Final Report to Israel Avocado Growers Association grant (2016-2018):

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

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Introduction

Euwallacea sp. near *fornicatus*, also known as the polyphagous shot hole borer (PSHB) is an ambrosia beetle (Coleoptera: Curculionidae: Scolytinae) that invaded California as early as 2003, and shortly thereafter Israel (Eskalen et al. 2012, Mendel et al. 2012, Freeman et al. 2012). PSHB appears to be the same species in both California and Israel and is closely related to *Euwallacea fornicatus* (Eichhoff) from Sri Lanka (the tea shot hole borer, TSHB) that has become a pest in Florida (Carrillo et al. 2015, 2016; Cooperband et al. 2016). These two species originated in Southeast Asia and are morphologically identical but differ enough in DNA to be considered distinct species (Eskalen et al. 2013, O'Donnell et al. 2015, Stouthamer et al. 2017). PSHB has a relatively broad host range of woody shrubs and trees and can be a serious pest because it may carry Fusarium dieback disease (Freeman et al. 2012, Eskalen et al. 2013, Cooperband et al. 2016, Lynch et al. 2016). In Israel, PSHB (O'Donnell et al. 2015) is potentially a serious problem in avocado *Persea americana* Mill. because, although the tree does not usually die from the disease, the beetle does kill infested limbs and reduces tree growth over a period of years (Freeman et al. 2012, Mendel et al. 2012). Like many scolytid beetles, this ambrosia beetle carries symbiotic fungal species that grow in the adult and larval tunnels in the sapwood and serve as food (Wood 1982, Freeman et al. 2012, Hulcr and Stelinski 2017). Only the females of PSHB and TSHB appear to leave the brood tree after mating, as the males are not capable of flight (Calnaido 1965, Carrillo et al. 2015). Our observations of PSHB and that of others (Eskalen et al. 2013) suggest that females generally do not bore alone into their host avocado tree but are commonly found together in an aggregation of females in a relatively concentrated area of a branch or branches. We demonstrated for the first time that PSHB is attracted to aggregations of females on limbs of avocado trees (Byers et al. 2017, 2018).

Ethanol is commonly reported to be attractive to ambrosia beetles and some bark beetles (Byers 1989, 1992). Carrillo et al. (2015) based on catch data from Florida suggested that ethanol together with a monoterpene alcohol (quercivorol) are weakly attractive to TSHB. However, in Carrillo et al.'s study ethanol was released in all formulations, so it is not clear whether quercivorol alone is attractive or enhances attraction to ethanol. Quercivorol, (1S,4R)-p-menth-2-en-1-ol, is known as the aggregation pheromone of another ambrosia beetle *Platypus quercivorus* (Murayama) that colonizes oaks in Japan (Tokoro et al. 2007). Therefore, we tested quercivorol on PSHB in Israel in 2016 and showed the chemical was highly attractive (Byers et al. 2017). We also calculated the "strength" of the attraction of a quercivorol-baited trap by means of a method called the EAR (effective attraction radius). The EAR gives the capture power of a specific lure-trap that is equivalent to what the size of a spherical sticky trap (with no attractant) must be in order to capture the same number of insects. A small radius EAR relative to other EAR of insect pheromones indicates a weak lure, while a relatively large radius EAR indicates a potent lure. The EAR of a specific lure-trap is important to know when determining the practical number of baited traps needed to reduce a population of pest insects by mass trapping (Byers 2009, 2011). We also conducted a dose-response relationship between increasing release of quercivorol and resulting attraction (numbers caught) of PSHB (Byers et al. 2017). This allowed us to predict the best release rate of quercivorol in order to attract the most PSHB. We also used quercivorol in sticky traps at six heights from 0.25 m to 5.75 m to determine the mean flight height of PSHB (1.24 m) and SD of the vertical flight distribution (0.88 m). The SD is used to convert EAR to EARc (effective attraction radius in two dimensions) for use in computer simulations of mass trapping (Byers et al. 2017).

In 2017, we continued with an expanded dose-response curve (0, 0.01, 0.1, 1x = 0.126 mg/day, 10x, and 108x) of quercivorol (Byers et al. 2018). The dose-response curve fit a kinetic formation function, which means that any release rate can predict the relative number of PSHB attracted to a trap. We wrapped naturally infested limbs of living avocado trees 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, shown attractive to TSHB (Carrillo et al. 2015), was released over a 64- fold range (lowest rate of 7.5 mg/day) and proved to be moderately inhibitory of PSHB attraction to 1× quercivorol. β-caryophyllene and eucalyptol did not appear to affect attraction of PSHB at the rates tested. We performed a field test of potential inhibitors of 1× quercivorol using ~1 mg/day releases of monoterpene ketones: (−)-(S)-verbenone, (+)-(R)-verbenone, 3-methyl-2-cyclo-hexen-1-one (also called MCH or seudenone), piperitone, (+)-(S)-carvone, and racemic cryptone. Only piperitone and the two enantiomers of verbenone were strongly inhibitory (Byers et al. 2018). 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 (Byers et al. 2018). 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. Push-pull computer simulations of moving beetles were performed in 1 ha plots with 2, 4, or 16 traps of 10× EARc and 400 trees (0, 1, or 3 inhibitors per tree) of which ten had an infested limb (EARc = 0.5 m). The simulations indicate that push-pull methods would be more effective in reducing PSHB mating than simply using mass-trapping alone (Byers et al. 2018).

Based on the previous work above, a mass-trapping test was conducted by Tapazol Chemical Works Ltd. (Tapazol) and analyzed in part below. Also, a push-pull test in 2018 was conducted by Tapazol Co. with our consultation. In addition, we wanted to test multiple repellents nearby an attractive source of quercivorol. This was to investigate the effects of such repellents in a practical setting of infested limbs and repellents placed in the avocado tree. Another experiment was to test a dose-response curve of piperitone repellent in order to optimize its release rate in push-pull control treatments. A preliminary investigation into the volatiles emitted by PSHB-infested avocado limbs was also desired using absorbents that collect odors that can then be extracted by solvent and analyzed by gas chromatograph mass spectrometry. These experiments of 2018 are reported below.

Experiment I - (12 Sept. – 5 Nov. 2018)

Effect of nearby piperitone repellents on attraction of PSHB to quercivorol. The purpose of this experiment was to determine the effect of an increasing number of nearby piperitone inhibitors on the attraction of PSHB to a 1x dose of quercivorol.

Methods: A 1x quercivorol lure (20 µl in 3.29 mm i.d. × 30.6 mm glass tube releasing 0.126 mg/day at 25 °C; Byers et al. 2017) was placed inside an inverted plastic cup covered with aluminum foil to protect from sun and rain. The cup was attached within a vertically-aligned tubular sticky-screen trap (6-mm mesh, 25 cm high x 25.5 cm diam.) placed at 1.2 m height as described earlier (Byers et al. 2017, 2018). The piperitone inhibitors were also fixed inside foil-covered plastic cups at 1.2 m height but without sticky traps; and either one, two, or three inhibitors were placed 0.75 m away from a quercivorol-baited trap (inhibitors were maximally spaced to surround the attractant trap, Fig. 1). A quercivorol-baited trap alone served as a control. The 1x piperitone inhibitor (Byers et al. 2018) was released at 0.52 mg/day (25 °C) from a 2-ml glass vial containing 60 µl neat piperitone (95% pure, 75% *R*-enantiomer, Synergy Semiochemicals). Each of the four treatment arrangements was replicated three times for a total of twelve groups randomized by position in a line with treatments separated by 15 m in a Hass-variety avocado orchard 2.5 km east of Nahsholim, Israel (32°,36′,31” N; 34°,56′,49” E). PSHB were picked from the traps every week to 10 days and replicate positions re-randomized by position in the line (12 Sept. – 5 November 2018). The catch on traps of each collection was adjusted to catch per week for a total of 15 replicates for each treatment and analyzed by ANOVA with significant differences between pairs of treatments indicated by Tukey’s HSD at α = 0.05 (JMP 4.0.4, SAS Institute Inc., USA). Non-linear regression software (TableCurve 2D version 5.01, Systat Software Inc., Chicago, USA) was used to find a decay function that fit the dose-response data well (Byers 2013).

Fig. 1. Central trap with 1x quercivorol surrounded by piperitone repellents (0, 1, 2, or 3) in avocado.

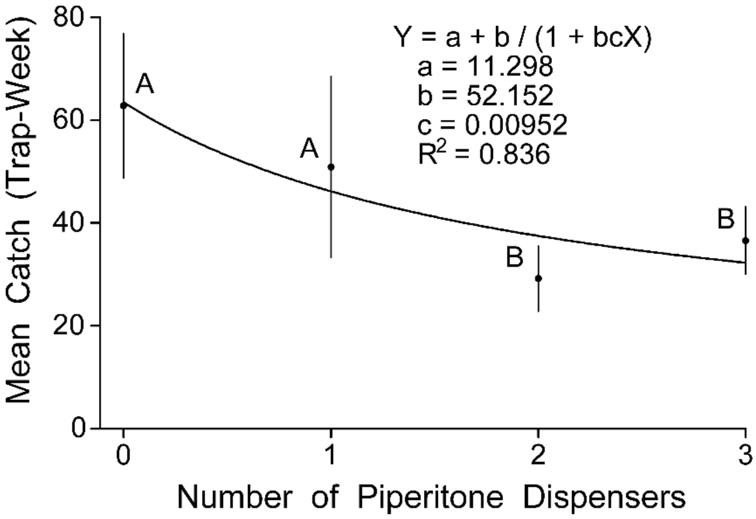

Fig. 2. Reduction in attraction of PSHB to quercivorol in relation to the number of piperitone repellent dispensers at 0.75 m distance. Error bars are ±95% CL (N = 15). Means with the same letters were not significantly different (α = 0.05, ANOVA and Tukey’s HSD).

Results: The mean catch per trap per week on the control quercivorol bait without a nearby piperitone repellent caught the most PSHB, while the presence of one repellent 0.75 m away caused a decline in mean catch - but this was not significant statistically (Fig. 2). However, two or three piperitone repellents did significantly decrease the mean catch (Fig. 2). A second order decay function fit the data well (R^2 = 0.836, adjusted R^2 = 0.507) but several other decay curves also fit the data well, although they gave similar curves.

Discussion: The use of more piperitone dispensers surrounding the 1x quercivorol attractant would likely further decrease catch but probably in diminishing amounts. The curve (Fig. 2) predicts that three piperitone dispensers would give a mean catch per week of 32 under the experimental trapping conditions, but even with 8 such dispensers the catch is predicted to decline to 22 per trap-week. This indicates that multiple repellent dispensers at a distance of 0.75 m have limited efficiency in the “push” of PSHB away from an attractive source. It would seem more effective to place the repellent on branches right next to where any attraction might begin. We will attempt to apply a splat mixture on avocado branches if funded next year. A splat mixture containing verbenone (a repellent of PSHB about as effective as piperitone, Byers et al. 2018) is commercially available for pine bark beetle suppression in North America.

Tapazol (contact Mr. Barak Cohen) set up a push-pull control test (in consultation with Byers and Maoz) that used attractive quercivorol traps as well as three repellent piperitone dispensers spaced vertically at each tree’s center. This vertical arrangement may or may not be as effective as the triangular arrangement above. However, it is somewhat probable that any arrangement will be only marginally effective if the repellents are placed some distance away from the attractive sources of PSHB. While other arrangements and numbers of repellents can be tested (as proposed in Dec. 2018), it is probably more productive to test verbenone (commercially available) instead of piperitone in a splat mixture directly on the main trunks and limbs where any source of PSHB attraction would occur naturally. A verbenone splat mixture mentioned above is relatively inexpensive and would be easy to test on avocado trees, presuming that unattacked trees can be found to treat.

Experiment II – (16 Sept. – 21 Nov. 2018).

Dose-response of repellent piperitone on attraction of PSHB to quercivorol. From our earlier work (Byers et al. 2018), we showed that 1x piperitone together with 1x quercivorol caused the attraction of PSHB to decrease to only 10%. The question arises then, what effect does a lower or higher release rate of piperitone have on the attraction to a quercivorol trap? We expected a 10x higher dose to be somewhat more effective, while it is possible that a one-tenth release would still be rather effective.

Fig. 3. Sticky trap with 1x quercivorol and different dosages of repellent piperitone (0, 0.01, 0.1, 1, 10x).

Methods: To investigate this, piperitone was released at 0, 0.01x, 0.1x, 1x, and 10x doses together with a 1x dose of quercivorol within a plastic cup inside a sticky trap as above. The 0.01 and 0.1x doses were prepared by dilution of piperitone with decanol based on a diffusion-dilution method (Byers 1988) that produces lower release rates than that with a neat (undiluted or pure) chemical for a particular dispenser. The method uses the molecular weights and densities of respective semiochemical and solvent to obtain the desired mole proportion that corresponds to the proportion of the release rate of the neat semiochemical. Thus, based on MW 158.28 for decanol (density 0.829 g/ml) and MW 152.23 for piperitone (density 0.933 g/ml) then a 0.1 mole proportion requires 100 µl piperitone and 1.053 ml decanol to give a low

Fig. 4. View from under the cup with 10 vials of Piperitone and quercivorol tube inside a sticky trap screen.

inhibitor release rate. The still lower 0.01 dose was then made by serial dilution (100 µl + 900 µl decanol). The 1x (standard experimental dose) and 10x doses (10 times higher) were made from neat piperitone (95% pure, 50% ee (R)-enantiomer, Synergy Semiochemicals) using one to ten 2-ml vials each with 60 µl giving a 1x release rate of 0.52 mg/day and 10x of 5.2 mg/day (as above). The 0.01 and 0.1 doses had 60 µl of their respective solutions in 2-ml glass vials, while the control had only the same volume of neat decanol in a vial.

The Sticky traps and foil cups described above were used with the different doses each replicated three times for a total of 15 traps. The traps were placed in a line with 15 m separating each trap (16 Sept. – 21 November 2018) in the same avocado orchard but 150 m away from the first experiment. PSHB were collected from sticky traps every week to 10 days and adjusted for catch per week for a total of 21 replicates per treatment. Catch was analyzed by ANOVA as above. Non-linear regression software (TableCurve 2D) was again used to find a decay function that fit the dose-response data well and the chosen curve plotted on a log scale according to previous methods (eqn. 2 in Byers 2013).

Results: The mean catch per trap per week on the control quercivorol bait was highest and appears to decline with even a small amount of piperitone (at 0.01 dose but not significant). However, at 0.1x dose of piperitone released together with quercivorol caused the mean catch to decline significantly (Fig. 5). The release of 1x and 10x piperitone caused the lowest mean catches although these doses do not appear to be different in repellency (Fig. 5). It appears that 1x piperitone is sufficient to repel PSHB if placed at the source of attraction. The reduction by 1x piperitone was 79%. A second order decay function fit the data well (R^2 = 0.997, adjusted R^2 = 0.987).

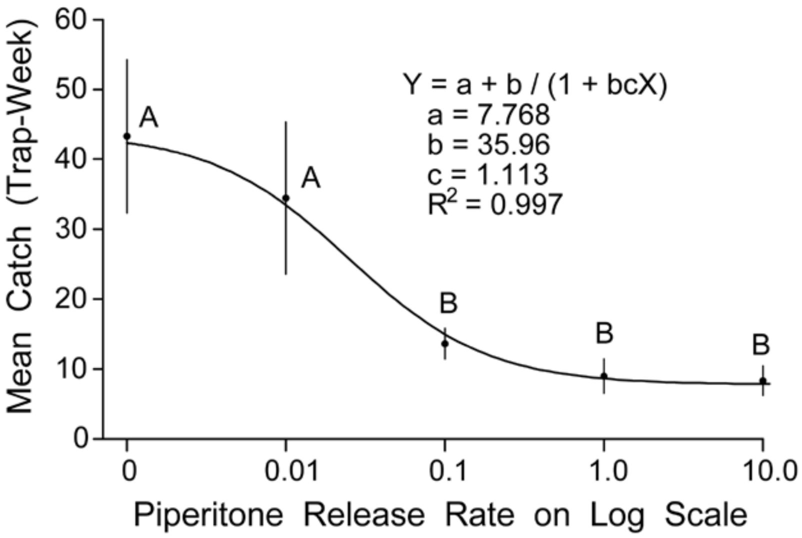

Fig. 5. Reduction in attraction of PSHB to 1x quercivorol in relations to 10-fold increasing release rates of repellent piperitone on a logarithmic scale. Error bars are ±95% CL (N = 21). Means with the same letters were not significantly different (α = 0.05, ANOVA and Tukey's HSD).

Discussion: Byers et al. (2018) found that piperitone [75% (R)-enantiomer] released at 0.52 mg/day, as well as (S)-verbenone and (R)-verbenone each released at 0.8 mg/day are among the most potent of inhibitors tested on any species of scolytid beetle, reducing response of PSHB to quercivorol by 90, 78, and 85%, respectively. In the present study, the same source of piperitone released at 1x and 10x rates (0.52 and 5.2 mg/day) reduced response of PSHB to quercivorol by 79 and 81%, respectively. The reduction at 1x piperitone in the present tests was slightly less than in tests the previous year, but similar to the repellency rates of verbenone above (Byers et al. 2018). A reduction of 92% in attraction of North American five-spine engraver *Ips paraconfusus* Lanier to a 50-male infested log was shown for (S)-verbenone released at 4 mg/day (Byers and Wood 1980). Bedard et al. (1980) also released (S)-verbenone at 4 mg/day and this reduced response of western pine beetle *Dendroctonus brevicomis* LeConte by 56% to its synthetic pheromone components. The response of pine shoot beetle

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 found that verbenone release increased from infested logs as they aged and suggested this was probably due to microorganisms as the beetles contained little verbenone. The repellency of piperitone and verbenone to PSHB can be due as well to microorganisms in decaying tissues, or may represent non-host volatiles to avoid.

Experiment III:

Collection of volatiles released from uninfested and PSHB-infested limbs on avocado trees. Our earlier work in the summer-autumn of 2016 and 2017 suggested that natural infestations surrounded by sticky screens caught several times more PSHB than uninfested limbs in avocado (Byers et al. 2017, 2018). The apparent attraction of PSHB to infested limbs could be due to quercivorol (possibly from the beetle's symbiotic fungi) or other pheromone compounds.

Methods: A limb of a Hass-variety avocado naturally infested with multiple attacks of PSHB (image) was selected and wrapped with a 25 cm × 35 cm long section of metal screen (6 mm mesh) to prevent collapse of surrounding plastic under slight vacuum. The plastic was cut from Reynolds® oven bags (482 mm × 596 mm) cut along its end and on one side. The plastic sheet was wrapped around the limb (about 9 cm diam.) and metal screen several times and taped along the outer seam with the plastic ends appressed to the bark by tying with string. At one end of the wrapped sheet, a charcoal filter (1 cm diam. × 20 cm) filtered ambient air that was drawn over the surface of the infested limb within the sheet's interior to the opposite end and into an adsorbent filter. This filter was made from a 20×2.3-mm ID Teflon tube filled with Porapak Q powder (80/100 mesh, Alltech Associates) and fit to a brass Swagelok fitting. This fitting was connected to a 2-m long Teflon tube (0.125 mm diam.) that exited the sheet-formed chamber and went to a flow meter on the ground and then into a 12V-1A vacuum pump powered by 12V lead-acid battery (Fig. 6). Volatiles from the infested limb were collected on the filter for 54 min beginning at 9:56 AM (16 Sept. 2018, about 27 °C) and on a second filter for 60 min beginning at 10:58 AM. The infested limb had 10-20 attacks that had produced "sugary" wound responses. A third and fourth collections (each 60 min) on other filters were done from an uninfested limb on a nearby tree beginning at 12:03 and at 13:07 PM. At the end of each collection period, each absorbent filter was fit to 1/8 to 1/4 brass fitting adapter and a 0.25 inch glass tube (5 cm long) was filled with 200 µl distilled hexane. A rubber bulb was place over the glass tube and pressed to express the hexane through the Porapak Q filter and dripped into a 2-ml glass vial with Teflon lined lid for transport to the laboratory and refrigerated until analysis by gas chromatography – mass spectrometry (GC-MS).

Fig. 6. Collection of volatiles from inside plastic oven bag with air drawn through charcoal filter (top right) and absorbed by Porapak Q filter using 12V vacuum pump.

GC-MS analyses of 1 µl of each liquid sample were performed on an Agilent 6890N GC instrument interfaced with an Agilent 5973 MS detector equipped with a nonpolar RTX-5SilMS (Restek, Bellefonte, PA) column (30 m × 0.25 mm ID × 0.25 µm film). The column was kept at 50 °C for 3 min, then 10°/min to 240 °C and held for 20 min. Column helium flow was 1.5 ml/min and GC–MS inlet temperature was kept at 230 °C with an injection time of 6 min in splitless mode (4-mm i.d. liner). The GC–MS was equipped with a commercial autosampler (MPS2-Twister, Gerstel, M. Snir Technological Services Ltd, K. Ata, Israel) and injections were done by a 10- µl syringe.

Results: Analyses showed a number of monoterpene and sesquiterpene volatiles (Table 1) released by both the PSHB-infested and uninfested (control) limbs. The second hour of each collection source contained some terpenoid alcohols (retention times 12.21 and 12.29 min) and ketones (13.3 min) with MS very similar to several known bark beetle pheromones (e.g. *cis*-verbenol, *trans*-verbenol, and verbenone) as well as compounds similar to quercivorol (Table 1). The *cis*- and *trans*-verbenol compounds tentatively identified could instead be monoterpene alcohols similar to quercivorol, *cis*-p-menth-2-en-1-ol (CAS 35376-39-7), such as cis-p-mentha-1(7),8-dien-2-ol (CAS 35907-10-9; and 2102-62-7), *cis*-p-mentha-2,8-dien-1-ol (CAS 3886-78-0), and *trans*-p-mentha-2,8-dien-1-ol (CAS 7212-40-0). A synthetic sample of quercivorol (Synergy Semiochemicals, Canada) had a retention time of about 12.17 min which is very similar to that above. However, the MS of the terpenoid alcohols from the infested limb had a base peak of 109, which is not found in quercivorol but is found in both *cis*- and *trans*-verbenol as well as the *cis*- and *trans*-p-mentha-compounds mentioned above. The volatile collections were also run on a polar column, which separates compounds differently than the Restek non-polar column, but results have not yet been analyzed.

Table 1. Preliminary GC-MS analysis on a non-polar column of volatiles collected in second hour from PSHB infested limb on avocado tree (compounds and amounts were similar for the second hour of the control limb). Possible attractive volatiles that are oxygenated monoterpenes are in **bold**.

Retention time	Compound	Compound Area	% of compounds	Certainty (1000 highest)
7.98	(+/-)-alpha-pinene (0.86 ng)	80354	0.501	936
8.993	(E)-B-ocimene (plant)	96630	0.603	809
9.613	alpha-phellandrene (8.2 ng)	243704	1.52	928
9.665	(E)-ocimene (trans, 28.05 mi	21292	0.133	791
9.68	(+)-B-pinene (24.305 min c2-	58841	0.367	834
10.424	E-ocimene (5 ng, Jackie B)	1.62E+06	10.114	923
10.491	3-Carene	169926	1.06	914
10.688	Cyclohexanol, 5-methyl-2-(1-	32677	0.204	728
11.713	Pulegone	147528	0.92	713
11.992	trans-4a-Methyl-decahydronap	118425	0.739	906
12.211	**cis-verbenol**	74390	0.464	780
12.284	**(S)-trans-verbenol (c2: S=30**	137676	0.859	868
12.292	**(S)-trans-verbenol (c2: S=30**	72307	0.451	855
12.919	Azulene	33881	0.211	897
12.93	Naphthalene	18342	0.114	929
13.305	**(S)-(-)-verbenone**	797752	4.977	944
15.312	alpha-cubebene (32.02 min co	199288	1.243	931

15.646	.alfa.-Copaene	408087	2.546	910
15.73	alpha-copaene (32.905 min co	292300	1.824	945
15.888	1H-Cyclopenta[1,3]cyclopropa	142471	0.889	864
16.195	Bicyclo[3.1.1]hept-2-ene, 2,	171158	1.068	806
16.345	(E)-B-caryophyllene (0.85 ng	856544	5.344	942
16.458	1H-3a,7-Methanoazulene, 2,3,	452938	2.826	806
16.511	.alpha.-Guaiene	24194	0.151	796
16.593	(-)-Aristolene	104875	0.654	868
16.706	1H-3a,7-Methanoazulene, octa	74085	0.462	857
16.782	No Match	170730	1.065	----
16.804	1,4,7,-Cycloundecatriene, 1,	152831	0.953	875
17.019	.gamma.-Muurolene	42585	0.266	865
17.126	Germacrene D (47.43 min c2ms	321047	2.003	946

Discussion: The *cis*-verbenol and *trans*-verbenol compounds indicated on the non-polar column are only tentatively identified by matching MS-library spectra. Some standards are available (*cis*-verbenol, verbenone, quercivorol) to help identify the PSHB-avocado voaltiles using GC retention times and MS. PSHB response has not been tested in a laboratory olfactometer (Byers et al. 2013) to any of the volatile collections nor on any of the possible monoterpene alcohols indicated by GC-MS analyses. It is possible that one or more of these monoterpene alcohols, if found consistently in more sampling of volatiles, would be as attractive, or more so, than quercivorol. The age of the natural infestation from which the volatiles were collected was not known - so it is necessary to repeat the volatile collection procedure with additional infestations of different ages and densities of attacks in avocado. The age of an infestation is usually important to the amounts of attractants/pheromones that are collected as shown in previous studies of bark beetles (Byers 1989). Other GC-MS columns such as polar and chiral columns might help resolve the identity of the monoterpene alcohols and ketones. One note of caution, verbenone (a repellent shown by Byers et al. 2018) was clearly matched in the volatiles from the second hour of each odor source. Verbenone can indicate fungal degradation of the host tree tissues (especially in PSHB infested limbs). However, it is surprising to find this compound in the control limb, and thus there may have been an external source of contamination. More sampling of volatiles from various naturally infested limbs of avocado (compared to control uninfested limbs) will be necessary to understand the implications and better identify the compounds emitted by PSHB-infested avocado limbs.

Experiment IV:

Mass trapping of PSHB in 2017.

Methods: A mass trapping experiment (1 July – 4 Dec. 2017) was conducted by Tapazol using two densities of barrier traps with bubble-cap lures of quercivorol (Synergy Semiochemicals Corp., Burnaby, Canada, each containing 300 ml of the compound). The bubble-cap released about 4 mg/day (Byers et al. 2017). Two densities of traps, 50 and 100 traps per ha, were set up that consisted of 25 and 50 traps, respectively, in each of three areas (Beit-Haemek, Nahsholim, and Kfar-Masrik, Israel). The traps were placed in a 5 × 5 grid at the lower density and in a 10 × 5 grid at the higher density in both Beit-Haemek and Nahsholim. However, while the same trap densities were used, a smaller 5 × 4 and a 10 × 4 grid was used for the two trap densities at Kfar-Masrik. Traps were picked of PSHB four times at Beit-Haemek (about every month from 1 July – 4 Dec. 2017), nine times at Nahsholim (about every week to two weeks from 24 June – 1 Oct. 2017), and four times at Kfar-Masrik (variable periods from 10 days to 65 days during 10 Aug – 4 Dec. 2017). For the lower density trapping grids, the average catch per trap was calculated from the 16 outer traps on the grid periphery and compared to the average catch per trap for the nine inner traps in all collections in the first two areas. The same calculations were made for the higher density traps that had 26 outer traps and 24 inner traps. In Kfar-Masrik with smaller trapping grids, the outer traps consisted of 14 and 24 traps for the lower and higher density areas, or 6 and 16 for the inner traps at the two densities, respectively. An average of the total catches during the summer in each area was made similarly for the outer and inner traps. The average catch per trap per week in each area was calculated from the raw catch data to normalize results of trapping periods of variable length when comparing trends in catch over time. In both the low and high density trapping grids in each of the three locations, the observed frequencies of catch on outer and inner traps were compared to the expected frequencies based on the proportion of each trap type by a Chi-square goodness of fit test (R-Statistics).

A second year of mass trapping in 2018 in the same plots at Beit-Haemek was conducted by Tapazol, but has not been analyzed yet.

Results: The normalized mean catch per trap-week in Beit-Haemek increased during the trapping period in both the low and high density grids. Traps in the low density grid increased from 0.35 PSHB/trap-week to 4.47 at the end of the experiment, while in the high density grid the results were similar with an increase from 0.57 to 6.26 at the end. In Nahsholim, there was no trend in catch in either low or high density grids. The low density grid had 1.8 PSHB/trap-week in the beginning and 1.7 in the end, while the high density grid attracted 1.05 PSHB/trap-week in the beginning and 1.5 in the end. In Kfar-Masrik, both the low and high density trap grids had a decline in catch during the trapping period. This area had more beetles initially than the other two locations, and declined from 12 to 1.9 PSHB per trap-week in the low density as well as from 6.4 to 1.45 in the high density grid.

The most definitive results are in the comparison of the outer trap catches with the inner trap catches in each of the low and high density trap grids in the three locations. In Beit-Haemek, all four of the low density grids, and all four of the high density grids had more mean catch per outer trap than on corresponding inner traps during the same periods. During the entire period, the low density inner trap on average caught 62% as much as the outer traps (total catch on nine inner traps was 268 compared to 764 on 16 outer traps: $X^2 = 45.1$, df = 1, P<0.0001). The high density grid inner trap on average caught 73% as much as the outer traps (1345 caught on 24 inner traps compared to 1998 on 26 outer traps: $X^2 = 80.8$, df = 1, P<0.0001). In Nahsolim, the outer traps in the low density grid had a higher mean catch than the inner traps on eight of nine occasions, while the outer traps in the high density grid had a higher mean catch than inner traps in six of nine occasions. The low density inner trap on average caught 70% as much as the outer traps (total catch on nine inner traps was 231 compared to 587 on 16 outer traps: $X^2 = 21.4$, df = 1, P<0.0001). The inner trap in the high density grid at Nahsolim caught on average 88% as much as the outer traps (462 on 24 inner traps compared to 569 on 26 outer traps: $X^2 = 4.2$, df = 1, P=0.04). In Kfar-Masrik, the outer traps in the low density grid had a higher mean catch than the inner traps on all four occasions, while the outer traps in the high density grid had a higher mean catch than inner traps on three of the four occasions. The low density inner trap on average caught 68% as much as the outer traps (total catch on six inner traps was 74 compared to 269 on 14 outer traps: $X^2 = 11.6$, df = 1, P<0.0001). The inner trap in the high density grid caught on average 93% as much as the outer traps (713 compared to 1154 on outer traps: $X^2 = 2.55$, df = 1, P=0.11) which was not significantly more.

Discussion: There were no control areas with monitor traps in sufficient numbers to determine if population trends increased during the summer and thus, the decline at Kfar-Masrik and the lack of an increase at Nahsholim cannot yet be declared a definite success due to mass trapping. In two locations (Beit-Haemek and Nahsholim) in both low and high density trap grids, the mean trap catch on the outer traps (along the grid periphery) was higher than on the corresponding inner traps. In Kfar-Masrik, grids with fewer traps were used, and at the low density, but not the high density grids, the outer traps caught significantly more per trap. These results suggest that PSHB were flying into the grid areas to be trapped in higher numbers on the peripheral, outer traps. This also indicates that the PSHB population was being significantly depleted within the grids, and at least in Nahsholim and Kfar-Masrik the flying population of PSHB was decreasing. In other experiments in 2017 (Byers et al. 2018) in Nahsholim, traps with quercivorol caught increasing numbers of PSHB from about 1.7 per trap-week to 15 per trap-week compared to the mass-trapping area where catches remained rather constant at about 1.5 to 2 per trap-week.

As mentioned above, the mass trapping experiment was continued in 2018 at Beit-Haemek. The overall impression according to Tapazol is that the population of PSHB in the mass trapping areas in the second year were significantly reduced compared to control areas and to populations in 2017.

A study in Israel of mass trapping may be relevant to work on PSHB. Mass trapping using pheromone of the lesser date moth pest of dates is currently being done in date plantations in Southern Arava area beginning in 2016 (managed by Avi Sadowsky in cooperation with Dr Anat Zada who identified the pheromone earlier and Dr Byers) and results have been published (Levi-Zada et al. 2018). Sadowsky and coworkers are continuing this work and found recently that the population (and damage) of lesser date moth under mass trapping declined over a three year period compared with untreated plots. Similarly, it can be that mass trapping control of PSHB needs more than one year to be shown effective. Models under development and largely theoretical by Byers are suggesting how even only 70% control each year from mass trapping (or push-pull below) can lead to successive years with population declines leading to low/endemic levels after a few years. However, these are preliminary models with assumptions and are not a substitute for field results.

Experiment V in 2018 (conducted by Tapazol with consultation):

Push-pull control test using piperitone dispensers inside avocado trees (“push”) and quercivorol baited barrier traps (“pull”). Models of push-pull for PSHB suggested that this method would be successful for control of this pest (Byers et al. 2018).

Methods: A push-pull experiment (June - Dec 2018) was initiated by Tapazol using three piperitone plastic bubble-cap dispensers (Synergy Semiochemicals, Canada) placed vertically at about 1, 1.5, and 2 m height within each avocado tree in an orchard near Beit-Haemek. At constant 25 °C, the piperitone bubble-cap dispenser released about 10.8 mg/day, while the quercivorol bubble-cap dispenser released about 4.8 mg/day. Fifty avocado trees were treated with the piperitone dispensers and 50 quercivorol barrier traps were placed in areas just outside of trees. Control areas of the orchard adjacent to the treatment area were monitored for PSHB with quercivorol traps. Numbers of infestations on avocado trees were counted within the treated area and in the control areas. (These methods are not final and need information from Tapazol).

Results: Data from the push-pull experiment is expected to be analyzed in the coming months. We will be looking for any trends, like a decrease in catches/attacks per trap collection in the treatment areas compared to control areas. A correlation of lower damage in treatment areas compared to control areas would also be of interest. According to Tapazol (verbal communication) the catches in both control and treatment areas were quite low and similar.

Discussion: The catches were lower than expected in the areas treated with piperitone repellent and quercivorol traps as well as in nearby untreated (control) areas. This could be due to a lower population in the area or that there was so much piperitone released (which could be smelled by humans throughout the treated areas) that perhaps PSHB were repelled from the entire area including nearby control areas.

It must be noted that the lesser date moth mentioned above is often in isolated plantations surrounded by desert (so less migration), while avocado orchards have other host trees of PSHB that are not avocado nearby, such as castor bean. Due to this situation, it probably means that control using a push-pull system for PSHB needs to be larger in area (than at present) in order to sweep up migrating populations coming into the protected and treated area. Also, it cannot be stressed enough that the mass trapping or the push-pull system must be started shortly before the first beetles begin to fly in the spring. The early spring treatments are needed to repel and then trap PSHB before they can start new aggregation centers in the avocado orchards that would compete with the repellents and attractive traps placed away from the trees but within or on periphery of orchard.

The push-pull experiment should be continued in place and limb damages in the treated and untreated orchards assessed. An enlargement of one area over that of 2018, and possible increase in density of push (repellent sources) and pull (quercivorol traps) would be desirable. Due to limitations in budget/manpower of Tapazol, I would suggest not to spread this experiment into more different areas but rather focus on the existing area (possibly enlarged) and monitor the surrounding untreated avocado areas versus the treated area. The results of experiments I and II above indicated that the "push" or repellent part of the push-pull system must be improved by directly placing a slow-release substance containing verbenone and/or piperitone on the trunks and major limbs of the avocado tree where PHSB prefer to breed. Experiment III has a chance to provide a better attractive blend for control, and also give an explanation as to why natural infestations were found to attract more PSHB than control uninfested limbs in 2016 and 2017 (Byers et al. 2017, 2018).

The barrier trap with quercivorol is currently expensive and the trap probably can be improved regarding price. There are many types of bark beetle traps reported in the scientific literature that would also work on ambrosia beetles, so this area can be investigated using 1x baits of quercivorol. For example, there are two-panel and four-panel barrier traps, multiple funnel traps, sticky traps, pipe-funnel traps, and other designs (some seen in Google search "bark beetle traps") that can be compared to the current one being used by Tapazol.

Summary and Conclusions

The use of semiochemicals in traps to attract insects for the purpose of monitoring their population is central to management of pest insects. This important tool presumes that a potent attract is chemically identified and characterized. An extension of monitoring, in which a relatively high density of monitoring traps are used, is termed mass trapping. In both methods, the relative strength of the attraction should be measured in order to understand whether the known attractive chemicals are effective. Computer simulations can aid in this determination but these depend on a field measurement of the potency of the lure-trap combination. A useful way to measure attractive strength is the EAR (effective attraction radius) method. The mean flight height of a pest insect needs to be determined to place traps at the optimal height, and the standard deviation of this mean height is used to convert EAR to EARc for use in simulation models of mass trapping. In our first publication from the grant (Byers et al. 2017) we determined mean flight height, SD, EAR, and EARc for quercivorol that attracts PSHB. It was found that the EAR for a 1x release of quercivorol is similar to the strength of some aggregation pheromones of bark beetles. A 10x release rate caught even more PSHB. In the second year of the grant, we tested several ketones of monoterpenes and found that both enantiomers of verbenone and piperitone were highly effective in repelling PSHB away from the attractant quercivorol (Byers et al. 2018). We further demonstrated that the repellents are most effective when they are released very close to the attractant (mimicking a natural infestation on a limb). We found that natural aggregations of PSHB on limbs of avocado trees had EAR that were similar to a 1x release of quercivorol. In the third year of the grant, our preliminary data on volatiles released from PSHB-infested limbs on avocado gave intriguing results of monoterpene alcohols and ketones that were collected from the natural odors. One or more of these chemicals may account for the attraction of PSHB to infestations on avocado, and could have attractive potency similar or better than quercivorol, but more analyses are needed to confirm this result. The results of the second and third year also suggest that repellents may need to be applied directly at the source of attraction to be effective. Finally, the repellents and attractant quercivorol, along with computer simulations, suggested that a push-pull test should be undertaken (Byers et al. 2018). This was begun in 2018 by Tapazol. We propose that future work optimize placement of repellents within avocado trees to maximize the effectiveness of push-pull control. Also, assessment of damage and/or infestation numbers in treatment plots and control areas nearby is needed to evaluate the push-pull method. The push-pull method (or mass trapping) should be initiated before PSHB fly in the spring in order to out-compete the newly forming aggregations that begin with just a female. Finally, either the mass trapping method or the push-pull method should be carried out for several years in the same areas because theoretical analyses indicate that even marginal reductions in females along with other natural mortality agents can reduce PSHB populations to low levels after a few years. The advantage of the PSHB system is that all the breeding females are targeted by mass trapping or push-pull as compared to moth systems where only males are removed.

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