole borer (PSHB), an ambrosia beetle infesting avocado limbs, *Persea americana* Mill. Synthetic quercivorol, a monoterpene alcohol, is known to attract females (males are flightless) over a range of release rates spanning three orders of magnitude. The upper release dose was extended 10-fold using sticky traps baited with quercivorol released at 1× (0.126 mg/day), 10×, and 108× relative rates to obtain a dose–response curve fitting a kinetic formation function. Naturally infested limbs of living avocado trees were wrapped with netting to exclude the possibility of catching emerging beetles on the encircling sticky traps. The results indicate PSHB are significantly attracted to infested limbs. Ethanol released over a 64-fold range (lowest rate of 7.5 mg/day) was moderately inhibitory of PSHB attraction to 1× quercivorol. β-caryophyllene and eucalyptol did not appear to affect attraction at the rates tested. A field test of potential inhibitors of 1× quercivorol was done using ~1 mg/day releases of monoterpene ketones: (–)-(S)-verbenone, (+)-(R)-verbenone, 3-methyl-2-cyclo-hexen-1-one (MCH or seudenone), piperitone, (+)-(S)-carvone, and racemic cryptone. Only piperitone and the two enantiomers of verbenone were strongly inhibitory. A blend of piperitone and verbenone tested together at different distances (0, 0.5, 1, 2, and 4 m) from a 1× quercivorol baited sticky trap became increasingly ineffective in inhibiting the attractant as separation distance increased. Due to the relatively short-range repellency (<1 m), the inhibitors would need to be released from several places on each tree to effectively repel PSHB from avocado trees. Effective attraction radii, EAR, and circular EAR_c are estimated for the quercivorol baits released at 1×, 10× and 108× rates. Push-pull simulations of moving beetles were performed in 1 ha plots with 2, 4, or 16 traps of 10× EAR_c and 400 trees (0, 1, or 3 inhibitors per tree) of which ten had an infested limb (EAR_c = 0.5 m). The simulations indicate that push-pull methods would be more effective in reducing PSHB mating than simply using mass-trapping alone.

Keywords Push-pull · Repellent · Attractant · Effective attraction radius · Semiochemicals · Mass trapping

Introduction

The ambrosia beetle, *Euwallacea fornicatus* (Eichhoff) (Curculionidae: Scolytinae) comprises a species complex of at least three cryptic species that includes the polyphagous

shot hole borer (PSHB) which originated in Southeast Asia. PSHB invaded California about 2003, and shortly thereafter Israel (Eskalen et al. 2012; Freeman et al. 2012; Mendel et al. 2012). Another important species of the *E. fornicatus* complex is the tea shot hole borer (TSHB), originally from Sri Lanka, which has become a pest in Florida (Carrillo et al. 2015, 2016; Cooperband et al. 2016). These two species and a third, Kuroshio shot hole borer (KSHB), are morphologically indistinguishable but differ in host preferences, fungal symbionts, and DNA geographically (Eskalen et al. 2013; Freeman et al. 2012; O'Donnell et al. 2015; Stouthamer et al. 2017). PSHB has a relatively broad host range of woody shrubs and trees and may carry *Fusarium* dieback disease (Cooperband et al. 2016; Eskalen et al. 2013; Freeman et al. 2012; Lynch et al. 2016). In Israel, PSHB is a pest of avocado because beetle infested limbs die reducing tree growth over time (Freeman et al. 2012; Mendel et al. 2012). Like other ambrosia beetles,

✉ John A. Byers
John.a.byers@gmail.com

¹ Department of Entomology, Robert H. Smith Faculty of Agriculture, Food and Environment, The Hebrew University of Jerusalem, Rehovot, Israel

² The Israel Fruit Growers Association, Yahud, Israel

³ Synergy Semiochemicals, Burnaby, British Columbia, Canada

⁴ Institute of Plant Protection, Agricultural Research Organization, Volcani Center, Rishon LeZion, Israel

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 complex leave the brood tree after mating since the males are flightless (Calnaido 1965; Carrillo et al. 2015). Our observations of PSHB and that of others suggest that females usually 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 (Byers et al. 2017; Eskalen et al. 2013).

Scolytid 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, 2004). 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. They synthesized racemic quercivorol that was predominantly the *cis*-isomer of which half was the (1*S*,4*R*) enantiomer. Quercivorol is commercially available (Synergy Semiochemicals, Burnaby, Canada) and contains racemic 85% *cis*-isomer that was shown to be attractive to TSHB in Florida (Carrillo et al. 2015; Kendra et al. 2017) and to PSHB in Israel (Byers et al. 2017). Quercivorol either acts as a kairomone released by the fungal infection in the host (Carrillo et al. 2015), elicits cross-attraction of PSHB in the ancestral range, is a pheromone mimic, or is a pheromone component of PSHB.

It is clear that synthetic quercivorol is highly attractive to PSHB females in the field as shown by a standardized comparative method termed the effective attraction radius (EAR) developed earlier (Byers 2012a, b; Byers et al. 2017). Quercivorol released at a 1× rate (0.126 mg/day) had an EAR of 1.02, 1.18, and 1.28 m in three tests while the 10× rate (1.26 mg/day) had an EAR of 2.0 m (Byers et al. 2017). The mean flight height of PSHB was calculated as 1.24 m with a standard deviation (*SD*) of the vertical flight distribution of 0.88 m (Byers et al. 2017). Using 0.88 m *SD* and 1.18 m EAR (1× rate), then the EAR_c (circular effective attraction radius in two dimensions for simulation models) was calculated to be 0.99 m (the 10× rate gave an EAR_c of 2.86 m). Simulations (Byers et al. 2017) suggested that mass trapping would be effective in reducing infestations of PSHB if the trapping was begun when the beetle begins to fly in June. A dose-response curve for quercivorol released at 10-fold increasing rates (0, 0.01×, 0.1×, 1×, and 10×) in the field fit a kinetic formation function (Byers 2013; Byers et al. 2017).

Many conifer-infesting bark beetle species are inhibited by verbenone, a ketone and oxidation product of the common monoterpene α -pinene that may indicate a degrading and unsuitable host tree (Byers et al. 1989; Byers 1989, 2004). Hughes et al. (2017) found verbenone (unreported release rate and enantiomer mix) to inhibit the attraction of redbay ambrosia beetles, *Xyleborus glabratus* Eichhoff, to manuka tree oil.

Since *E. fornicatus* was formerly designated in the genus *Xyleborus* (Calnaido 1965), we hypothesized that verbenone and other monoterpene ketones might be inhibitory to PSHB. Some monoterpene ketones, likely oxidation products of tree monoterpenes, have structures similar to quercivorol such as piperitone and cryptone. Another candidate inhibitor was MCH (3-methyl-2-cyclohexen-1-one) that inhibits Douglas-fir bark beetles (Furniss et al. 1972, 1976; Rudinsky et al. 1975). Inhibitors are revealed by a reduction in catch when the attractant is released together with the inhibitor as compared with the attractant alone (Byers and Wood 1980). Eucalyptol is found in hosts of *X. glabratus* (Hanula and Sullivan 2008) and is attractive to this ambrosia beetle species (Kuhns et al. 2014), but the behavioral activity of eucalyptol is not known regarding PSHB. Several sesquiterpenes including β -caryophyllene, α -copaene, and α -cubebene from host tree species of *X. glabratus* are possible kairomone candidates for PSHB (Hanula and Sullivan 2008; Kendra et al. 2011, 2012, 2014). Recently, TSHB in Florida was shown similarly attracted to lures releasing either α -copaene or quercivorol, and when both were released together the attraction was approximately doubled (Kendra et al. 2017).

Ethanol is commonly reported to be attractive to ambrosia beetles and some bark beetles (Byers 1989, 1992, 2004). Ethanol together with quercivorol appears weakly attractive to TSHB in Florida, and while ethanol alone caught more than controls this was not statistically significant (Carrillo et al. 2015). We hypothesized that ethanol may enhance the attraction of PSHB to quercivorol. Attractive response to host monoterpenes of some bark beetles depends on release rate of ethanol (Klimetzek et al. 1986; Schroeder and Lindelöw 1989; Byers 1992) and also for attraction of some ambrosia beetles to their aggregation pheromone (Byers 1989, 2004).

We reported (Byers et al. 2017) that artificially infesting cut avocado logs with PSHB females did not create an attractive source in the field. However, avocado trees with limbs naturally infested by PSHB when surrounded by sticky traps caught significantly higher numbers compared to control limbs on nearby avocado trees. This suggests that infested limbs connected to a living tree emit an aggregation signal that is more attractive. However, the infested limbs were not wrapped with fine screen to prevent the possibility of emerging brood beetles being caught on the sticky traps thereby possibly creating a false impression of attraction. Thus, our first objective was to confirm that naturally-infested limbs of avocado are attractive to PSHB as suggested from catches on sticky traps surrounding the limbs (Byers et al. 2017). This test was improved by use of fine screen to cover the infested limbs and prevent any emerging beetles from being caught as they flew outward toward the surrounding sticky mesh trap. A second objective was to expand the dose-response curve of quercivorol ten-fold to include a

108× high-release rate. A third objective was to test various volatiles such as verbenone and piperitone as potential inhibitors of PSHB response to quercivorol, which could be used later in a push-pull management strategy (Cook et al. 2007; Miller and Cowles 1990; Pyke et al. 1987). We also wanted to test computer simulations of a push-pull semiochemical method to see if these would be more effective than mass trapping alone. Assuming potent inhibitors could be found, we wanted to investigate the effect of placing inhibitors at increasing distances from the quercivorol attractant source, again to gain knowledge to better simulate a push-pull strategy as well as implement such a method in the field. We wanted to test the effect of ethanol over a 64-fold range of release rates in combination with the attractant to see if this combination would be more attractive than quercivorol alone. A few other volatiles were tested to see if they increased or decreased the response of PSHB to quercivorol.

Methods and Materials

Attraction of PSHB to Infested Limbs of Avocado Earlier work suggested that sticky screens surrounding PSHB-infested avocado limbs caught higher numbers of beetles (Byers et al. 2017), but this result could have been influenced by reemerging and emerging brood adults. Thus we located avocado trees with an infested limb ($N=8$) or without infested limbs ($N=8$) in an orchard at Beit HaEmek, Israel (32°58'20.2"N 35°08'41.4"E). The limbs of each type were wrapped with nylon screen that excluded beetles and then a sticky cylinder trap (25 cm long × 25.5 cm diam. of 6 mm mesh wire screen) was aligned to surround each limb. The sticky screens were covered with adhesive (80% polyisobutene, Rimifoot, Rimi, Petah Tikva, Israel) and exposed from 12 Sept. to 20 Nov. 2017. The sticky traps were collected and PSHB counted in the laboratory and compared with a *Mann–Whitney U test* (R Foundation for Statistical Computing version 3.1.2).

Quercivorol Dose-Response Curve of PSHB Attraction The previous dose-response curve for PSHB attraction to quercivorol used doses of 0.01× and 0.1× made by mole-percentage dilution with decanol (Byers 1988) as well as neat 1× and 10× rates (Byers et al. 2017). We used the previous data for the two lowest rates with new results from 1×, 10×, and 108× rates obtained during the second half of the flight season in the same Hass variety avocado orchard (2.5 km east of Nahsholim, Israel, 32°36',31" N; 34°56',49" E) where all subsequent experiments were done. The 1× 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, USA) that gives an almost constant release rate at a specified temperature (Byers 1988). The release at 25 °C for the 1× dispenser was 0.126 mg/day according to previous weight loss measurements (Byers et al. 2017). Either one (1×) or 10 dispenser tubes (10×) were Scotch taped upright inside an inverted aluminum foil-covered plastic cup (8 cm diameter × 10 cm) to protect from sun and rain. For the 108× high dose we placed four bubble cap dispensers (#3250 Synergy Semiochemicals) inside the plastic cup. Each bubble cap dispenser released 3.4 mg/day at 25 °C as measured by linear regression of the weight losses ($N=3$) determined by a 0.1 mg precision microbalance over 5 weeks in the laboratory. The tubes or bubble caps were then surrounded by a sticky screen trap (cylinder, 28 cm diameter × 33 cm high). Two replicates of the three higher release rates were placed in the avocado orchard (20 m between traps) and beetles collected each week for 8 weeks (27 Sept. – 26 Nov.). Traps were randomized among positions each week. Non-linear regression software (TableCurve 2D version 5.01, Systat Software Inc., Chicago, USA) was used to find a function that fit the catch data best (Byers 2013). The three-dimensional EAR was calculated from catches at the three higher doses according to $EAR = [Ca \times S / (\pi \times Cb)]^{0.5}$, 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 m²) of the cylinder trap. A two-dimensional EAR_c for simulation models was calculated from the respective EAR using the standard deviation (SD) of the vertical flight distribution of PSHB (0.88 m) according to $EAR_c = \pi \times EAR^2 / [2 \times SD \times (2 \times \pi)^{0.5}]$ (Byers 2011; Byers 2012a, b; Byers et al. 2017).

Inhibitory Effects of Various Volatiles on Attraction of PSHB to Quercivorol In the first experiment, the candidate inhibitors, MCH (3-methyl-2-cyclohexen-1-one, also known as seudenone, 99% chemical purity, Acros Organics), (±)-cryptone (4-isopropyl-2-cyclohexen-1-one, 91% pure, Synergy Semiochemicals), (–)-(*S*)-verbenone (99% pure, 85% ee, enantiomeric excess, Acros Organics), and piperitone (95% pure, 50% ee (*R*)-enantiomer, Synergy Semiochemicals) were each released together with 1× quercivorol and compared to 1× quercivorol alone. Chemical purity of compounds was analyzed by GC-MS (Levi-Zada et al. 2017). Sixty μl of each volatile was placed in the bottom of a 2-ml glass vial (5.2 mm top opening, 32 mm long; Chrom4 GmbH, Thüringen, Germany) and Scotch taped on the inside of a foil-covered plastic cup next to a 1× tube of quercivorol as described above. The treatments were placed in the center of cylinder sticky traps (25 cm long × 25.5 cm diam., described above) at 1.2 m height in the avocado orchard near Nahsholim, Israel (10 July – 7 August 2017). Three replicates of each treatment were set up randomly for a total of 15 traps each separated 10 m apart in two parallel lines (20 m between

lines). Traps were picked of PSHB and randomized by position every week for 4 weeks. Trap catches of PSHB were square root transformed and analyzed by ANOVA with significant differences between pairs of treatments indicated by *Tukey's HSD* at $\alpha = 0.05$ (JMP 4.0.4, SAS Institute Inc., USA). Release rates of compounds ($N = 3$ each volatile) were determined by linear regression of the weight losses as above at 25 °C. The rates were 2.45, 1.22, 0.80, and 0.52 mg/day for MCH, cryptone, (*S*)-verbenone, and piperitone, respectively.

Effect of Spacing Inhibitors Away from Quercivorol on PSHB Attraction Because (*S*)-verbenone and piperitone each exhibited a strong inhibitory effect on PSHB attraction to quercivorol (Results), we tested the effects of increasing the distance between the attractant and these two inhibitors together. The distance of separation was 0 m [$1\times$ quercivorol dispenser together with 2-ml vials of (*S*)-verbenone and piperitone (as above) inside a cup in a 25.5 cm diameter sticky trap described above at 1.2 m height], or either 0.5, 1, 2, or 4 m apart. In these latter four cases, the $1\times$ quercivorol was alone in a cup and placed at 1.2 m height inside a sticky trap while the two inhibitors were placed inside a cup at the same height on a pole without a sticky trap. Trap pairs of the five distances were replicated twice at random in two lines with separation of 20 m between pairs and lines. The sticky traps were picked each week of beetles and randomized for 6 weeks (10 August – 27 September 2017). Non-linear regression using TableCurve 2D software was used to find a function that fit the relationship best.

Tests of Possible Synergists or Inhibitors on Attraction of PSHB to Quercivorol These tests were similar to the earlier tests for the candidate inhibitors and included the opposite enantiomer (+)-(*R*)-verbenone (97% pure, 78% ee, Synergy Semiochemicals) and (+)-(*S*)-carvone (98% pure, 93% ee, Acros Organics) as possible inhibitors. Eucalyptol (1,8-cineol, 99% Acros Organics) and (–)- β -caryophyllene (>98.5%, $[\alpha]_D^{20} = -10^\circ$, Fluka) were tested as quercivorol synergists. Sixty μ l of each volatile was placed in the bottom of a 2-ml glass vial (described above) and Scotch taped on the inside of a foil-covered plastic cup next to a $1\times$ tube of quercivorol as described above. However, four 2-ml glass vials were used for testing β -caryophyllene because of its low volatility and its release rate appeared negative possibly due to oxidation or water vapor absorption. The treatments were placed in the center of the 25 cm long \times 25.5 cm diam. Sticky traps at 1.2 m height in the avocado orchard near Nahsholim, Israel (27 Sept. – 26 Nov. 2017). Three replicates of each treatment were set up randomly for a total of 15 traps each separated 10 m apart in two parallel lines (20 m between lines). Trap catches of PSHB were analyzed statistically as in the first test for inhibitors. Release rates of the dispensers ($N = 3$ each

volatile) were determined by linear regression of the weight losses determined by the microbalance over 5 weeks in the laboratory at 25 °C. The rates were 0.80, 0.61, and 8.2 mg/day for (*R*)-verbenone, (*S*)-carvone, and eucalyptol, respectively.

Effect of Ethanol Release Rates on Attraction of PSHB to Quercivorol We employed a range of ethanol release rates spanning a 64-fold range together with a $1\times$ rate of quercivorol. This was done using 10 ml glass vials (16.8 mm diam. \times 61.3 mm long, Chrom4 GmbH) filled half full with ethanol and drilling their caps with different sized holes. The holes were 0.5, 1, 2, and 4 mm diameter. According to theory (Byers 1988), the release rate from a tube depends on the vapor pressure of the compound and is proportional to the area of the top opening and the distance the liquid surface is from the opening. For the 2-mm diameter hole, the rate initially would be proportional to this area of $\pi(2/2)^2 = 3.141 \text{ mm}^2$ that released 120 mg/day of ethanol at 25 °C as measured by weight loss. Thus, the 0.5, 1, 2, and 4 mm diameter openings had areas of 0.196, 0.785, 3.141, and 12.566 mm^2 , respectively, releasing 7.5, 30, 120, and 480 mg ethanol/day. Each release rate vial of ethanol was clipped into brackets inside the cups next to a $1\times$ quercivorol dispenser and filled with ethanol weekly if needed (three replicates of each rate). The inverted foil-covered cups were placed in the sticky traps (25 cm long \times 25.5 cm diam., described above) at 1.2 m height in the avocado orchard near Nahsholim, Israel (10 August – 27 September 2017). PSHB were collected from the sticky traps each week and then randomized by position for 6 weeks and analyzed statistically by ANOVA and *Tukey's HSD* as above.

Simulation of Push-Pull in Avocado Orchard Computer simulations programmed in Java 1.6 similar to that described earlier (Byers 2012b; Byers et al. 2017) were performed to estimate the effect of inhibitors in a push-pull system compared to mass trapping alone in an avocado orchard. Simulated avocado trees were spaced 4 m apart within a row, with parallel rows 6 m apart, and the radius of each tree was 2 m (dimensions suggested by growers in Israel). Thus, in a 1 hr area (100 m sides) 400 trees could be placed. The model used either 2, 4 or 16 traps each of 2.8 m *EARc* for the $10\times$ bait of quercivorol (from Results). One thousand initial beetles were simulated each moving in a correlated random walk with steps of 0.5 m in which their forward direction could vary at each step by an angular deviation randomly selected from a normal distribution with a standard deviation of 6° . Ten infestations were placed at random in the orchard in randomly selected avocado trees but within 1 m of the trunk to account for PSHB preference for larger branches. The model allows changing the *EARc* of PSHB infestations (a mature infestation may have an *EARc* = 0.98 m, Byers et al. 2017) but the *EARc* used in the simulations was assumed to be 0.5 m to coincide

with a smaller aggregation beginning earlier in the season. Beetles wandered up to 14,400 steps until usually encountering either a trap or an infestation. Either 0, 1, or 3 inhibitors of 0.6 m EARc (solving for X at $a/2$ in Fig. 3) were spaced uniformly along a radius of 1 m inside the tree in order to be close to any aggregations. When a beetle encountered the circle of an inhibitor they then ignored aggregations (inhibited from landing) for 3 steps (1.5 m of flight) without changing its random walk. The numbers of beetles attracted to aggregations on trees or caught in traps were counted and converted to a percentage of the initial population. The number of steps for all beetles to encounter a trap or aggregation also was recorded. The simulations at each of the nine parameter combinations (number traps x number inhibitors per tree) were repeated eight times.

Results

Attraction of PSHB to Infested Limbs of Avocado The average catch on traps surrounding infested limbs was 219.6 ± 40.5 ($\pm SE$) which was significantly higher than on traps around control limbs of 73.1 ± 17.9 ($U = 5$, $N1 = N2 = 8$, $P = 0.0027$ two-tailed), indicating that the naturally infested limbs were producing volatiles attractive to female PSHB. The results of 2016 with unwrapped limbs (Byers et al. 2017) are supported by the results here in which the infested and control limbs were wrapped with fine screen to preclude catch of any reemerging adults and brood.

Quercivorol Dose-Response Curve of PSHB Attraction The dose-response curve of mean catch per trap per week (Y) increased with increasing release rate (X) and fit perfectly a second-order hyperbolic kinetic function ($R^2 = 1$; Fig. 1). The data also fit as well a second-order kinetic formation function, $Y = a(1 - (1/(1 + abX)))$, where $a = 83.53$ and $b = 0.00495$. Both curves can be plotted on the x-axis with either a logarithmic scale (giving a sigmoidal shape) or on a linear scale (Fig. 1). To convert X as a relative rate to absolute rate of release, simply multiply X by 0.126 mg/day. The dose-response tests indicate that the $108\times$ dose catches the most, although $10\times$ (1.26 mg/day) is almost as potent as $108\times$ (13.6 mg/day) catching about 84% as much as the highest dose (Fig. 1). In 2016, the EAR for $1\times$ and $10\times$ were calculated as 1.02 and 2.00 m, respectively, with EARc of 0.73 and 2.86 m (Byers et al. 2017). In the present study in 2017, the EAR for $1\times$, $10\times$, and $108\times$ were 1.18, 1.98, and 2.17 m, respectively, giving EARc of 0.99, 2.80, and 3.35 m, respectively.

Inhibitory Effects of Various Volatiles on Attraction of PSHB to Quercivorol It is clear that the monoterpene ketones (S)-verbenone and piperitone are both inhibitors of the attraction

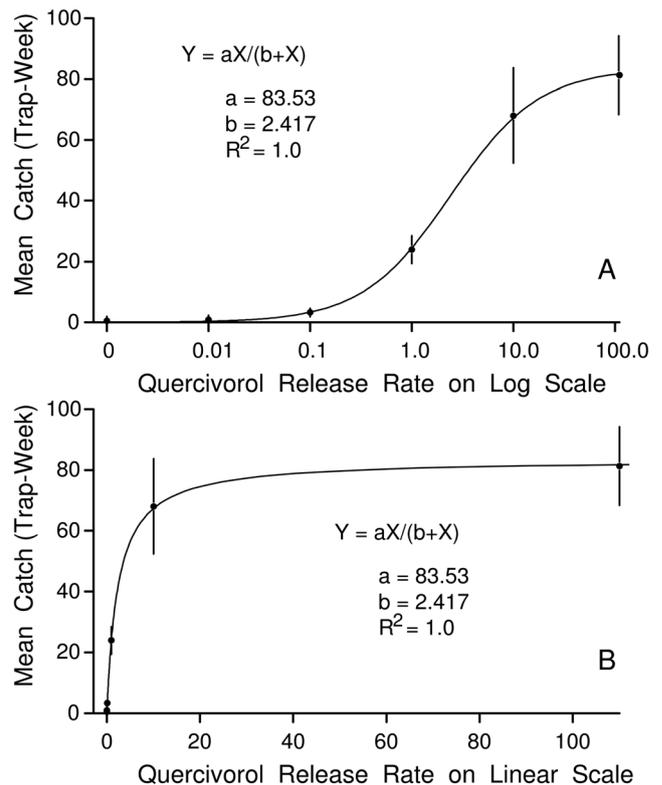


Fig. 1 Mean catch per trap-week of PSHB attracted to 10-fold increasing release rates of quercivorol on a logarithmic scale (a) or on a linear scale (b). Error bars are $\pm SE$ ($N = 16$)

of PSHB to quercivorol (Fig. 2). (S)-verbenone reduced the response to quercivorol by 78% while piperitone reduced response 90% compared to quercivorol alone. There was no significant reduction in PSHB response to quercivorol when either cryptone or MCH were tested (Fig. 2).

Effect of Spacing Inhibitors Away from Quercivorol on PSHB Attraction The reduction in response to quercivorol by the combination of the two inhibitors, piperitone and (S)-verbenone, identified above became gradually less as the distance between inhibitors and attractant was increased (Fig. 3). The relationship of distance and mean catch followed an exponential function of separation distance ($Y = a + b(\exp(-X))$). This means that as the distance is increased at X then the mean catch Y will more slowly increase at a rate proportional to X . The value $a = 21.159$ (Fig. 3) suggests the maximum mean catch at maximum separation, and taking half this value with $b = -19.247$ and solving for X gives:

$$X = -\ln \left[\frac{(Y-a)}{b} \right] = 0.6\text{m} \quad (1)$$

(the distance where half the mean catch is expected). This value was used as an approximation for the size of the inhibitory radius in the push-pull model presented subsequently.

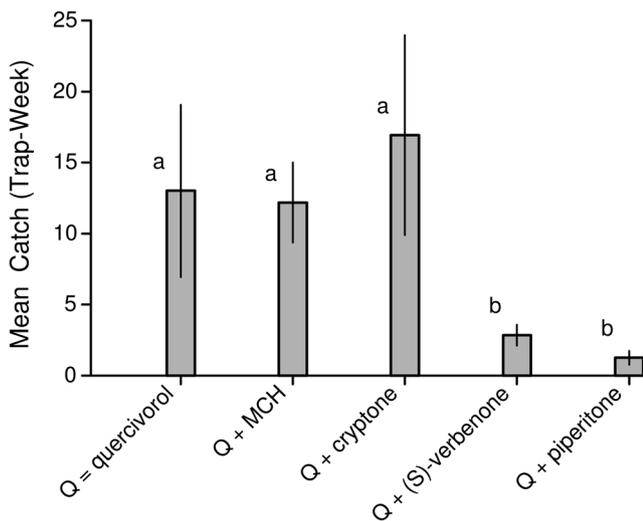


Fig. 2 Effect of potential inhibitors on attraction of PSHB to quercivorol at 1× release rate from sticky traps in an avocado orchard (10 July – 7 August 2017, Nahsholim, Israel). Vertical lines on bars represent 95% CI of means ($N=12$ each bar). Bars with the same letter were not significantly different ($\alpha=0.05$, Tukey's HSD)

Tests of Possible Synergists or Inhibitors on Attraction of PSHB to Quercivorol Eucalyptol and (*S*)-carvone did not enhance or reduce attraction of PSHB to quercivorol, but (*R*)-verbenone did significantly reduce the response by 85% (Fig. 4). Thus, both enantiomers of verbenone appear to reduce attraction of PSHB to quercivorol. The catch on traps baited with quercivorol plus β -caryophyllene did attract more than any other treatment containing quercivorol, but this was not significant except when compared to (*R*)-verbenone (Fig. 4).

Effect of Ethanol Release Rates on Attraction of PSHB to Quercivorol All rates of ethanol decreased the response to quercivorol, but only the 120 mg/day rate was significantly

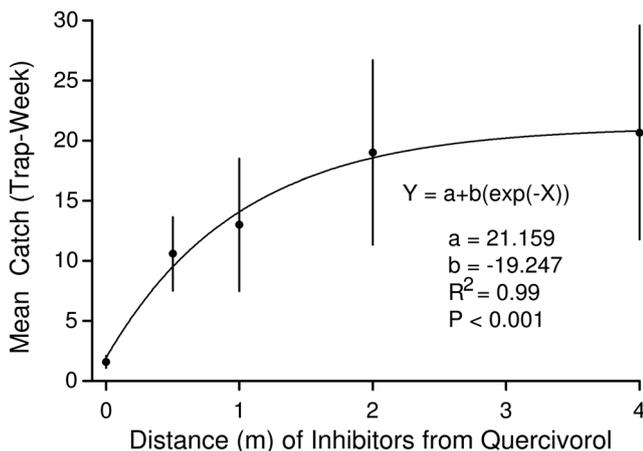


Fig. 3 Effect of increasing distance of combined inhibitors piperitone and (*S*)-verbenone on attraction of PSHB to 1× attractant quercivorol in sticky traps (10 August – 27 September 2017, Nahsholim, Israel). Vertical lines above and below points represent 95% CI of means ($N=12$ each bar)

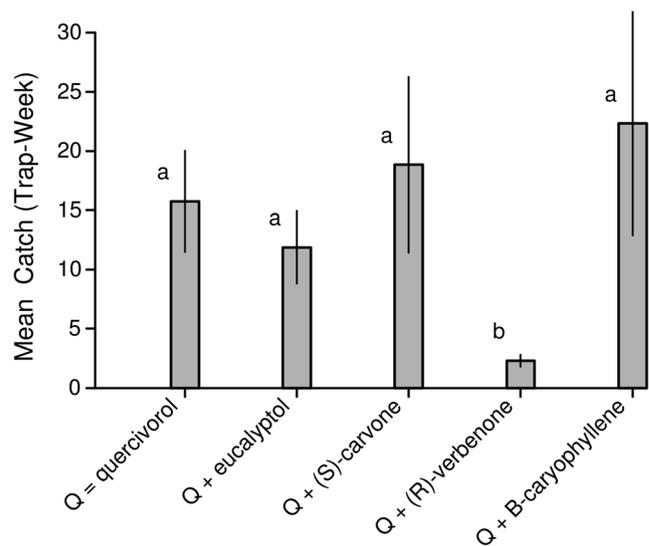


Fig. 4 Effect of volatiles on attraction of PSHB to quercivorol at 1× release rate from sticky traps in an avocado orchard (27 Sept. – 26 Nov. 2017, Nahsholim, Israel). Vertical lines on bars represent 95% CI of means ($N=12$ each bar). Bars with the same letter were not significantly different ($\alpha=0.05$, Tukey's HSD)

less than quercivorol alone (Fig. 5). An increase in release rate may have increasingly reduced the attraction to quercivorol but the highest rate does not support this conclusion. In any case, there is no evidence that ethanol can enhance attraction of PSHB to quercivorol and therefore adding ethanol is not justified.

Simulation of Push-Pull in Avocado Orchard The case of mass trapping alone was simulated by zero inhibitors per tree, while one or three inhibitors per tree caused an increase in trap catch regardless of trap density (Fig. 6). Of more interest is that at all trap densities, an increase in inhibitors per tree caused a decrease in numbers of beetles finding aggregations and reproducing. Mass trapping alone with 16 traps and no inhibitors per tree allowed $8.9 \pm 0.6\%$ ($\pm 95\%$ CI) of the females to find one of the 10 aggregations while the same trap density with three inhibitors per tree allowed only $2.5 \pm 0.3\%$ of females to mate. At three inhibitors per tree, increasing trap density from two to 16 per ha decreased mating percentage from $20.7 \pm 2.1\%$ down to $2.5 \pm 0.3\%$ (Fig. 6). As the number of traps per ha was increased to four or 16, all beetles were either caught or found aggregations during the simulation period (up to 7200 m flight or 14,400 steps). Only in two of eight simulations at 2 traps/ha were a few beetles still flying at the end of a simulation. The number of inhibitors extended the time for all beetles to be caught or to find aggregations, regardless of trap density. This was due to the 3-step or 1.5 m flight where aggregations were ignored after encountering an inhibitor radius. With an increase in trap density, the time for all beetles to be caught or find aggregations was reduced significantly. For example, at three inhibitors per tree, the mean number of steps

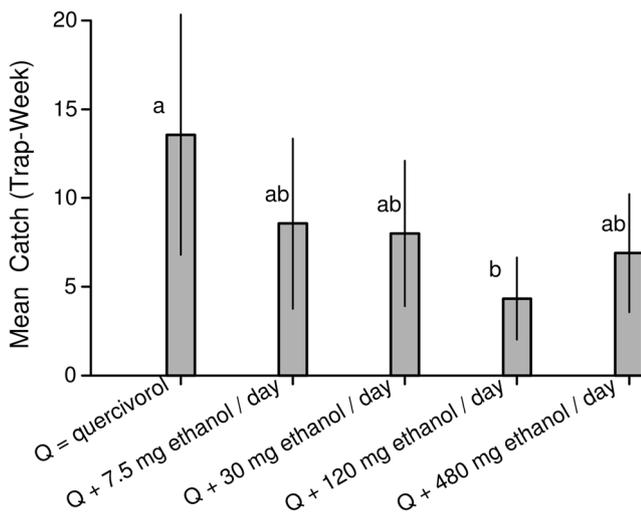


Fig. 5 Effect of ethanol at four release rates (64-fold range) on attraction of PSHB to quercivorol at $1\times$ release rate from sticky traps in an avocado orchard (10 August – 27 September 2017, Nahsholim, Israel). Vertical lines on bars represent 95% CI of means ($N = 12$ each bar). Bars with the same letter were not significantly different ($\alpha = 0.05$, Tukey's HSD)

for all beetles to have encounters (trap or aggregation) was $11,370 \pm 1678$, 5673 ± 919 , or 1559 ± 133 ($\pm 95\%$ CI) at 2, 4, or 16 traps, respectively.

Discussion

In 2016, sticky traps surrounding infested limbs that were not wrapped with fine screen caught a mean of 140 PSHB that

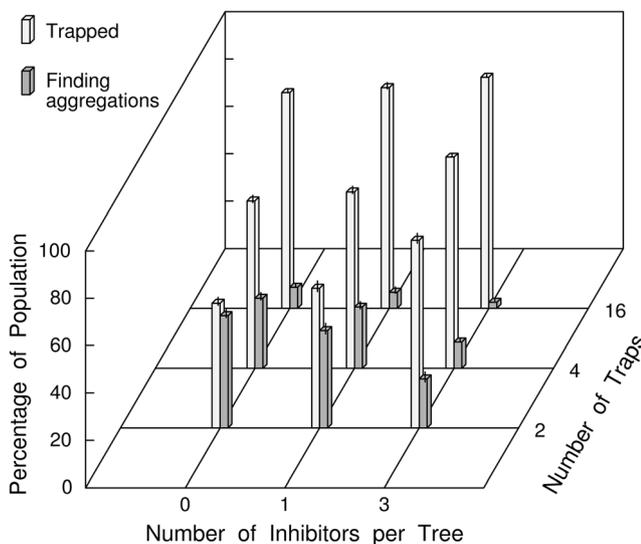


Fig. 6 Mean percentages of initial populations of 1000 simulated beetles that were trapped by various numbers of traps ($EARc = 2.8$ m, lighter bars) or found one of 10 aggregations ($EARc = 0.5$ m, darker bars) in a 1 ha area. Inhibitors had a radius of 0.6 m and were placed on all 400 trees (see Methods for details). Each bar mean of eight simulations, and vertical error lines on top of each bar represents 95% CI (bars are significantly different if error lines do not overlap)

was about four times the catch on unwrapped control limbs with a mean of 35 (Byers et al. 2017). In the present study, the mean catch on the wrapped infested limbs caught about three times more than wrapped control limbs. The catch on the control limbs was probably due to interception of flying beetles searching for nearby infested areas. These results suggest that collection of volatiles from infested limbs in avocado trees should be attempted and subjected to chemical analysis by GCMS. Recently we argued that females are attracted to aggregations on avocado limbs because females have an interest that their daughters and sons mate with the progeny of non-related females similarly attracted to the aggregation in order to reduce harmful genetic inbreeding (Byers et al. 2017). In addition, there may be reproductive benefits for PSHB females, as in other bark beetle species, to cooperate in a simultaneous attack of a living host that otherwise might resist a single attacking beetle. Furthermore, attractive volatiles would indicate a food and habitat resource to exploit.

A dose-response curve performed in 2016 indicated that the mean catch per trap per week (Y) increased with increasing release rate (X) and fit a first-order kinetic formation function (Byers 2013; Byers et al. 2017). However, the expanded dose-response curve that included a $108\times$ high dose did not fit this function as well but rather fit perfectly both a second-order hyperbolic kinetic formation function (Fig. 1) or a second-order kinetic formation function $Y = a(1 - (1/(1 + abX)))$. The earlier dose-response curve for quercivorol (Byers et al. 2017) or the two curves mentioned here are all examples of kinetic formation functions that fit many data sets from dose-response studies reported earlier (Byers 2013). In both second-order kinetic formation functions above, $a = 83.5$ represents the predicted maximum mean catch/trap/week possible, which is close to 81.25 catch for the $108\times$ dose. However, since the $10\times$ dose caught 84% as much as $108\times$, the $10\times$ rate appears the most cost-effective. Catches on the $10\times$ dose were used to calculate an effective attraction radius, $EAR = 1.98$ m, which can be converted using the SD of the vertical flight distribution (0.88 m, Byers et al. 2017) to a circular $EARc = 2.80$ m. This latter radius is very close to that calculated in 2016 for the $10\times$ dose giving an $EARc = 2.86$ m (Byers et al. 2017). In the push-pull simulations discussed shortly, we used the 2.80 m value.

The EAR calculation proposed in 1989 (and $EARc$ in 2008) is a construct that unfortunately is little used when designing mass-trapping or push-pull control strategies (Byers 2012a, b). Many studies, too numerous to mention, have presented statistically significant catches that indicate attractive pheromone compounds in field tests. However, some of the conclusions of these studies have implied exaggerated attractive potency for specific semiochemicals since a lure could appear strongly attractive at high population densities while weakly attractive at low densities. Therefore, in order to appropriately measure the catching strength of a lure and trap

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 in a trap is presented. The EAR appears like a spherical three-dimensional “sticky ball” that, if large enough, would catch the same as a trap releasing a pheromone plume (Byers 2012a, 2012b; Byers et al. 2017). 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 EAR_c (see Methods).

The relationship between EAR and EAR_c depends on the “thickness” of the vertical flight distribution reflected in its standard deviation, *SD*. An insect with a large *SD* of its vertical flight distribution (e.g., bark beetles of tall pine trees) would tend to have a smaller EAR_c than EAR, while a more shallow *SD* (e.g., moths flying just above ground crops) would have an EAR_c several times larger than their EAR. For example, the European spruce bark beetle, *Ips typographus* (L.) has an *SD* of its vertical flight layer of 2.75 m, and its standard pheromone bait gives an EAR = 1.55 m and a smaller EAR_c = 0.55 m; the California 5-spined ips, *Ips paraconfusus* Lanier, has an *SD* = 5.14 m and a 50 male-infested pine log an EAR = 3.17 m and smaller EAR_c = 1.23 m (Byers 2012a). In contrast, the pink bollworm moth, *Pectinophora gossypiella* (Saunders), of cotton has an *SD* = 0.26 m with an EAR = 1.03 m and larger EAR_c = 2.61 m (Byers and Naranjo 2014), while the lesser date moth, *Batrachedra amydraula* Meyrick, has a flight *SD* = 1.53 m and monitoring delta traps were estimated to have an EAR = 2.89 m and larger EAR_c = 3.43 m (Levi-Zada et al. 2017).

Piperitone [75% (*R*)-enantiomer], (*S*)-verbenone and (*R*)-verbenone are among the most potent of inhibitors tested on any species of scolytid beetle, reducing response of PSHB to quercivorol in our study by 90, 78, and 85%, respectively. In comparison, the aggregation response to synthetic pheromone components of the western pine beetle, *Dendroctonus brevicomis* LeConte, was reduced 56% by verbenone released at 4 mg/day (Bedard et al. 1980). The response of *Tomicus piniperda* L. to host monoterpenes was reduced by 75 or 80% by 0.25 mg/day of (*R*)-verbenone or (*S*)-verbenone, respectively (Byers et al. 1989). They also showed increasing verbenone release from infested logs as they aged and suggested this was probably due to microorganisms as the beetles contained little verbenone. These intraspecific reductions can be compared to an interspecific response in which 4 mg/day release of (*S*)-verbenone (known from male *D. brevicomis*) reduced the attraction of *Ips paraconfusus* to its own aggregation pheromone (pine log with 50 males) by 92% (i.e., only 8.4% catch compared to 50-male log alone; Byers and Wood 1980).

(*S*)- and (*R*)-verbenone are monoterpene ketone oxidation products of (*S*)- and (*R*)- α -pinene. The quantities of verbenone in hindguts of some bark beetles as well as

increasing release of verbenone from bark beetle colonized and degrading pine logs over several days (and low constant amounts of verbenone from control logs) led to the hypothesis that verbenone indicates an unsuitable host tree (Byers 1989; Byers et al. 1989). Piperitone appears to be a ketone of α -terpinene. Cryptone can be chemically converted to quercivorol (named *cis*-1-hydroxy-menth-2-ene) and quercivorol can be oxidized to piperitone (Thomas et al. 1967). Because of the structural similarity of piperitone with quercivorol, piperitone may block the PSHB’s antennal receptor for quercivorol and perhaps result in the inhibition of attractive response. Rudinsky et al. (1975) demonstrated piperitone was inhibitory to Douglas-fir beetles and suggested it might be a pheromone mimic of MCH. Perhaps the presence of piperitone is signaling that the local host area is no longer suitable for colonization.

MCH is well known to reduce response of Douglas fir beetle (Furniss et al. 1972, 1976; Rudinsky et al. 1975) but at the dose we tested did not affect PSHB response. (+)-(*S*)-carvone is a ketone oxidation product of the monoterpene (+)-(*R*)-limonene while eucalyptol is not an alcohol but rather a cyclic ketal of α - or γ -terpinene. Cryptone would be a direct oxidation product of quercivorol. However, there was no significant reduction in PSHB responses when either (\pm)-cryptone, (+)-(*S*)-carvone, or eucalyptol were tested. Eucalyptol is much more volatile than the monoterpene ketones and thus may not have been released at the rate expected during the entire test period. The release of β -caryophyllene could not be determined due to weight gain in the laboratory. This weight gain might be due to oxidation and polymerization and/or water vapor absorption (Turek and Stintzing 2013). Assuming the vapor pressures of β -caryophyllene (0.0128 Torr, 25 °C) and carvone (0.0656 Torr, 25 °C) are correct (SciFinderⁿ database) then we estimate the release of β -caryophyllene per vial was 0.12 mg/day or 0.48 mg/day for four vials.

Earlier observations of small bark beetles *Pityogenes bidentatus* (Herbst) approaching within 0.5 to 1 m from a source of aggregation pheromone and inhibitory non-host tree volatiles were described as “repelled” and they “turned away” (Byers et al. 2004). Thus, we suspected that inhibitors many function primarily over a relatively short distance. Because piperitone and (*S*)-verbenone in the earlier test were highly inhibitory when placed together with quercivorol (Fig. 2) we separated these inhibitors together at increasing distances from an attractive source of quercivorol and found a relationship of catch as a function of separation distance (Fig. 3). The effect of the two potent inhibitors appears close-range where the reduction in catch lessens to about half at 0.6 m away, and they are hardly effective by 2 m away. Therefore, a push system of control would need to use several inhibitors spaced around the avocado tree (as used in the model). We are not aware of any previous work that has investigated the effect of

increasing the distance of repellents/inhibitors away from an attractant, despite many such studies showing various compounds inhibit the attractants that are usually pheromones (Byers 1989, 2004). Byers (1987) separated bark beetle aggregation synergists *exo*-brevicommin and frontalin of *D. brevicomis* and found that the attraction rapidly decreased with an increase in separation distance. However, the effects of inhibitors such as verbenone are usually investigated by increasing densities of the inhibitor to “blanket” an area for protection of trees (Furniss et al. 1976; Gillette et al. 2012).

The release of ethanol over a 64-fold range did not enhance attraction of PSHB to quercivorol, on the contrary, ethanol at all rates reduced response (Fig. 4). Only in one case, however, was the reduction significant. The responses decrease with dose but at the highest dose the reduction was intermediate. However, the 4-mm hole allowed ethanol to evaporate the most of any dose causing the meniscus to drop substantially and reduce release rate during the latter part of the week until refilling. Thus, the dose-response at the highest ethanol dose may have been compromised. It appears ethanol over the doses tested is not enhancing attraction to quercivorol. Ethanol together with quercivorol as well as ethanol alone was shown by Carrillo et al. (2015) to attract low numbers of TSHB in Florida. We did not test ethanol alone.

The simulation model of push-pull can accommodate different (a) sizes of inhibitor radius, (b) numbers of inhibitors per tree, (c) numbers of traps, (d) EARc size of traps, (e) numbers of aggregations, and (f) EARc size of aggregations. The modelling of attraction to traps and aggregations in our view is appropriate, while the modeling of inhibitors and their effect on behavior of beetles is debatable. This is because the behavior of insects after encountering inhibitors is poorly known. There seems to be a change of path, at least briefly, and for the insect to leave the area of highest concentrations of inhibitor (Byers et al. 2004). In our model, this was accomplished by “blinding” the beetle or inhibiting her from landing in aggregations encountered immediately after exposure to the inhibitor radius. The number of steps of landing inhibition can be changed in the model but at least two steps are needed to affect encounter rates (Byers unpublished), so three steps were used in the model. More than three steps may be an exaggeration. The size of the inhibitor in the model was 0.6 m (the distance experimentally found with piperitone and verbenone causing half catch level at quercivorol). This distance for the inhibitor radius in the model is somewhat arbitrary but does reflect the position and size of the inhibition effect at the dose tested in the field.

One major parameter that is poorly known is how far females fly during their search for an aggregation on an avocado tree. Female PSHB probably have the same flight capacity as TSHB that were observed to fly for up to 24 min in the laboratory at 0.3 to 0.6 m/s (or up to 864 m without aid of wind; Calnaido 1965). Females could rest between flights and wind

would increase their potential dispersal range. However, the 864 m distance may be an underestimate. Jactel and Gaillard (1991) flew a larger bark beetle, *I. sexdentatus* Boern, on rotary flight mills and found that 50% of the beetles could fly more than 20 km and 10% more than 45 km based on about 50 interrupted flights. Forsse and Solbreck (1985) placed *I. typographus* on a flight mill and the longest continuous flight was 6 hr and 20 min, suggesting that a few of these beetles can fly up to 45.6 km at a ground speed of 2 m/s. Other reports indicate that *Scolytus multistriatus* (Marshall), a beetle (2–3 mm long) similar in size to PSHB (2–2.5 mm), were caught on pheromone traps 30 km from the nearest elm tree in a ‘desert’ valley bordered by the Sierra Nevada mountains of California (Paine et al. 1984). In our simulations, however, the size and number of aggregations as well as the number of traps was the main limit on reproduction and not the length of the search flight of a maximum 7.2 km. The model only considers the maximum mean flight distance possible for females (7.2 km) which would result in nature from the female’s inherent mean speed plus wind speed (equations in Byers 2012a), thus the simulated time of maximum flight (14,400 steps) would represent less than 4 hr of flight in nature. The model of push-pull does not purport to predict outcomes specifically but rather has the goal of better understanding the important parameters and effects of different densities and sizes (EARc) of traps, aggregations, and inhibitors.

Byers (2012b) conducted simulations that showed male-searching flight for stationary females was as efficient as female-searching flight for stationary males. However, in the presence of mass trapping, female-searching flight was less effective and caused less mating. In the case of PSHB, females are searching for aggregations of females in competition with pheromone traps. In this scenario, mass trapping would reduce female mating more so than for a male-searching system as in moths. The simulations indicate that inhibitors (push) in the model as well as traps (pull) were able to enhance efficacy of control compared to mass trapping alone.

In practical respects, a 1 to 3 mg/day release rate of quercivorol should attract significant numbers of PSHB and be suitable for monitoring, mass trapping or push-pull control. The 10× rate of quercivorol (1.26 mg/day) caught about 2.6 times more PSHB than the 1× rate in 2016 and 2.8 times more in 2017. In selecting the optimal density of traps for mass trapping it is necessary to consider that an overly high density of traps is expensive and can cause multiple odor plumes to disrupt orientation of PSHB and thereby lower trap efficiency. On the other hand, a too low density will not give good coverage and take longer to trap out the population allowing many females to find suitable host branches/trees. In addition, the density of competing attractive sources (baited traps and natural sources) is very important to the success of mass trapping (Byers et al. 2017). Therefore, 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 (enlarging their EARc). In the push part, the more dispensers of inhibitor the better, and although only dispersers and not traps are needed, costs of chemical must be considered. The great advantage with PSHB is that females, the reproductive sex, are caught and not males alone as with moths (Byers 2012b). Thus we believe that PSHB is a good candidate for mass trapping and push-pull methods.

Acknowledgments 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 Israel Avocado Growers Association #0390320 of 2017.

References

- Bedard WD, Tilden PE, Wood DL, Lindahl KQ, Rauch PA (1980) Effects of verbenone and *trans*-verbenol on the response of *Dendroctonus brevicomis* to natural and synthetic attractant in the field. *J Chem Ecol* 6:997–1013
- Byers JA (1987) Interactions of pheromone component odor plumes of western pine beetle. *J Chem Ecol* 13:2143–2157
- Byers JA (1988) Novel diffusion-dilution method for release of semiochemicals: Testing pheromone component ratios on western pine beetle. *J Chem Ecol* 14:199–212
- Byers JA (1989) Chemical ecology of bark beetles. *Experientia* 45:271–283
- Byers JA (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 JA (2004) Chemical ecology of bark beetles in a complex olfactory landscape. In: Lieutier F, Day KR, Battisti A, Grégoire JC, Evans H (eds) *Bark and Wood Boring Insects in Living Trees in Europe*, a Synthesis. Kluwer Academic Publishers, Dordrecht, pp 89–134
- Byers JA (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 JA (2012a) Estimating insect flight densities from attractive trap catches and flight height distributions. *J Chem Ecol* 38:592–601
- Byers JA (2012b) Modelling female mating success during mass trapping and natural competitive attraction of searching males or females. *Entomol Exp Appl* 145:228–237
- Byers JA (2013) Modeling and regression analysis of semiochemical dose–response curves of insect antennal reception and behavior. *J Chem Ecol* 39:1081–1089
- Byers JA, Naranjo SE (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 JA, Wood DL (1980) Interspecific inhibition of the response of the bark beetles, *Dendroctonus brevicomis* and *Ips paraconfusus*, to their pheromones in the field. *J Chem Ecol* 6:149–164
- Byers JA, Lanne BS, Löfqvist J (1989) Host-tree unsuitability recognized by pine shoot beetles in flight. *Experientia* 45:489–492
- Byers JA, Zhang QH, Birgersson G (2004) Avoidance of nonhost plants by a bark beetle, *Pityogenes bidentatus*, in a forest of odors. *Naturwissenschaften* 91:215–219
- Byers JA, Maoz Y, Levi-Zada A (2017) Attraction of the *Euwallacea* sp. near *formicatus* (Coleoptera: Curculionidae) to quercivorol and to infestations in avocado. *J Econ Entomol* 110:1512–1517
- Calnaido D (1965) The flight and dispersal of shot-hole borer of tea (*Xyleborus formicatus* Eichh., Coleoptera: Scolytidae). *Entomol Exp Appl* 8:249–262
- Carrillo D, Narvaez T, Cossé AA, Stouthamer R, Cooperband M (2015) Attraction of *Euwallacea* nr. *formicatus* (Coleoptera: Curculionidae: Scolytinae) to lures containing quercivorol. *Fla Entomol* 98:780–782
- Carrillo D, Cruz LF, Kendra PE, Narvaez TI, Montgomery WS, Monterroso A, De Grave C, Cooperband MF (2016) Distribution, pest status and fungal associates of *Euwallacea* nr. *formicatus* in Florida avocado groves. *Insects* 7:55, 11 p. <https://doi.org/10.3390/Insects7040055>
- Cook SM, Khan ZR, Pickett JA (2007) The use of pushpull strategies in integrated pest management. *Annu Rev Entomol* 52:375–400
- Cooperband MF, Stouthamer R, Carrillo D, Eskalen A, Thibault T, Cossé AA, Castrillo LA, Vandenberg JD, Rugman-Jones PF (2016) Biology of two members of the *Euwallacea formicatus* 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, Gonzalez A, Wang DH, Twizeyimana M, Mayorquin JS (2012) First report of a *Fusarium* sp. and its vector tea shot hole borer (*Euwallacea* nr. *formicatus*) causing *Fusarium* dieback on avocado in California. *Plant Dis* 96:1070
- Eskalen A, Stouthamer R, Lynch SC, Rugman-Jones PF, Twizeyimana M, Gonzalez A, Thibault T (2013) Host range of *Fusarium* dieback and its ambrosia beetle (Coleoptera: Scolytinae) vector in southern California. *Plant Dis* 97:938–951
- Forsse E, Solbreck CH (1985) Migration in the bark beetle *Ips typographus* L.: Duration, timing and height of flight. *Z Angew Entomol* 100:47–57
- Freeman S, Protasov A, Sharon M, Mohotti K, Eliyahu M, Okon-Levy N, Maymon M, Mendel Z (2012) Obligate feed requirement of *Fusarium* sp. nov., an avocado wilting agent, by the ambrosia beetle *Euwallacea* aff. *formicata*. *Symbiosis* 58:245–251
- Furniss MM, Kline LN, Schmitz RF, Rudinsky JA (1972) Tests of three pheromones to induce or disrupt aggregation of Douglas-fir beetles (Coleoptera: Scolytidae) on live trees. *Ann Entomol Soc Am* 65:1227–1232
- Furniss MM, Baker BH, Hostetler BB (1976) Aggregation of spruce beetles (Coleoptera) to seudenol and repression of attraction by methylcyclohexenone in Alaska. *Can Entomol* 108:1297–1302
- Gillette NE, Mehmel CJ, Mori SR, Webster JN, Wood DL, Erbilgin N, Owen DR (2012) The push–pull tactic for mitigation of mountain pine beetle (Coleoptera: Curculionidae) damage in lodgepole and whitebark pines. *Environ Entomol* 41:1575–1586
- Hanula JL, Sullivan B (2008) Manuka oil and phoebe oil are attractive baits for *Xyleborus glabratus* (Coleoptera Scolytinae), the vector of laurel wilt. *Environ Entomol* 37:1403–1409
- Hughes MA, Martini X, Kuhns E, Colee J, Mafra-Neto A, Stelinski LL, Smith JA (2017) Evaluation of repellents for the redbay ambrosia beetle, *Xyleborus glabratus*, vector of the laurel wilt pathogen. *J Appl Entomol*. <https://doi.org/10.1111/jen.12387>
- Hulcr J, Stelinski LL (2017) The ambrosia symbiosis: From evolutionary ecology to practical management. *Annu Rev Entomol* 62:285–303
- Jactel H, Gaillard J (1991) A preliminary study of the dispersal potential of *Ips sexdentatus* Boern (Coleoptera: Scolytidae) with an automatically recording flight mill. *J Appl Entomol* 112:138–145
- Kendra PE, Montgomery WS, Niogret J, Peña JE, Capinera JL, Brar G, Epsky ND, Heath RR (2011) Attraction of the redbay ambrosia beetle, *Xyleborus glabratus*, to avocado, lychee, and essential oil lures. *J Chem Ecol* 37:932–942
- Kendra PE, Niogret J, Montgomery WS, Sanchez JS, Deyrup MA, Pruett GE, Ploetz RC, Epsky ND, Heath RR (2012) Temporal analysis of sesquiterpene emissions from manuka and phoebe oil lures and

- efficacy for attraction of *Xyleborus glabratus* (Coleoptera: Curculionidae: Scolytinae). *J Econ Entomol* 105:659–669
- Kendra PE, Montgomery WS, Niogret J, Pruett GE, Mayfield AE III, MacKenzie M, Deyrup MA, Bauchan GR, Ploetz RC, Epsky ND (2014) North American Lauraceae: Terpenoid emissions, relative attraction and boring preferences of redbay ambrosia beetle, *Xyleborus glabratus* (Coleoptera: Curculionidae: Scolytinae). *PLoS One* 9:e102086
- Kendra PE, Owens D, Montgomery WS, Narvaez TI, Bauchan GR, Schnell EQ, Tabanca N, Carrillo D (2017) α -Copaene is an attractant, synergistic with quercivorol, for improved detection of Euwallacean *forficatus* (Coleoptera: Curculionidae:Scolytinae). *PLoS One* 12:e0179416
- Klimetzek D, Köhler J, Vité JP (1986) Dosage response to ethanol mediates host selection by “secondary” bark beetles. *Naturwissenschaften* 73:270–272
- Kuhns EH, Martini X, Tribuiani Y, Coy M, Gibbard C, Peña J, Hulcr J, Stelinski LL (2014) Eucalyptol is an attractant of the redbay ambrosia beetle, *Xyleborus glabratus*. *J Chem Ecol* 40:355–362
- Levi-Zada A, Sadowsky A, Dobrinin S, Ticuchinski T, David M, Fefer D, Dunkelblum E, Byers JA (2017) Monitoring and mass-trapping methodologies using pheromones: the lesser date moth *Batrachedra amydracula*. *Bull Entomol Res* 108:58–68
- Lynch SC, Twizeyimana M, Mayorquin JS, Wang DH, Na F, Kayim M, Kasson MT, Thu PQ, Bateman C, Rugman-Jones P, Hulcr J, Stouthamer R, Eskalen A (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, Protasov A, Sharon M, Zveibil A, Yehuda SB, O’Donnell K, Rabaglia R, Wysoki M, Freeman S (2012) An Asian ambrosia beetle *Euwallacea* nr. *forficatus* and its novel symbiotic fungus *Fusarium* sp. pose a serious threat to the Israeli avocado industry. *Phytoparasitica* 40:235–238
- Miller JR, Cowles RS (1990) Stimulo-deterrent diversion: a concept and its possible application to onion maggot control. *J Chem Ecol* 16: 3197–3212
- O’Donnell K, Sink S, Libeskind-Hadas R, Hulcr J, Kasson MR, Ploetz RC, Konkol JL, Ploetz JN, Carrillo D, Campbell A, Duncan RE, Liyanage PNH, Eskalen A, Na F, Geiser DM, Bateman C, Freeman S, Mendel Z, Sharon M, Aoki T, Cossé AA, Rooney AP (2015) Discordant phylogenies suggest repeated host shifts in the *Fusarium–Euwallacea* ambrosia beetle mutualism. *Fungal Genet Biol* 82:277–290
- Paine TD, Birch MC, Miller JC (1984) Use of pheromone traps to suppress populations of *Scolytus multistriatus* (Marsham) (Coleoptera: Scolytidae) in three isolated communities of elms. *Agric Ecosyst Environ* 11:309–318
- Pyke B, Rice M, Sabine B, Zalucki MP (1987) The push-pull strategy – behavioural control of *Heliothis*. *Aust Cottongrower* 7:9
- Rudinsky JA, Kline LN, Diekman JD (1975) Response–inhibition by four analogues of MCH, an antaggregative pheromone of the Douglas-fir beetle. *J Econ Entomol* 68:527–528
- Schroeder LM, Lindelöw Å (1989) Attraction of scolytids and associated beetles by different absolute amounts and proportions of α -pinene and ethanol. *J Chem Ecol* 15:807–817
- Stouthamer R, Rugman-Jones P, Thu PQ, Eskalen A, Thibault T, Hulcr J, Wang LJ, Jordal BH, Chen CY, Cooperband M, Lin CS, Kamata N, Lu SS, Masuya H, Mendel Z, Rabaglia R, Sanguansub S, Shih HH, Sittichaya W, Zong S (2017) Tracing the origin of a cryptic invader: phylogeography of the *Euwallacea fornicatus* (Coleoptera: Curculionidae: Scolytinae) species complex. *Agric For Entomol*. <https://doi.org/10.1111/afe.12215>
- Thomas AF, Willhalm B, Bowie JH (1967) Mass spectra and organic synthesis. Part VIII. The mass spectra of piperitone, the piperitols, and related products. *J Chem Soc B* 392–400. <https://doi.org/10.1039/J29670000392>
- Tokoro M, Kobayashi M, Saito S, Kinuura H, Nakashima T, Shoda-Kagaya E, Kashiwagi T, Tebayashi S, Kim C, Mori K (2007) Novel aggregation pheromone, (1*S*,4*R*)-p-menth-2-en-1-ol, of the ambrosia beetle, *Platypus quercivorus* (Coleoptera: Platypodidae). *Bull For For Prod Res Inst* 6:49–57
- Turek C, Stintzing FC (2013) Stability of essential oils: A review. *Compr Rev Food Sci Food Saf* 12(1):40–53
- Wood SL (1982) The bark and ambrosia beetles of North and Central America (Coleoptera: Scolytidae), a taxonomic monograph. *Great Basin Naturalist Memoirs* 6:1–1359