Journal of Pest Science (2021) 94:991–1002
https://doi.org/10.1007/s10340-020-01310-x

ORIGINAL PAPER

Protecting avocado trees from ambrosia beetles by repellents and mass trapping (push–pull): experiments and simulations

John A. Byers[1] · Yonatan Maoz[2] · Barak Cohen[3] · Maayan Golani[4] · Daniela Fefer[5] · Anat Levi-Zada[5]

Received: 30 July 2020 / Revised: 22 October 2020 / Accepted: 27 November 2020 / Published online: 3 January 2021

Abstract

The polyphagous shot hole borer (PSHB), *Euwallacea fornicatus* (Eichhoff), is an ambrosia beetle (Coleoptera: Curculionidae) infesting avocado branches *Persea americana* Mill. in North America, South Africa and Israel. Field experiments were conducted with attractive quercivorol traps and repellents on trees to develop push–pull control methods. PSHBs were collected over the summer from multiple-funnel traps baited with quercivorol in grids of low-, medium- and high-density traps to assess mass trapping interactions and capture trends. Mean catch/trap-week was higher on peripheral outer traps compared to the next inner ring of traps in grids. These ratios and grids were simulated to estimate the circular effective attraction radius (EARc) of multiple-funnel traps and compared to a previous relationship for sticky traps yielding EARc from release rate of quercivorol. The results indicate that multiple-funnel traps have smaller EARc in higher-density grids, likely due to interactions between traps. Tests with verbenone compared to methyl salicylate (MeSA) indicate both are repellents and no evidence for synergism. Dollops of verbenone–MeSA–SPLAT (5% each volatile) were applied monthly at 10- and 40-cm spacings along branched trunks of avocado trees, with release of volatiles declining exponentially over a month. These treatments caused a reduction in both numbers of aggregations/tree and attacks/aggregation to about half that of untreated control trees. Verbenone–MeSA–SPLAT dollops caused localized phytotoxicity on avocado bark, suggesting 8 cm^2 plastic bag dispensers containing 0.25 g verbenone are preferred at 40-cm spacing. Push–pull should be done just before flight season to overwhelm natural attraction of single females initiating aggregations.

Keywords Quercivorol · Verbenone · Methyl salicylate · *Euwallacea fornicatus* · Inhibitors · Chemical ecology

Key Message

- Methods of push–pull control of an ambrosia beetle were developed including protection of avocado trees with repellents and quercivorol-attractant mass trapping.
- Ratios of catch on outer/inner trap rings of trapping grids suggest beetles flew into the grids. Simulations of corresponding grids and catch ratios gave estimates of effective attraction radius of capture strength of quercivorol traps.
- Methyl salicylate (MeSA) and verbenone repelled ambrosia beetles; and branches of avocado trees treated with verbenone–MeSA–SPLAT dollops received half the attacks as control trees.

Communicated by Donald Weber.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s10340-020-01310-x.

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

[1] Semiochemical Solutions, 7030476 Beer Yaakov, Israel

[2] The Israel Fruit Growers Association, Yahud, Israel

[3] Tapazol Chemical Works Ltd, Hasolela 1, Beit Shemesh, Israel

[4] Luxembourg Industries LTD, 27 Hamered, Tel Aviv, Israel

[5] Department of Entomology, Institute of Plant Protection, Agricultural Research Organization, Volcani Center, Rishon LeZion, Israel

Introduction

The polyphagous shot hole borer (PSHB), *Euwallacea fornicatus* (Eichhoff), is an ambrosia beetle (Coleoptera: Curculionidae: Scolytinae) from Southeast Asia that invaded California about 2003 and shortly thereafter Israel. The female beetle bores into branches of many woody plants and is an important pest of avocado *Persea americana* Mill. (Eskalen et al. 2012, 2013; Mendel et al. 2012, 2017; Freeman et al. 2012). A sibling species, the tea shot hole borer (TSHB) from India and Sri Lanka, feeds on avocado bark in Florida and previously was named *E. fornicatus* (Carrillo et al. 2015, 2016; Cooperband et al. 2016). However, both species differ in DNA and are considered separate species (Eskalen et al. 2013; O'Donnell et al. 2015; Stouthamer et al. 2017; Smith et al. 2019). Recently, Smith et al. located a type specimen of *E. fornicatus* which was identical to PSHB, requiring TSHB to be renamed *E. perbrevis* (Schedl). Only females of these species leave broodtree limbs after mating since males are flightless (Fig. S1 in Supplementary material; Calnaido 1965; Carrillo et al. 2015). In Israel, PSHB females aggregate in scattered infestations in avocado limbs that die due to beetle feeding and vectoring of *Fusarium* dieback disease, reducing tree growth (Eskalen et al. 2012, 2013; Freeman et al. 2012; Mendel et al. 2012, 2017; Lynch et al. 2016). Like other ambrosia beetles, PSHB bores into sapwood and introduces symbiotic fungi that the beetle feeds upon in their tunnels (Wood 1982; Freeman et al. 2012; Hulcr and Stelinski 2017). Insecticides are not used to control PSHB in Israel because (i) the beetles are protected under the bark and (ii) domestic and export markets desire avocados free of residues. Therefore, our goal was to investigate control of PSHB using environmentally benign semiochemicals such as repellents and attractants to develop push–pull methods (Miller et al. 1990; Borden et al. 2006; Cook et al. 2007; Byers et al. 2018, 2020) to protect avocado limbs from attack.

Scolytine beetles including many bark beetles and ambrosia beetles usually are attracted from tens of meters to aggregation pheromones that consist of one to three chemicals produced by either males or females, or both, depending on the species (Byers 1989). Quercivorol, (1S,4R) − p − menth − 2 − en − 1 − ol, is the aggregation pheromone of the oak-killing ambrosia beetle *Platypus quercivorus* (Murayama) of Japan (Tokoro et al. 2007). Quercivorol is commercially available and attractive to TSHB (Carrillo et al. 2015; Kendra et al. 2017) and to PSHB (Byers et al. 2017, 2018). However, quercivorol has not been shown to be a pheromone of either species.

Monitoring traps releasing semiochemicals such as pheromones to attract and trap responding insects is a well-known management tool for assessing populations of adult pests. An extension of monitoring is mass trapping, whereby many attractive traps in an area are used to reduce populations of adults before they mate and reproduce (El-Sayed et al. 2006; Miller et al. 2015; Levi-Zada et al. 2018). In both monitoring and mass trapping, the trap's capture rate is due to the following factors: (a) blend of attractive components, (b) chemical release rates, (c) trap size and efficiency, (d) population density of flying insects and (e) competitive sources of natural attraction. Thus, the most desirable trap lure is strongly attractive, efficient in pest capture, of low cost and easy to maintain and service. A strongly attractive lure of pests at low population density would likely catch few and erroneously appear ineffective, while a weak bait in the same trap at high pest densities might catch many insects and wrongly appear effective. Therefore, it is crucial to have a consistent measure of trap lure potency regardless of flight density.

The effective attraction radius (EAR) is such a measure of an attractive trap's capture strength (Byers 2012a, b). Given a catch of an insect species by a baited trap, the EAR of this trap lure is equivalent to the radius of an unattractive sticky sphere that would intercept the same number in the area during the trapping period (Byers et al. 2017, 2020). The calculation of EAR requires comparison of attractive trap catch (Ca) to passive (blank) trap catch (Cb) in the same area and the silhouette interception area (S) of the passive trap (S is mean of all interception areas as the trap is rotated). For example, a tubular sticky screen trap 0.25 m diam. × 0.3 m high has $S = 0.075\ \mathrm{m}^2$ (same interception area from any direction). The passive trap should have a high capture efficiency such as a sticky screen, while the attractive trap can be any design type. The equation: $\mathrm{EAR} = [\mathrm{Ca} \times S/(\mathrm{Cb} \times \pi)]^{0.5}$ gives a consistent value for a specific bait and trap regardless of insect density during the field test, as shown by simulations (Byers 2009). The size of the spherical radius of the EAR indicates the strength of the combination of lure and trap.

Although insects fly in three dimensions (3D), flight of insects searching for mates or resources over large areas occurs essentially in two dimensions (2D). Because simulations in 2D are less complex and faster than in 3D, the spherical EAR can be converted to a circular effective attraction radius (EARc) in 2D by estimating the vertical flight distribution of a species using sticky traps at several heights above ground (Byers 2011). 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). An EAR is converted to EARc (Byers 2012a) with the equation:

$$\mathrm{EARc} = \pi \times \mathrm{EAR}^2 / [2 \times \mathrm{SD} \times (2 \times \pi)^{0.5}] \quad (1)$$

(Eq. 1) for use in simulation models (Byers et al. 2017, 2018). Our previous experiments showed that flying PSHB females are attracted to their aggregations on limbs of avocado trees and a typical aggregation has an EAR of 1.17 m and EARc of 0.98 m, about the same as a trap releasing a 1 × dose of quercivorol (0.126 mg/day) (Byers et al. 2017, 2018). A 10 × higher release rate of quercivorol (1.26 mg/day) from traps gave an EAR of 1.98 m which is similar to EAR of aggregation pheromones of bark beetles (Byers 2012a; Byers et al. 2018).

Measurement of EAR/EARc of a specific baited trap is important to estimate how many such traps might be needed in mass trapping to reduce a population of pest insects (Byers 2009, 2011, 2012a; Byers et al. 2017; Levi-Zada et al. 2018). A curve of quercivorol dose related to trap catch/week was recently converted to curves of quercivorol release rate yielding EAR or EARc for predicting sticky trap capture potency (Byers et al. 2020). In addition to the above method of obtaining EAR and SD to calculate EARc, EARc can be estimated from ratios of catch on rings of pheromone traps of insects dispersing from a central release point (Byers 1999) or into a trap grid (Byers 1993). Our first objective, regarding the "pull" of push–pull, was to use 0.5 ha plots with 3 × 4, 5 × 5 or 5 × 10 grids of quercivorol-baited barrier traps (multiple-funnel) to attract PSHB. The ratios of mean catch on traps in outer grid positions to mean catch on traps in the next inner ring positions will be compared to corresponding ratios from simulations to estimate EARc of the particular bait, trap and grid.

As mentioned above, push–pull requires chemicals that are repellent. Volatiles are termed inhibitors if they reduce response of insects to attractants such as pheromones (Byers and Wood 1980, 1981; Byers et al. 1989, 2004). An inhibitor is indicated if its release along with an attractant semiochemical causes significantly lower captures compared to control traps with attractants alone. The behavioral mechanism of inhibitors resulting in lower catch seems to involve repellency (turning away) near the odor source. Byers et al. (2004) reported that bark beetles in late afternoon sun flew several meters upwind directly into a trap releasing aggregation pheromone, but when inhibitors were added, beetles turned away at 0.5 to 1 m from the trap. One of the best-known inhibitors of scolytine beetles is verbenone, a monoterpene ketone, that may indicate a degrading and unsuitable host tree (Byers and Wood 1980; Byers et al. 1989; Byers 1989, 1992; Borden et al. 2006; Burbano et al. 2012; Hughes et al. 2017). Byers et al. (2018) reported that either piperitone or verbenone strongly reduced attraction of PSHB to quercivorol traps and that when both inhibitors (hereafter also called repellents) together were placed at increasing distances from the quercivorol trap, their repellent effect became apparent only at distances within ~ 1 m from the trap. In subsequent experiments, as verbenone (0.8 mg/day) was moved increasingly closer to a quercivorol trap, the catch of PSHB declined to 50% at 0.5 m from the trap (Byers et al. 2020). Increasing tenfold doses (0.01–10x) of either verbenone or piperitone released at 1 × quercivorol traps caused a sigmoidal kinetic–decay relationship in catch (Byers et al. 2020). They suggested these repellents should be placed regularly along avocado trunks in spacings < 0.5 m where PSHB aggregations occur and preferably before the flight season.

In order to develop the "push" of push–pull, our second objective was to treat two plots of 16 avocado trees each with commercial 10% verbenone–SPLAT at two spacings, 10 cm and 40 cm along major branches, compared to adjacent untreated trees and two plots without treatments. However, after the avocado trees were treated with commercial SPLAT we found it contained 5% verbenone and 5% methyl salicylate (MeSA). Therefore, we subsequently tested MeSA and verbenone individually and together for inhibitory effects on PSHB attraction to quercivorol traps.

Materials and methods

Mass trapping of PSHB in avocado

Mass trapping experiments were conducted in three Hass variety avocado orchards in Israel at Beit-Haemek (32°58′26.6"N 35°08′05.4"E), Nahsholim (32°36′25″N 34°56′44″E) and Kfar-Masrik (32°52′09.0"N 35°07′32.3"E). Multiple-funnel traps were hung at 1.5 m height and consisted of 12 black plastic funnels (diameter 19 cm top, 5.5 cm bottom) stacked 1 m vertically (Organi Sheli Ltd., Israel). In Beit-Haemek and Nahsholim, traps were placed inside rows of trees (~ 4 m tree spacing) in grids of 12 (3 traps/row × 4 rows), 25 (5 traps/row × 5 rows) and 50 traps (5 traps/row × 10 rows) each placed in 0.5 ha plots, while at Kfar-Masrik grids consisted of 12 (2 × 6), 20 (4 × 5) and 40 (4 × 10) traps in 0.4 ha plots. For 3 × 4 grids (lower density traps), the traps were placed in rows of trees every 24 m (horizontal spacing) and in every fourth row (~ 18 m vertical spacing). For the 5 × 5 grids (medium-density traps), traps were placed in rows of trees every 12 m (horizontal spacing) and in every other row (~ 12 m vertical spacing). For the 5 × 10 grids (higher-density traps), traps were spaced apart by 12 m in each row and by ~ 6 m vertical spacing in adjacent rows. The same relative spacings between traps for the medium- and higher-density traps were used for the 4 × 5 and 4 × 10 grids at Kfar-Masrik.

Traps were baited with thin-film polyethylene bags (bubble caps) initially filled with 300 μL of quercivorol (racemic 85% cis, 15% trans; Synergy Semiochemicals, Burnaby, Canada). Bubble-cap dispensers release constant amounts of volatiles at constant temperature, and weight loss measurements on a microbalance indicated quercivorol release rate was 4.5 mg/day at 25 °C. Traps were picked of PSHB four times at Beit-Haemek (about every month from July1 to December 4, 2017), nine times at Nahsholim (every 1–2 weeks from June 24 to October 1, 2017) and four times at Kfar-Masrik (variable periods from 10 to 65 days during August 10 to December 4, 2017). The mean catch/trap-week in each area was calculated from the raw catch to normalize results of trapping periods of variable length when comparing trends in catch over time and between the three trapping densities. Seasonal trends in mean catch/trap-week collection in each grid in the three areas were assessed by linear regression (TableCurve 2D version 5.01, Systat Software Inc., Chicago, USA). To determine if traps compete more as the trap density increases (low-, medium- and high-density grids), mean catch/trap-week over the entire season in each of three areas was compared by one-way ANOVA with significant differences between pairs of density grids indicated by Tukey's HSD at $\alpha = 0.05$ (JMP 5.0.1a, SAS Institute Inc., USA).

In the first two areas above for the low-density grids (3 × 4), the average catch per trap calculated from the 10 traps on the rectangular "ring" of peripheral traps (outer traps) was divided by the average catch/trap for the two traps on the next inner rectangular ring of traps (inner traps) to obtain ratios for all collections. The same calculations were made for the medium- and higher-density trap grids that had 16 outer and 8 inner traps, or 26 outer and 18 inner traps, respectively. In Kfar-Masrik with the same medium- and higher-density grids, but in 0.4 ha, the medium-density traps had 14 outer and 6 inner traps, while the higher-density traps had 24 outer and 16 inner traps. The 2 × 6 grids at Kfar-Masrik were not analyzed because there were no inner traps. We postulated that a mean ratio of outer to inner trap catch > 1 would suggest movement of flying beetles into the trapping grids encountering outer traps first and causing fewer beetles to pass through to the inner ring of traps. Means of the ratios ± SE were calculated for each collection in each grid density and area. Ratios of the total outer and inner catches during the season for each grid and area were also calculated. These ratios were considered significantly different from a ratio of 1 (no difference between mean catch on outer and inner traps) if a chi-square test was significant ($P < 0.01$) when comparing observed catches (outer/inner) to expected catches at a ratio of 1 (*R* Statistics, Version 3.2.3). The ratio results also were compared to simulations of beetles captured by trap grids of the same dimensions (presented in the next section).

Simulation of mass trapping

Individual-based movement of beetles in two-dimensions was simulated in the respective trapping grids described above to investigate effects on the outer/inner trap catch ratio due to increasing trap EARc. The computer simulations were programmed in Java 1.6 using movement and trap capture algorithms described earlier (Byers 2012b; Byers et al. 2018). The 3 × 4, 5 × 5 and 5 × 10 trap grids were centrally located in areas with 70.7 m sides (0.5 ha), while the 4 × 5 and 4 × 10 grids were placed inside areas with 56.6 × 70.7 sides (0.4 ha). The 5 × 5 and 4 × 5 trap grids had an inter-trap spacing of 12 m in the *x*- and *y*-directions, while the 5 × 10 and 4 × 10 grids had a spacing of 12 m in the *x*-direction and 6 m in the *y*-direction. The 3 × 4 grid had 24-by-18 m spacing within 0.5 ha area. In each simulation, 1000 beetles were equally distributed along the edges of the area and initially moved in a random direction inward. Thereafter, each individual moved in a correlated random walk with 1-m steps in which their forward direction at each step could vary from the previous direction either right or left according to a random deviation of mean zero and probability function of a normal distribution with standard deviation of angles (SDA) of 10°. This distribution produces movement that appears consistent with flight of insects searching for host plants or mates (Byers 2012b; Byers et al. 2018). Individuals attempting to move outside the simulation area rebounded inward at a random angle. In the simulation series, the circular radius (EARc) of traps placed in the 3 × 4, 4 × 5, or 5 × 5 grids was increased from 0.2 to 4 m in increments of 0.2. Each increment of EARc was simulated eight times (total 8000 beetles). For the 4 × 10 and 5 × 10 grids, the EARc was incremented similarly from 0.2 to 2.6 m to avoid overlap of the radii. Beetles moved until all individuals intercepted a trap and were captured whereupon a new simulation was initiated. The ratio of mean catch/trap for the outer traps to the mean catch/trap for the inner traps in the grids and areas above was recorded for each simulation ($N = 8$), and the mean and 95% CI were recorded at each increment of EARc.

An inverse quadratic regression (Byers 1993) was used to model the resulting simulated data (X = EARc and Y = ratio of outer/inner mean trap catch) using nonlinear regression software (TableCurve 2D). The regression functions for the simulation results of each grid were then solved for X (EARc), and ratios of outer/inner catches from the field trapping experiments were used to estimate the EARc of the respective baited traps in the field. These results will be compared to EARc obtained previously with the standard method based on comparison of catches on baited and blank cylinder sticky traps that gave a function: $\text{EARc} = aX/(b + X)$ where $a = 3.44387$ and $b = 0.30459$ ($R^2 = 0.999$) and X is mg quercivorol released per day (Byers et al. 2020). Using this function, the release of 4.5 mg/day from the bubble-cap dispenser gives an EARc of 3.23 m for a tubular sticky screen trap (25 cm diam × 30 cm high).

Release rates of SPLAT volatiles

Amounts and release rates of methyl salicylate (MeSA) and verbenone in samples of SPLAT (ISCA Technologies Inc, Riverside, CA) were tested each week over 45 days in the laboratory at 25 °C and analyzed by GC–MS. Verbenone's enantiomeric percentages were also determined by chiral GC analysis. Release rates of the two volatiles were determined by drawing air through an activated charcoal filter into a 50-ml glass tube containing a ~ 1 g sample of SPLAT (weighed in mg, $N = 4$) and then through a 0.4 cm diam × 10 cm Porapak SuperQ glass column into a vacuum pump at 0.2 L/min. After 24 h, the Porapak was washed with 1 mL hexane and diluted (× 10) for GC–MS-FID analyses. These analyses were performed on an Agilent 7890A GC instrument interfaced with an Agilent 5975C MS detector and FID detector working in parallel (Agilent, Santa Clara, CA, USA). The GC was equipped with a chiral Hydrodex-β-6TBDMS column (25 m × 0.25 µm, Macherey-Nagel, Germany) kept at 70 °C for 5 min, then increased at 5 °C min^{-1} to 140 °C and held for 10 min. Column helium flow was 1.5 ml min^{-1}, and the GC–MS inlet temperature was 230 °C with an injection time of 1 min in splitless mode. Amounts were calculated by calibration curves with commercial standards of verbenone (Acros) and MeSA (Fluka).

Field testing repellents in SPLAT

Since the chemical analysis of SPLAT revealed both verbenone and MeSA, but the latter's behavioral effect was unknown, we conducted inhibition tests of these two compounds in Hass avocado orchards near Nahsholim, Israel. Traps consisted of sticky cylinders (25 cm long × 25.5 cm diam.) of 6-mm mesh wire screen covered with adhesive (80% polyisobutene, Rimifoot, Rimi, Petah Tikva, Israel). Traps were baited with a small glass test-tube dispenser (3.29 mm i.d. × 30.6 mm long) containing 20 µL of quercivorol (1 × dose) releasing an almost constant rate of 0.126 mg quercivorol/day at 25 °C (Byers et al. 2017). Verbenone (Sigma-Aldrich) was > 95% pure and a mixture of 74% (*S*)- and 26% (*R*)-enantiomers, while MeSA (Fluka) was > 99% pure. The verbenone and MeSA dispensers were 2-mL glass vials (5.2 mm top opening, 32 mm long; Chrom4 GmbH, Thüringen, Germany) containing 60 µL of either compound (1 × dose) releasing 0.8 or 1.3 mg/day, respectively, at 25 °C as determined by weight loss on microbalance. Treatments with dispensers of either (1) quercivorol, (2) verbenone and quercivorol or (3) MeSA and quercivorol were scotch-taped inside an inverted plastic cup covered with aluminum foil and placed centrally within a sticky trap. Each of the three treatments was replicated three times with sticky traps separated by 15 m in a line. PSHBs were collected from traps every 9 to 14 days and replicate positions re-randomized (August 19–October 2019). Trap catches of each collection were adjusted to catch/week ($N = 12$) and treatment differences analyzed by ANOVA and Tukey's HSD as described above.

In order to determine if verbenone and MeSA are synergistic in inhibiting attraction of PSHB to quercivorol, we tested a lower (one tenth) dose of both MeSA and verbenone with 1 × quercivorol compared to a lower (one tenth) dose of verbenone with 1 × quercivorol. Because 1 × doses of verbenone reduced responses to quercivorol to only ~ 10%, while a 0.1 × dose reduced response to ~ 50% (Byers et al. 2020), we hypothesized that synergism would be easier to observe at lower 0.1 doses of verbenone and MeSA. The 0.1 × dose dispensers of each compound consisted of 2-mL glass vials filled with 60 µL diluted solutions. The solutions were made by diffusion–dilution method that mixes the semiochemical in a solvent of similar volatility to obtain a mole proportion giving the desired proportion of the release rate of the neat semiochemical (Byers et al. 2020). Thus, 0.1 × verbenone (0.08 mg/day) was prepared from 102.25 µL verbenone (MW 150.21, density 0.978 g/mL) and 1.128 mL decanol (MW 158.28, density 0.829 g/mL), while 0.1 × MeSA (0.13 mg/day) was a mixture of 85.17 µL MeSA (MW 152.15, density 1.174 g/mL) and 1.1425 mL decanol. Treatments of (1) verbenone with quercivorol and (2) verbenone and MeSA with quercivorol were compared in sticky traps with three replicates of each treatment and four collections of trapped PSHB (October 3–November 24, 2019) as described above.

The test for synergism above compares 0.1 dose verbenone versus 0.1 doses of verbenone and MeSA (twice as much inhibitor). Another comparison would be to compare verbenone + MeSA to a 0.2 dose of verbenone. We did not do this test, but earlier results (Byers et al. 2020, Fig. 4) of mean catch (Y) as a function of verbenone dose (X) gave the kinetic decay relationship $Y = a + b / (1 + bcX)$, where $a = 12.02$, $b = 205.03$ and $c = 0.035$ ($R^2 = 0.99$). This function can be applied to mean catch on 0.1 dose verbenone to predict catch on 0.2 dose verbenone in order to detect synergism.

SPLAT treatments on avocado limbs

A pilot test to protect avocado trees from PSHB attack intended to use commercial 10% verbenone–SPLAT, but our analysis of the delivered material showed that it contained 5% verbenone (*S* isomer 11%, *R* isomer 89%) and 5% MeSA. Two control areas of 16 untreated trees each and two SPLAT-treated areas of 16 treated trees each were designated. Separation of areas was 100–150 m. Treated trees (4 rows of 4 trees per row) had two untreated trees in between, with one of the untreated trees selected at random as a control. On July 16, 2019, we applied ~ 1 mL dollops of SPLAT along the major trunks (> 4 cm diam.) of treated trees, with treatment areas having either 10- or 40-cm spacing between dollops. A second application of SPLAT was done on August 19, 2019, and a third application on October 7, 2019 (dollops placed on or next to previous ones). The number of aggregations per tree and number of attack holes per aggregation on treated and control trees were recorded on November 19, 2019.

Results

Mass trapping of PSHB in avocado

Mass trapping over time should cause a pest population to decline as indicated by progressively fewer captures; however, such trends could be counteracted by immigration and population buildup/emergence during the season. At Beit-Haemek, the normalized mean catch/trap-week increased linearly during the 156-day trapping period ($N=4$ collections) in the low- (3×4), medium- (5×5) and high-density (5×10) grids (all $R^2>0.8$). For example, traps in the medium-density grid increased from 0.35 ± 0.12 (±95% CI) PSHB/trap-week to 4.64 ± 1.39 at the end of the experiment, while in the high-density grid the results were similar with an increase from 0.57 ± 0.15 to 6.49 ± 1.33 at the end. In Nahsholim (July 1–October 1; $N=9$), there were no significant linear trends in catch during the season in any of the three grid densities ($R^2=0.02$, 0.08 and 0.25 in low-, medium- and high-density grids, respectively). At Nahsholim, the medium-density grid had 2.44 ± 0.93 PSHB/trap-week in the beginning and 1.66 ± 0.61 in the end, while the high-density grid attracted 1.20 ± 0.31 PSHB/trap-week in the beginning and 1.58 ± 0.33 in the end. In Kfar-Masrik (August 10–December 4; $N=4$), all three trap densities declined linearly in catch during the trapping period ($R^2=0.48$, 0.71 and 0.73, respectively). The medium-density grid had 12.01 ± 3.77 PSHB/trap-week in the beginning and 1.94 ± 0.97 in the end, while the high-density grid attracted 8.02 ± 1.80 PSHB/trap-week in the beginning and 1.86 ± 0.46 in the end. Thus, in one area mean catch/trap trended upward during the summer, in a second area no trends were observed, while in the third area catch declined during the season.

One hypothesis is that traps in a grid tend to compete for the local PSHB population such that grids with more traps in the same area would catch fewer per trap compared to traps in grids with fewer traps. However, there was no significant difference in mean trap catch/week in either low- (1.65 ± 1.27, ±95% CI), medium- (1.97 ± 1.83) or high-density (3.10 ± 2.47) grids in Beit-Haemek over the entire season ($F_{2,9}=0.61$, $P=0.56$), nor in low- (5.30 ± 4.45), medium- (7.31 ± 6.58) or high-density (4.03 ± 3.27) grids in Kfar-Masrik ($F_{2,9}=0.43$, $P=0.66$). In Nahsholim, however, the hypothesis was supported because the low density caught most (3.28 ± 0.52), the medium intermediate (2.29 ± 0.44) and the high-density traps caught least (1.40 ± 0.19) ($F_{2,24}=20.43$, $P<0.0001$; each density different from others, Tukey's HSD at $\alpha=0.05$).

The hypothesis that flight of PSHB into grids would cause outer traps to catch more per trap than inner traps (next inner ring) giving catch ratios outer/inner > 1 was supported. In the three areas, outer/inner catch ratios were > 1 in 38 cases of the three trap grid densities, which was significantly more than in 9 cases where ratios were < 1 ($X^2=17.9$, $df=1$, $P<0.0001$). At Beit-Haemek, all eight ratios were > 1 for the medium- and high-density traps, while only one of four was > 1 for the low-density traps, perhaps because of higher variation with only two inner traps. At Nahsholim, 16 of 18 ratios were > 1 for the medium- and high-density traps, while 7 of 9 ratios were > 1 for the low-density traps. At Kfar-Masrik, 7 of 8 ratios were > 1 for the medium- and high-density traps (low density ratio undefined as no inner traps). The total catches on outer and inner traps (and respective numbers of traps) in each trap density grid in the three areas (Table 1) were compared with expected catches on outer and inner traps assuming that there was no difference in catch proportions (ratio = 1) by a chi-square test. In all comparisons, the observed catch ratios were significantly > 1 except for the 3×4 grid at Nahsholim where the ratio was not quite significant (Table 1). The table also shows the ratios of mean catch/trap for outer divided by inner traps for each collection during the experimental period. These ratios from collections as well as the overall ratios for each grid and area were then used to calculate EARc (explained in the next section).

Simulation of mass trapping

The paths of 100 simulated beetles are shown moving in a correlated random walk with steps of 1 m and SDA of 10° until all intercept a trap (Fig. 1). In each grid simulation, the mean catch/trap on the outer traps along the periphery was usually higher than on the inner traps (Fig. 1). This appears due to the filtering effect of the outer traps intercepting beetles as they move into the grid. It is reasonable that in a relatively small grid of traps, most beetles would fly from outside into the grid of traps. For a 5×5 trap grid in a 0.5 ha area, the larger the radius of the traps (EARc) the larger the ratio of mean catch of an outer trap to the mean catch of an inner trap (Fig. 2). This effect was seen for all the simulated trap grids. If the EARc is very small or nearly zero, then the ratio is expected to be about one, which is indicated by the curve in Fig. 2. An inverse quadratic function fit the

Table 1 Parameters calculated for each trap grid and area: total catches on outer and inner traps of various grids in three areas, chi-square *P* values of observed versus expected catches assuming equal catch proportions on traps, mean ratio of catches ± SE for several collections, EARc estimated from simulation-generated relationships (see text) and EAR calculated from EARc (Eq. 4)

Trap grid	Outer catch (traps)	Inner catch (traps)	X^2 P[a]	Mean ratio of catches[b]	EARc (m) simulations[c]	EAR (m) simulations[d]
Beit-Haemek						
3×4	322 (10)	88 (2)	0.009	0.954 ± 0.25	–	–
5×5	764 (16)	222 (8)	< 0.0001	1.596 ± 0.31	2.06 (2.26)	1.70 (1.78)
5×10	1998 (26)	1017 (18)	< 0.0001	1.339 ± 0.05	0.83 (0.85)	1.08 (1.09)
Nahsholim						
3×4	487 (10)	77 (2)	0.055	1.542 ± 0.25	3.95 (2.35)	2.36 (1.82)
5×5	587 (16)	215 (8)	< 0.0001	1.446 ± 0.12	1.76 (1.56)	1.57 (1.48)
5×10	595 (26)	339 (18)	0.004	1.268 ± 0.10	0.73 (0.64)	1.01 (0.95)
Kfar-Masrik						
4×5	1031 (14)	290 (6)	< 0.0001	1.425 ± 0.15	1.79 (2.02)	1.59 (1.68)
4×10	1249 (24)	703 (16)	0.0003	1.170 ± 0.12	0.57 (0.60)	0.89 (0.92)

[a] Chi-square of outer and inner catches and trap numbers compared to expected values assuming catches proportioned equally to all traps in the outer and inner rings (df = 1)

[b] Means ± SE were calculated from catches on trap grids collected four times at Beit-Haemek and at Kfar-Masrik and nine times at Nahsholim

[c] EARc calculated from corresponding mean ratios of catch (table column 5), or EARc in parentheses from corresponding ratios of total caches on outer and inner traps (columns 2 and 3)

[d] EAR calculated from corresponding EARc at left, or EAR in parentheses from corresponding EARc in parentheses at left, according to Eq. 4 and SD = 0.88 for PSHB (see text)

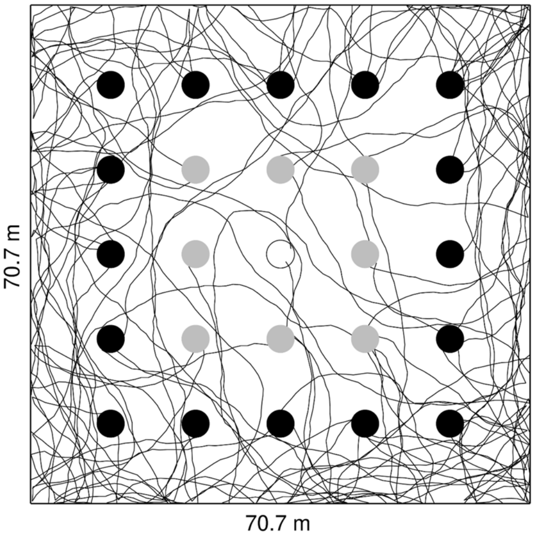

Fig. 1 Movement paths of 100 simulated beetles in flight moving at 1-m steps with possible turns from the previous direction at each step randomly selected from a normal distribution with SDA = 10° until all were captured by one of 25 circular traps (trap EARc = 2 m). Beetles initially began their flight from the periphery of the 0.5 ha area. A catch of 71 on 16 outer traps (black disks) compared to 27 caught on 8 inner traps (gray disks) yields a ratio of mean trap catches of outer to inner traps of 1.315 (giving an estimated EARc of 1.42 m from Eq. 1)

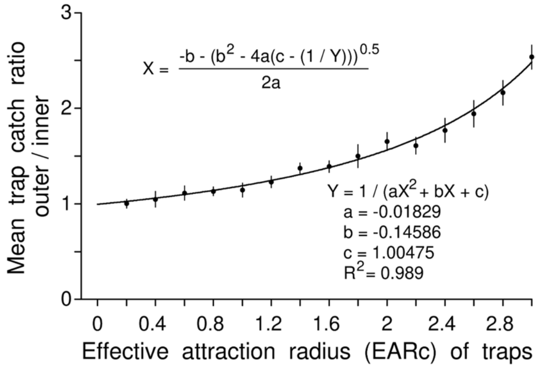

Fig. 2 Mean trap catch ratio of 16 outer to 8 inner simulated traps in a 5×5 grid within a 0.5 ha square area (70.7 m sides, Fig. 1) as a function of trap effective attraction radius (EARc). Points represent means of ratios of eight simulations of 1000 beetles each (individuals moved in 1-m steps with SDA of 10° until all were caught by one of the 25 traps, vertical lines through points are 95% CI)

data well, and in fact the opposite inner/outer ratio is fit by a quadratic function. The relationship between the outer/inner trap catch ratio (*Y*) and the trap EARc (*X*) based on the simulations is given by the following function (Eq. 2):

$$Y = \frac{1}{aX^2 + bX + c} \tag{2}$$

and solving for *X* gives (Eq. 3):

$$X = \frac{-b - \sqrt{b^2 - 4a\left(c - \frac{1}{Y}\right)}}{2a} \tag{3}$$

Regression results for X and Y for each of the simulated grids were as follows: 3 × 4 grid, $a = -0.001505$, $b = -0.0796$ and $c = 0.98602$ ($R^2 = 0.970$); 4 × 5 grid, $a = -0.1776$, $b = -0.13236$ and $c = 0.99596$ ($R^2 = 0.987$); 4 × 10 grid, $a = 0.01743$, $b = -0.39427$ and $c = 1.07433$ ($R^2 = 0.998$); 5 × 5 grid, $a = -0.01829$, $b = -0.14586$, $c = 1.00475$ ($R^2 = 0.989$); and 5 × 10 grid, $a = 0.05125$, $b = -0.49251$ and $c = 1.1188$ ($R^2 = 0.997$). The coefficients for each grid can be used in both Eqs. 2 and 3. However, Eq. 3 is useful to analyze the trap ratios of catch (Y) obtained in the field in various trap grids at different localities and trapping dates (Table 1) in order to estimate the EARc (X) of these baited traps. The outer/inner trap catch ratios from the field were used as Y in Eq. 3 with the appropriate coefficients above for simulated grids to estimate EARc for the barrier (multiple-funnel) traps baited with 4.5 mg/day quercivorol (Table 1). The EARc in 2D can be converted to EAR in 3D by solving Eq. 1 for EAR as shown in Eq. 4:

$$\mathrm{EAR} = \sqrt{\frac{\left(2 \cdot \mathrm{SD} \cdot \sqrt{2\pi}\right) \cdot \mathrm{EARc}}{\pi}} \tag{4}$$

Release rates of SPLAT volatiles

The release of both verbenone and MeSA from 1 g of verbenone–MeSA–SPLAT declined exponentially over 45 days at 25 °C (Fig. 3). Chiral GC–MS analysis of the SPLAT showed that its verbenone was composed of 11% S-(−)- and 89% R-(+)-enantiomers.

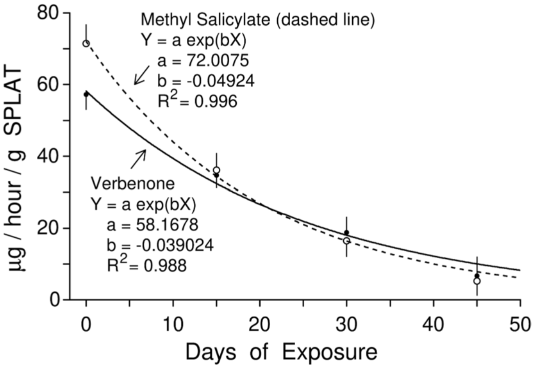

Fig. 3 Exponential decline in release rates of verbenone and methyl salicylate (MeSA) from 1 g verbenone–MeSA–SPLAT dollops in the laboratory at 25 °C over 45 days as determined by Porapak SuperQ adsorption, solvent extraction and analysis by simultaneous GC-FID and GC–MS. Vertical lines above or below points are SE

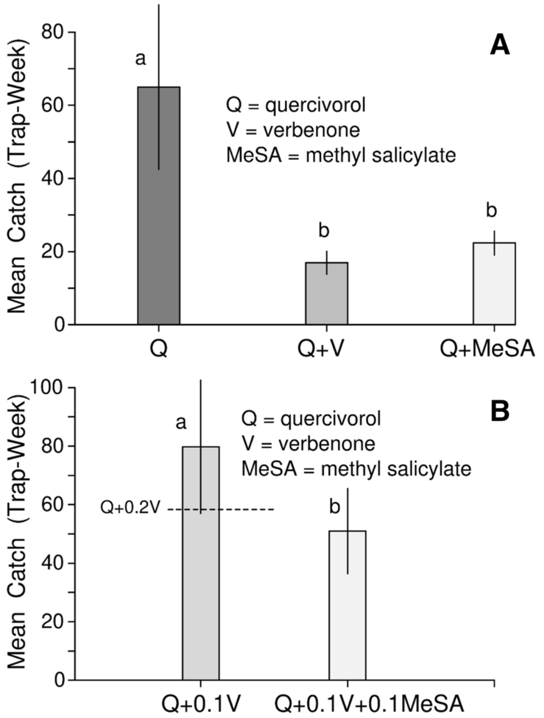

Fig. 4 **a** Inhibition of PSHB attraction to quercivorol by either verbenone or methyl salicylate ($F_{2,33} = 15.17$, $P < 0.0001$) in field test (August 19–October 3, 2019), Nahsholim, Israel. Error bars ± 95% CI of means ($N = 12$; means with the same letters were not significantly different, Tukey's HSD $\alpha = 0.05$). **b**. Test of synergism of verbenone and methyl salicylate ($F_{1,22} = 4.36$, $P = 0.0485$) in field test (October 3–November 24, 2019), Nahsholim, Israel. Means ($N = 12$) with error bars as above; the dashed line shows expected mean catch with a 0.2 dose of verbenone presented with quercivorol (see text for details)

Field testing repellents in SPLAT

The field tests of verbenone and MeSA provide strong evidence that MeSA is also an inhibitor of PSHB attraction to quercivorol (Fig. 4a). While verbenone reduced attraction of PSHB a little more than MeSA, the repellencies by either compound were not significantly different. Thus, it was fortuitous that the volatiles in ISCA SPLAT we tested had two active ingredients, but were they synergistic? Our tests of verbenone and MeSA indicated that there could be a weak synergism because both together gave a lower catch than verbenone alone and the difference was significant, but only just at $P = 0.0485$ (Fig. 4b). As mentioned earlier, a second way to test for synergism would be to compare the combined 0.1 doses of both inhibitors (total 0.2) versus a 0.2 dose verbenone using a previously documented relationship. This function predicts a mean catch $Y = 131.4$ at 0.1 dose verbenone and $Y = 96.2$ at 0.2 dose verbenone

(96.2 is 73.2% of 131.4). Therefore, if the predicted mean catch for 0.1 + 0.1 verbenone is 73.2% as much as at 0.1 verbenone (with mean 79.75 in Fig. 4b), then this gives a predicted mean catch of 58.4 for 0.2 dose of verbenone (dashed line in Fig. 4b). This suggests the difference in mean catches for 0.2 dose verbenone and both inhibitors together (total 0.2 dose) was not statistically significant, and thus synergism is not supported.

SPLAT treatments on avocado limbs

The two control areas C1 and C2 had 32 trees, and their mean aggregations per tree and mean attacks per aggregation were similar to the 16 control trees adjacent to either the 10-cm spacing or adjacent to the 40-cm dollop spacing treatment trees (Fig. 5a, b). The 10-cm dollop spacing treatment, however, had only about half as many aggregations per tree and half the number of attacks per aggregation compared to the 150 m distant C1 + C2 control trees (both $P < 0.01$) as well as to the C10 adjacent control trees (both $P < 0.05$, Fig. 5a). The 40-cm dollop spacing treatment (Fig. 5b) also had about half the mean aggregations per tree and half the attacks per aggregation compared to the distant C1 + C2 control trees ($P < 0.01$) and to the adjacent C40 trees, but this latter comparison with adjacent trees was not quite significantly different ($P = 0.06$). It appears that the 40-cm dollop spacing was nearly as effective as the 10-cm spacing in reducing aggregations per tree and number of attacks per aggregation. Unfortunately, during the second and third treatments we noted that many avocado trunks had extrusions of sawdust-like powder immediately surrounding each SPLAT dollop. Four months later, the bark under several dollops was cut away to reveal localized darkened areas extending halfway through the phloem layer.

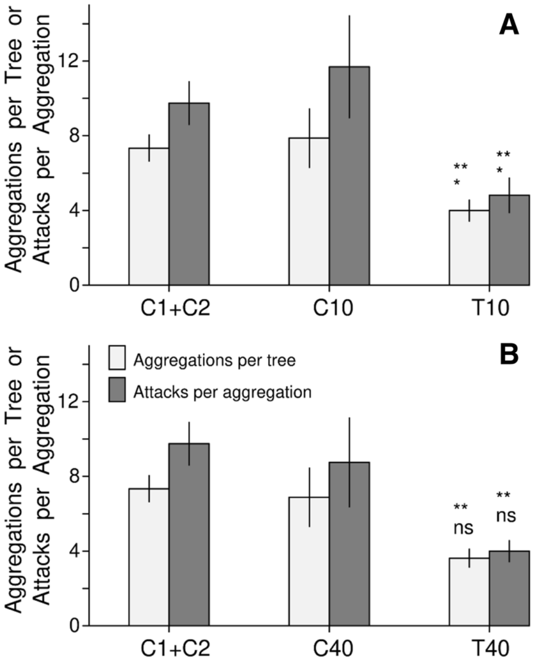

Fig. 5 **a**. Mean number of PSHB aggregations per avocado tree (light bars) and mean number of attacks per aggregation (shaded bars) on trees in two control areas (C1 + C2) about 100 m from treatment areas, on control trees (C10) next to treated trees and on treated trees (T10) with 10-cm spacing of verbenone + MeSA–SPLAT dollops along trunks and limbs (November 19, 2019, Nahsholim, Israel). **b**. Mean numbers of aggregations and attacks per aggregation on control trees in two 100-m distant areas (C1 + C2), on control trees (C40) adjacent to treated trees and on treated trees (T40) with 40-cm spacing of SPLAT along trunks (same date and place as above). Asterisks denote significantly less aggregations or number of attacks when the treatments were compared to controls C1 + C2 (upper asterisks) or to nearby control trees (lower asterisks). Vertical lines at top of bars represent ± SE ($N = 16$). Significant differences (*t* tests) are designated at *$P < 0.05$ and **$P < 0.01$

Discussion

Because the trap grids were relatively small (12, 25 and 50 traps), any local effects of mass trapping were likely overwhelmed by populations of PSHB moving into the grid areas. During the trapping season, flying populations of PSHB appeared to increase at Beit-Haemek but decrease at Kfar-Masrik, while no trend was apparent at Nahsholim (2.4 captures initially and ending at 1.7 captures per trap-week). No control plots in the three areas were monitored for population levels. However, other experiments at Nahsholim about 250 m away with quercivorol traps during the same period in 2017 indicate that populations were increasing from 1.7 to 15 beetles per trap-week (Byers et al. 2018), so the trapping grids may have decreased PSHB locally. The idea that grids with more traps at higher density would catch fewer beetles per trap due to competition among traps (Miller 2006; Jamieson et al. 2008; Miller et al. 2015) was not supported in two areas, but was supported at Nahsholim where the plot with the highest number and density of traps did catch significantly fewer PSHB per trap. The most definitive results were found between mean trap catches on outer traps that were significantly larger than on traps in the inner ring of the grids.

In all three locations, most grids of any trap density and collection date had higher mean catches on peripheral (outer) traps than on traps in the next inner ring (Table 1). This suggests that PSHBs were flying into the grid areas to be intercepted by the peripheral outer traps and that there were no significant sources of PSHB emerging within the grids. These observed catch ratios were compared to simulated results of respective grids and areas in which EARc was incrementally increased to change the outer/inner ratios from about 1 (no differences in catches between outer and inner traps) to a much > 1 ratio (Fig. 2). The relationship fit an inverse quadratic relationship that was unique for each grid (Eq. 2). By solving the relationships for EARc (Eq. 3), the observed ratios from the field trapping were used to estimate the EARc of the quercivorol-baited funnel traps. The simulation results with field catch ratios for the small 3 × 4 trap grid at Nahsholim estimated an EARc of 3.95 m (mean of collections) or 2.35 m (total catch) (Table 1). The medium-density grids (5 × 5) of 25 traps had estimated EARc that ranged from 1.76 to 2.06 m (mean) or 1.56 to 2.26 m (totals), while the high-density grids (5 × 10) of 50 traps had the lowest estimated EARc from 0.57 to 0.83 m (mean) or 0.6 to 0.85 m (totals). The relationship between quercivorol release and EARc for isolated sticky traps predicts that 4.5 mg/day has an EARc of 3.23 m (Byers et al. 2020), which is generally larger than the values above estimated from catch ratios. EAR and EARc should remain constant for isolated traps of a particular bait dose. However, these values may become smaller in higher-density grids because trap separation distance decreases causing greater interactions among pheromone plumes that increasingly disrupt PSHB orientation (Suckling et al. 2015). Smaller EARc estimates can additionally result because multiple-funnel traps are less efficient in catching PSHB. The 2D EARc values can be converted to 3D EAR (Table 1) using Eq. 4.

Attraction of PSHB to quercivorol was shown to be substantially reduced by either enantiomer of verbenone as well as by piperitone (Byers et al. 2018, 2020). In the present study, we compared (−)-verbenone and methyl salicylate (MeSA) for their ability to reduce attraction to quercivorol and found that each reduced catch to 26.1% and 34.4%, respectively, that of quercivorol alone. Both repellents together reduced response more than either alone, but when compared to the dose–response curve of verbenone at the same 0.2 dose, no synergism at the dose tested was evident (Fig. 4b). Several studies have reported that verbenone inhibits attraction of ambrosia beetles to ethanol or to host logs. For example, the attraction of ambrosia beetle *Trypodendron domesticum* to 800 mg ethanol/day was reduced to 48% by release of 0.25 mg (−)-verbenone/day (Byers 1992). The ambrosia beetles *Xylosandrus compactus*, *X. crassiusculus* and *Xyleborinus saxesenii* are attracted to ethanol, but 2 mg (−)-verbenone/day reduced this response to 58, 19.3 and 48.1%, respectively (Burbano et al. 2012). Hughes et al. (2017) found that attraction of ambrosia beetle *Xyleborus glabratus* to host redbay logs was reduced by 17.5 g of 10% verbenone–SPLAT to 5.5%, while 10% MeSA–SPLAT reduced catch to 36.7% that of the positive control. They also tested verbenone + MeSA (each 5%) SPLAT that reduced catch to 8.8% indicating a lack of synergism. Rivera et al. (2020) placed dollops of SPLAT of either 10% verbenone or 10% MeSA in avocado trees and placed sticky traps baited with ethanol (15 mg/day) either 10 cm or 1.2 m from repellents. Either MeSA– or verbenone–SPLAT significantly reduced catches of several ambrosia beetle species especially at 10 cm, although verbenone appeared more effective. Synergism of both repellents was not tested.

Although verbenone, piperitone and MeSA represent dissimilar chemical structures that probably excite different antennal receptors of PSHB, any of these signals to the brain may represent a non-host or unsuitable host to avoid. Similarly, the attraction of bark beetle *Pityogenes bidentatus* to aggregation pheromone is drastically reduced by volatiles from non-hosts birch and Norway spruce and even by host odors of freshly cut (un-aged) Scotch pine (Byers et al. 2004). They also showed that several synthetic conifer monoterpenes and green leaf volatiles reduced attraction, but no statistically significant effect of verbenone.

Our study indicates that 1 g dollops of verbenone–MeSA–SPLAT treated either at 10- or 40--cm spacings along major trunks of avocado trees significantly reduced both aggregations and attacks/aggregation to about half that on control trees (Fig. 5). As mentioned above, Hughes et al. (2017) also protected redbay logs from landing and boring by *X. glabratus* with MeSA– and verbenone–SPLAT. The disadvantage of SPLAT is that the active volatiles verbenone and MeSA diffuse to the surface to be released in an exponentially declining rate as shown by our chemical aeration analyses (Fig. 3). SPLAT releases volatiles similar to rubber septa that initially dispense insect pheromones at maximum amounts that then decline exponentially (Flint et al. 1978; McDonough et al. 1989; Downham et al. 1999). Our release curves for verbenone–MeSA–SPLAT explain why the repellency of *X. glabratus* declined over a month in the field (Hughes et al. 2017) and in our study likely resulted in less efficiency before replenishment. For example, the active volatiles from SPLAT declined to 15% after 50 days (Fig. 3).

Another drawback with SPLAT is that avocado limbs with applied dollops extruded a sawdust-like powder composed partly of D-mannoheptulose and perseitol (Tesfay et al. 2012) for about 1 cm around each dollop and the phloem beneath suggested phytotoxic effects. MeSA is considered as a phytohormone (Park et al. 2007) and reported to be phytotoxic to several plants (Ibáñez and Blázque 2019a, 2019b). Thus, research is needed to determine the source of phytotoxicity. Alternatively, plastic capsules/bags dispensing volatiles of verbenone/piperitone a few mm from avocado bark probably would not harm the tissues. After 10 days of exposure, a gram of SPLAT released verbenone at 39.4 µg/h or 0.95 mg/day (Fig. 3). Since completing our studies, we placed 200 µl verbenone inside heat-sealed polyethylene sleeves (5 × 2 cm × 0.15 mm film thickness) held at 25 °C and found a constant release of 0.14 mg/day/cm^2. Thus, a flat 2 × 2 cm bag (8 cm^2 surface) would release an active amount of 1.1 mg verbenone per day, with 250 mg lasting the entire flight season (May–November).

The push–pull method with mass trapping and repellents on avocado trunks where aggregations occur should be initiated in May to outcompete single females before larger aggregations can form. In Israel, PSHB populations are mitigated by removal of infested avocado limbs and alternative hosts within orchards, a practice that is complementary to a push–pull system. Natural enemies of PSHB are not known to be affected by push–pull with quercivorol and ideally repellent bags containing verbenone or piperitone. Mass trapping of PSHB where the egg-laying females are targeted has an advantage over mating disruption or mass trapping of moths in which only the males are captured. The disadvantage, however, is that PSHB is polyphagous, so avocado orchards may not be isolated as in some successful mass trapping programs for bark beetles and moths (Schlyter et al. 2000; Levi-Zada et al. 2018). Mass trapping is recognized as being more effective at lower population densities in reducing mating because searching individuals on average must travel farther to encounter the opposite sex or aggregations and thus are more likely to encounter traps (Miller 2006; El-Sayed et al. 2006; Byers 2012b; Miller et al. 2015). Push–pull becomes more effective on larger scales as explored by simulations (Byers et al. 2018, unpublished), and thus larger pilot tests and control treatments in the field are best conducted by growers and companies.

Acknowledgments We thank Kibbutz Ma'agan Michael avocado growers for assistance in setup and use of their avocado orchards for field experiments. We are grateful to ISCA, USA, that contributed the verbenone–methyl salicylate–SPLAT tubes. Also, thanks are due to Mr. Ithamar Zada and Mr. Amnon Magnus for field assistance with SPLAT applications.

Author contributions JB, AZ, BC and YM conceived and designed research. All authors conducted the experiments. JB and AZ analyzed the data and wrote the manuscript. JB generated simulations. All authors read and approved the manuscript.

Funding This research was supported in part by Israel Avocado Growers Association grant #0390320 (2018 and 2019).

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors.

References

Borden JH, Birmingham AL, Burleigh JS (2006) Evaluation of the push-pull tactic against the mountain pine beetle using verbenone and non-host volatiles in combination with pheromone-baited trees. Chron 82:562–590

Burbano EG, Wright MG, Gillette NE, Mori S, Dudley N, Jones T, Kaufmann M (2012) Efficacy of traps, lures and repellents for *Xylosandrus compactus* (Coleoptera: Curculionidae) and other ambrosia beetles on *Coffea arabica* plantations and *Acacia koa* nurseries in Hawaii. Environ Entomol 41:133–140

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 (1993) Orientation of bark beetles *Pityogenes chalcographus* and *Ips typographus* to pheromone-baited puddle traps placed in grids: a new trap for control of scolytids. J Chem Ecol 19:2297–2316

Byers JA (1999) Effects of attraction radius and flight paths on catch of scolytid beetles dispersing outward through rings of pheromone traps. J Chem Ecol 25:985–1005

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

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, 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, Wood DL (1981) Interspecific effects of pheromones on the attraction of the bark beetles, *Dendroctonus brevicomis* and *Ips paraconfusus* in the laboratory. J Chem Ecol 7:9–18

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 *fornicatus* (Coleoptera: Curculionidae) to quercivorol and to infestations in avocado. J Econ Entomol 110:1512–1517

Byers JA, Maoz Y, Wakarchuk D, Fefer D, Levi-Zada A (2018) Inhibitory effects of semiochemicals on the attraction of an ambrosia beetle *Euwallacea* nr. *fornicatus* to quercivorol. J Chem Ecol 44:565–575

Byers JA, Maoz Y, Fefer D, Levi-Zada A (2020) Semiochemicals affecting attraction of ambrosia beetle *Euwallacea fornicatus* (Coleoptera: Curculionidae: Scolytinae) to quercivorol: developing push-pull control. J Econ Entomol 113:2120–2127

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

Carrillo D, Narvaez T, Cossé AA, Stouthamer R, Cooperband M (2015) Attraction of *Euwallacea* nr. *fornicatus* (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. *fornicatus* in Florida avocado groves. Insects 7:55–11

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 fornicatus* species complex (Coleoptera: Curculionidae: Scolytinae), recently invasive in the U.S.A., reared on an ambrosia beetle artificial diet. Agric For Entomol 18:223–237

Downham MCA, Smit NEJM, Laboke PO, Hall DR, Farman DI, Braun A, Odongo B (1999) Specificity of response to sex pheromones among sweetpotato weevils, *Cylas puncticollis* and *C. brunneus*. J Chem Ecol 25:591–609

El-Sayed AM, Suckling DM, Wearing CH, Byers JA (2006) Potential of mass trapping for long-term pest management and eradication of invasive species. J Econ Entomol 99:1550–1564

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 fornicatus) 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

Flint HM, Butler L, McDonough LM, Smith RL, Forey DE (1978) Pink Bollworm: response to various emission rates of gossyplure in the field. Environ Entomol 7:57–61

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. *fornicata*. Symbiosis 58:245–251

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 141:653–664

Hulcr J, Stelinski LL (2017) The ambrosia symbiosis: from evolutionary ecology to practical management. Ann Rev Entomol 62:285–303

Ibáñez MD, Blázquez MA (2019a) Tea tree and wintergreen essential oils in the management of the invasive species *Cortaderia selloana* and *Nicotiana glauca*. J Plant Protect Res 59:160–169

Ibáñez MD, Blázquez MA (2019b) Phytotoxic effects of commercial *Eucalyptus citriodora*, *Lavandula angustifolia* and *Pinus sylvestris* essential oils on weeds, crops and invasive species. Molecules 24:2847

Jamieson LE, Suckling DM, Ramankutty P (2008) Mass trapping of *Prays nephelomima* (Lepidoptera: Yponomeutidae) in citrus orchards: optimizing trap design and density. J Econ Entomol 101:1295–1301

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 Euwallacea nr. fornicatus (Coleoptera: Curculionidae: Scolytinae). PLoS ONE 12(6):e0179416

Levi-Zada A, Sadowsky A, Dobrinin S, Ticuchinski T, David M, Fefer D, Dunkelblum E, Byers JA (2018) Monitoring and mass-trapping methodologies using pheromones: the lesser date moth *Batrachedra amydraula*. 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

McDonough LM, Browne DF, Aller WC (1989) Insect sex pheromones effect of temperature on evaporation rates of acetates from rubber septa. J Chem Ecol 15:779–790

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. *fornicatus* and its novel symbiotic fungus *Fusarium* sp. pose a serious threat to the Israeli avocado industry. Phytoparasitica 40:235–238

Mendel Z, Protasov A, Maoz Y, Maymon M, Miller G, Elazar M, Freeman S (2017) The role of *Euwallacea* nr. *fornicatus* (Coleoptera: Scolytinae) in the wilt syndrome of avocado trees in Israel. Phytoparasitica 45:341–359

Miller JR (2006) Differentiation of competitive vs. non-competitive mechanisms mediating disruption of moth sexual communication by point sources of sex pheromone (Part I): theory. J Chem Ecol 32:2089–2114

Miller JR, Cowles RS (1990) Stimulo-deterrent diversionary cropping: a concept and its possible application to onion maggot control. J Chem Ecol 16:3197–3212

Miller JR, Adams CG, Weston PA, Schenker JH (2015) Trapping of small organisms moving randomly. Springer, New York, p 114

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 Gen Biol 82:277–290

Park SW, Kaimoyo E, Kumar D, Mosher S, Klessig DF (2007) Methyl salicylate is a critical mobile signal for plant systemic acquired resistance. Science 318:113–116

Rivera MJ, Martini X, Conover D, Mafra-Neto A, Carrillo D, Stelinski LL (2020) Evaluation of semiochemical based push-pull strategy for population suppression of ambrosia beetle vectors of laurel wilt disease in avocado. Sci Rep 10:2670

Schlyter F, Zhang QH, Liu GT, Ji LZ (2001) A successful case of pheromone mass trapping of the bark beetle *Ips duplicatus* in a forest island, analyzed by 20-year time-series data. Integr Pest Manage Rev 6:185–196

Smith SM, Gomez DE, Beaver RA, Hulcr J, Cognato AI (2019) Reassessment of the species in the *Euwallacea fornicatus* (Coleoptera: Curculionidae: Scolytinae) complex after the rediscovery of the "lost" type specimen. Insects 10:261. https://doi.org/10.3390/insects10090261

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 19:366–375

Suckling DM, Stringer LD, Kean JM, Lo PL, Bell V, Walker JTS, Twidle AM, Jiménez-Pérez A, El-Sayed AM (2015) Spatial analysis of mass trapping: how close is close enough? Pest Manag Sci 71:1452–1461

Tesfay SZ, Bertling I, Bower JP (2012) D-mannoheptulose and perseitol in 'Hass' avocado: metabolism in seed and mesocarp tissue. South African J Bot 79:159–165

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

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