Trapping Optimization and Development of “attract and kill” strategy for the Polyphagous Shot Hole Borer in avocado

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Abstract:

Goal of the work was to improve our ability to trap the beetles for detection of the invasive shot-hole borers

Lures were successfully developed for attracting the two invasive shot hole borer species to traps

Different variants of the attractive chemicals were tested to determine which chemical production procedure, resulting in a reliable production method and a reliable availability of the lures.

The influence of release rates of the lures was tested for the attractant lure and results show that the release rates can be substantially lower than the present lures without loss of effectiveness.

Different trap types were tested to determine which traps and trap colors would optimize the trap catch of lured traps.

Different preservation materials were tested to determine how they influenced trap catch.

A materials were found that deters the beetles from being attracted to lured traps.

Population development as measured by trap catch was determined in two different avocado groves over time in Escondido.

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Introduction/Background:

The species morphologically identified as *Euwallacea fornicatus* has been present in Southern California for at least a decade (Eskalen et al 2013). It is unknown how this species arrived in California but transport in wooden packing material seems to be an obvious possibility. For the earliest detection in 2003 until 2012 little was known about the distribution of this beetle. Historically *Euwallacea fornicatus* was associated with problems in tea in Sri Lanka (Walgama, 2012), where the density of these beetles could reach 1,500,000 beetles per acre. While *E. fornicatus* in Asia was a big economic problem in tea crops, other tree species were also attacked and Danthana (Danthana, 1973) reported that up to 90 species of hosts were known.

Once we became involved in the research on these beetles it soon became clear that the beetle that had invaded the Los Angeles area was genetically different from the "Tea Shot Hole Borer", in order to distinguish it from that beetle we named it the "Polyphagous Shot Hole Borer" to indicate that this beetle attacks many different host plant species (Eskalen et al 2013). In 2014 a second invasion of a species morphologically identified as *E. fornicatus* was detected in El Cajon, CA and upon genetic fingerprinting it became clear that this again was a different cryptic species close to E. fornicatus. This species was found in its native range in Taiwan and Okinawa and was named Kuroshio Shot Hole Borer (KSHB) (Rugman-Jones and Stouthamer, 2016). Based on our phylogeographic work we now know that the PSHB occurs in Asia in Northern Taiwan, Southern China, Vietnam, Taiwan and Okinawa, while the KSHB occurs only in Taiwan and Okinawa (Stouthamer et al., submitted) . The DNA sequences of the PSHB would indicate that the invasion found in California originated from either southern China or from Vietnam. The KSHB sequences are identical to sequences found in beetles from Taiwan.

These SHB species carry with them a specific combination of fungi that they use to culture for food, inside the trees (O'Donnell et al., 2015, Eskalen et al., 2012, Freeman et al., 2013). Each generation females leaving their natal gallery will leave with a set of fungi in special organs inside their heads that they will use to seed their own galleries. The mode of reproduction of these SHB is similar to bees and ants, in that inseminated females can regulate the access of sperm to their eggs, and in allowing a sperm to fertilize an egg, the resulting offspring will become a female, while eggs that remain unfertilized develop into males. Thus the mother can control the sex ratio of her offspring. She does this in a very precise manner and produces mainly daughters and only a few sons. In addition to this unusual sex ratio, the siblings also mate with each other, this so called sibmating is common among ambrosia beetles. No negative effect of sibmating are known in these species. The result of this sibmating is that female beetles that emerge from galleries once they are adult will already have mated with their brother, and have stored his sperm in a special organ called spermatheca. Once such

females have created their own galleries they will be able to produce both sons and daughters.

This specific lifecycle –spending most of their time inside the tree, cultivating their fungi, where both the mother and her offspring exclusively feed on these fungi, leaves few opportunities to control these insects with insecticides/fungicides. Research both here in California and in Israel (Mendel et al., 2012) has not yet resulted in an effective control strategy. Control of these beetles is hampered by the fact that they spend most of their life inside their galleries inside trees. Thus reaching them with pesticides is difficult once they are inside the tree. During their dispersal phase they spend a short time outside their native tree, either to walk on the bark surface to find another spot to construct a new gallery or to fly away and find another suitable host.

Before we started our research little was known on how to effectively trap these beetles. For some beetle species aggregation pheromones are known that have been developed to attract them. For the species that have invaded California no such lures were known. During our research project, in cooperation with the USDA, we discovered a lure that attracts the beetle. The composition of this lure is based on chemicals found in the diet of the beetles after fungi have infected the diet. So the assumption is that the beetles respond to trees in which fungi are already growing. During this project our overall goal was to improve our ability to trap the beetles by optimizing the attractant lure and by determining the trap type that would be the most effective in catching beetles.

Objectives: the main objectives of the project.

1. Optimize trapping for detecting PSHB
2. Develop “attract and kill” strategy for the PSHB

Material and Methods:

Lure development.

When we discovered early on that quercivorol substantially improved our ability to attract beetles to a trap we immediately ran into the problem that the company producing the lure was not capable of producing a large number of these lures in an effective manner. The first spring after the discovery of the lure there i was a shortage of lures. The company (Synergy) figured out methods to improve the production of the lures but the resulting chemistry needed to be tested in the field.

Setup Field Testing

General set up of testing lures in the field. Funnel traps consisting or 12 stacked funnels with a collection cup at the bottom were used in the experiments. They were attached to an l-shaped pole with a 6 foot long side and a 1 foot side piece, all made out of one piece of metal conduit. Attached to the side piece was the funnel trap. The whole assembly was anchored into the soil using a 3 foot piece of rebar. Traps were located at least 50 ft apart from each other. The lure was attached to the second funnel from the bottom. Traps were placed in avocado groves. In general, these experiments will be done by testing three different treatments against each other, where the test will take place for a period of three weeks. The experimental design consists of blocks containing three trap locations, and each week the traps with a particular treatment will be rotated to a new position in the block. So that each treatment will rotate through all locations during the three week experimental period. We did at least 10 repetitions per treatment so these experiments will consist of a total of 30 traps placed in a field or orchard.

Optimizing traps:

Several physical factors may influence the ability of traps to attract and capture beetles. Including trap type (Lindgren with variable number of funnels vs sticky traps), color, size of the trap, treatment with fluon (=liquid teflon)(Crook et al, 2014, Francese et al 2013a,b, Furstenau 2014, McIntosh 2001). The placement of traps may also influence its effectiveness (Yamasaki et al 2014, Hanula et al 2011); we already know that the lured lindgren traps appear to catch the largest number of beetles when placed in open areas vs close to trees. Since it is known that the closely related species Tea Shot Hole Borer will fly up to several meters above the canopy of tea plantations (Calnaido, 1965), we will test to determine the optimal height for trap placement above the canopy.

Thus far we have used the standard lures provided by the (Synergy and Chemtica) companies, but we do not know if the lure strength is optimal for the trap catch (Kendra et al., 2014). We studied the relationship between lure strength and trap catch, to optimize the attractiveness of the lures.

Materials and traps tested.

Experiment number	Treatments compared
1	Quercivorol lure (Synergy) 3250 plus High release ethanol versus control
2	Three different types of quercivorol (3250,3039 and 3355)
3	Precursors of quercivorol and querivorol
4	Quercivorol lures 3250 vs 3361 vs 3362
5	Preservation fluid:DESS vs RV antifreeze vs Dry
6	DESS vs large funnel dry vs small funnel dry
7	12-funnel vs 3 funnel vs vane
8	Vane color: black vs orange vs white
9	Vane color: red vs silver vs blue
10	Vane color: green vs brown vs dark blue
11	Vane color: green vs silver vs orange
12	Black vane trap with Teflon vs black vane trap without Teflon
13	12 funnel trap vs bottle trap (half design) vs bottle trap (flat design)
14	Chemtica lure vs Synergy 3361 vs control
15	Number of lures per trap (Chemtica) 6 vs 2 vs 1
16	Concentration Chemtica lure: 1 vs ½ vs ¼
17	Concentration Synergy lure: 1 vs ½ vs ¼ (first set up) escondido
18	Concentration Synergy lure 1 vs ½ vs ¼ open area
19	Duration activity Chemtica lure, new lure vs aged lure vs control
20	3361 vs 3361 with verbenone vs control

Yearly Flight Activity

We do not know the flight activity of the beetles over the year, consequently we will establish 12 funnel traps equipped with quercivorol in various locations. The Huntington Library and Gardens in Pasadena, an avocado grove in Escondido and the San Diego Zoo in San Diego.

Results

Experiment 1

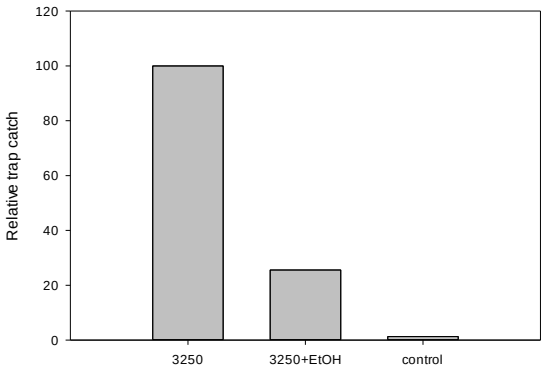


Figure 1. Influence of quercivorol lure (Synergy 3250) and high release ethanol on the trap catch of PSHB in an avocado grove in LaHabra.

Results of this experiment show two effects, 1 the synergy lure 3250 (quercivorol) is effective in attracting PSHB to the traps and 2. The addition of a high release ethanol lure to the quercivorol lure has a negative effect on the trap catch compared to the quercivorol lure alone.

Experiment 2

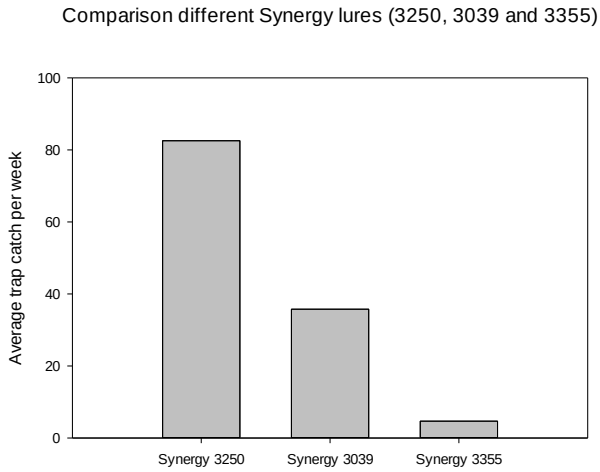


Figure 2. Comparison between different Synergy lures with different combinations of quercivorol stereo chemical forms

Quercivorol is a chemical with different chiral centers thus for the chemical different versions exist that are either left or right turning, and this experiment shows that the composition of lure 3250 attracts more PSHB than the two other lures with a different stereo chemistry of the quercivorol making up the lure

Experiment 3

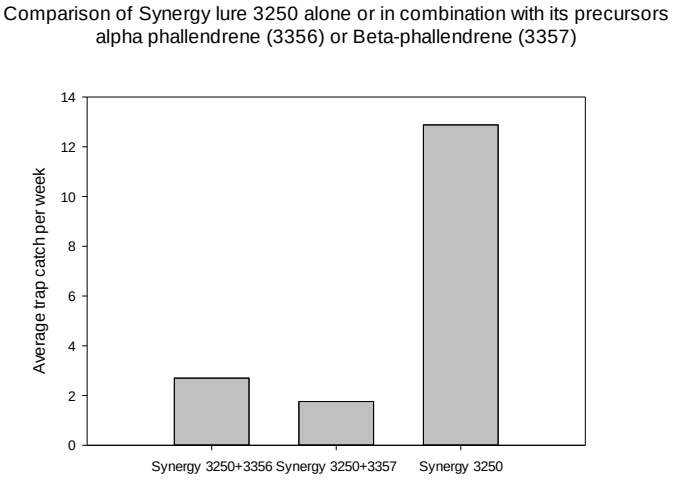
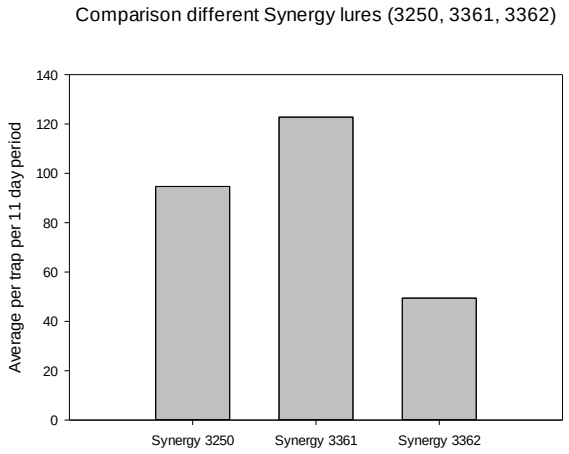


Figure 3. Comparison of trap catch of Synergy 3250 combined with either precursor of quercivorol alsph or beta phellendrene or Synergy 3250 alone.

In this experiment we test to see if addition of the two potential precursors of quercivorol to the effective lure 3250 improve the trap catch. Such patterns have been found in some bark beetle lures. Results of this experiment show no such additive effect.

Experiment 4



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To determine if the different processes used by the chemical company Synergy to produce the attractant lure for shot hole borers were equally effective in their attraction to the beetles, funnel traps with the original 3250 lure were compared with lures produced in two other methods. Results show that the lure 3361 showed at least an equal ability to lure 3250.

Experiment 5

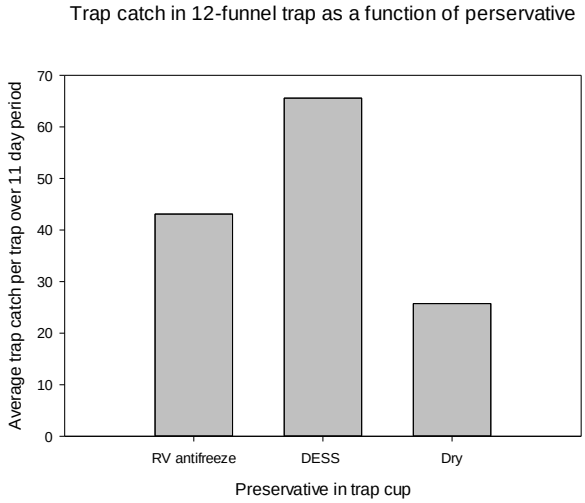


Figure 5. Influence of preservative (RV antifreeze, DESS or no preservative) on trap catch of 12-funnel traps lured with quercivorol (Synergy 3250)

Beetles once attracted to the lure drop down into the funnels and end up in the collection cup. Different preservatives were tested to determine their effectiveness in retaining beetles in the cup. From this experiment it is clear that the chemical DESS is the most efficient in retaining the caught beetles.

Experiment 6

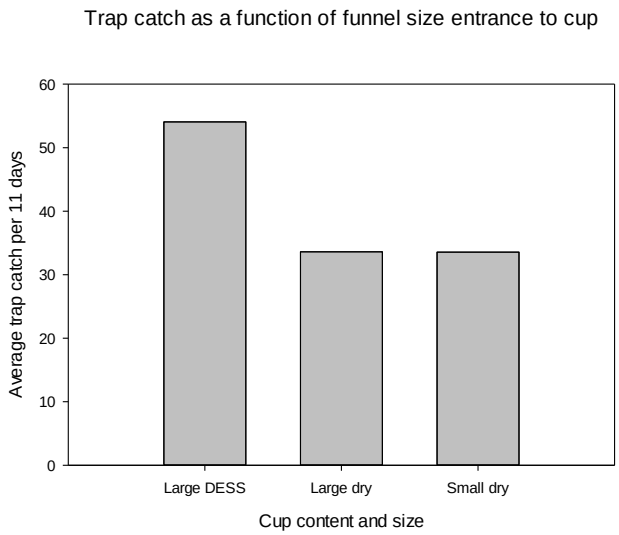


Figure 6. Influence of entrance size to collection cup on trap catch of 12-funnel traps lured with Synergy 3250 lure

In the previous experiment we showed that the chemical DESS was more efficient in retaining beetles in the collection cup than when no fluid was added to the collection cup. Here we determine if the size of the opening of the collection cup makes a difference, by either maintaining the collection cup opening size the same (large) or by restricting the size (small). Results show that the size of the entry hole to the cup do not influence the trap catch.

Experiment 7

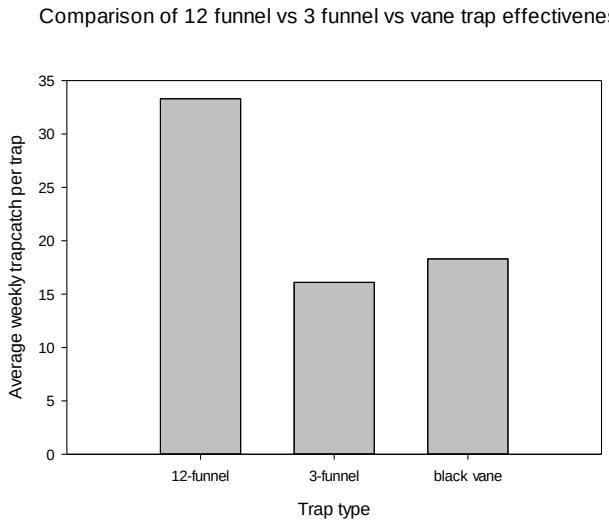


Figure 7. Influence of trap type on trap catch of traps lured with quercivorol lure Synergy 3361

Several trap types are available, in this experiment we compared the relative trap catch of a 12-funnel trap, compared to a 3 funnel trap and a vane trap. All traps were black in color and the 12-funnel trap was the most efficient in catching beetles.

Experiment 8

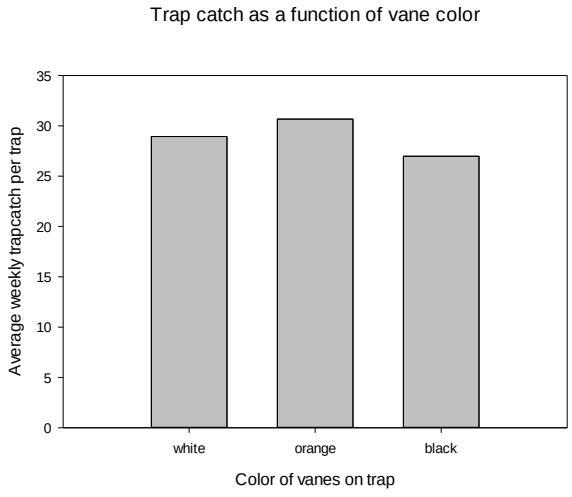


Figure 8. Influence of vane color (white, orange and black) on trap catch in vane traps lured with Chemtica quercivorol lures

Experiment 9

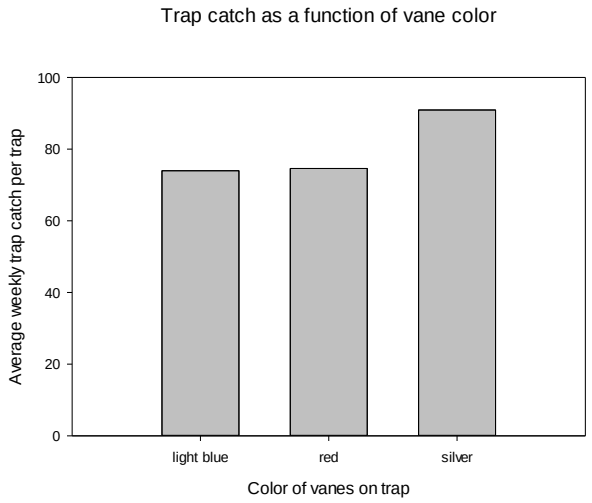


Figure 9. Influence of vane color (light blue, red and silver) on trap catch in vane traps lured with Chemtica quercivorol lures

Experiment 10

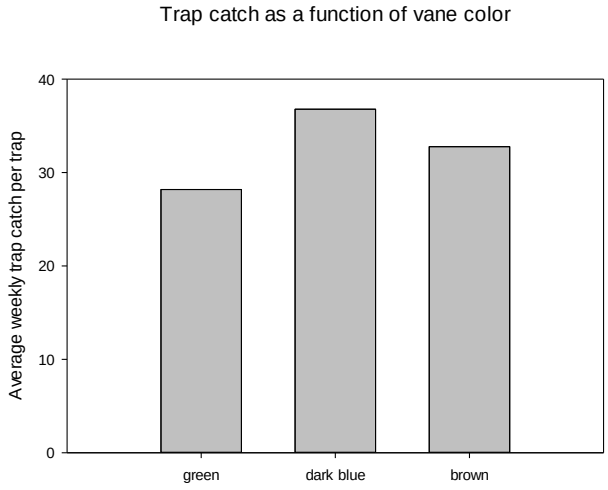


Figure 10. Influence of vane color (green, dark blue and brown) on trap catch in vane traps lured with Chemtica quercivorol lures

Experiment 11

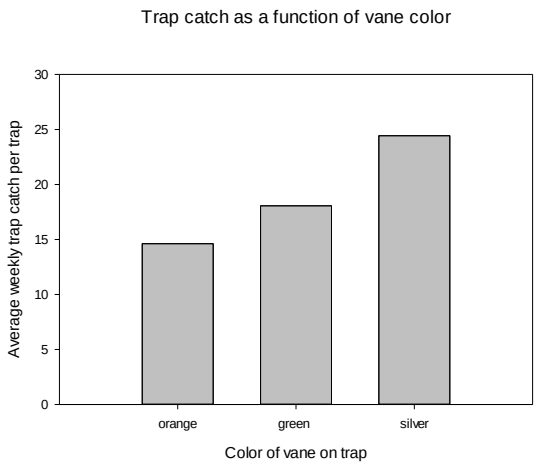


Figure 8. Influence of vane color (orange, green and silver) on trap catch in vane traps lured with Chemtica quercivorol lures

Vane traps can be equipped with different colored vanes and to determine if there is an effect of vane color on the trap catch we compared a total of nine colors, first in groups of three to determine which of the three colors caught the most beetles in the experiment, then the color that caught the highest number of beetles in the initial set of tests were compared with each other in a “playoff” to determine the color with may be best to use in these vane traps. In each of the first rounds of comparison, the color orange caught the most when compared to white and black, the color silver appeared to catch the most when compared to light blue and red, while dark blue did best when compared to green and brown. For the final round we compared silver with green and orange. In this comparison the color silver did the best.

Experiment 12

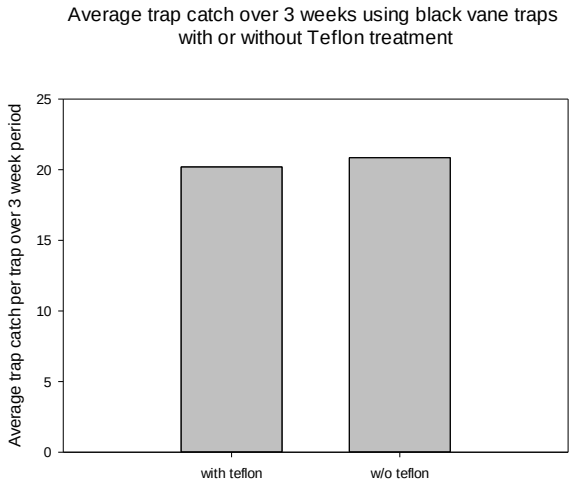


Figure 12 Influence of Teflon sprayed on vanes of vane trap on trap catch of traps equipped with Chemtica quercivorol lures.

The effect of spraying Teflon spray on the vanes was studied using black vane traps. No effect of treatment with Teflon was detected.

Experiment 13

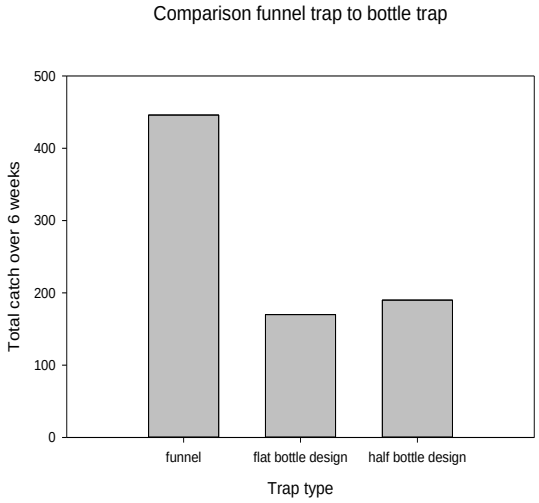
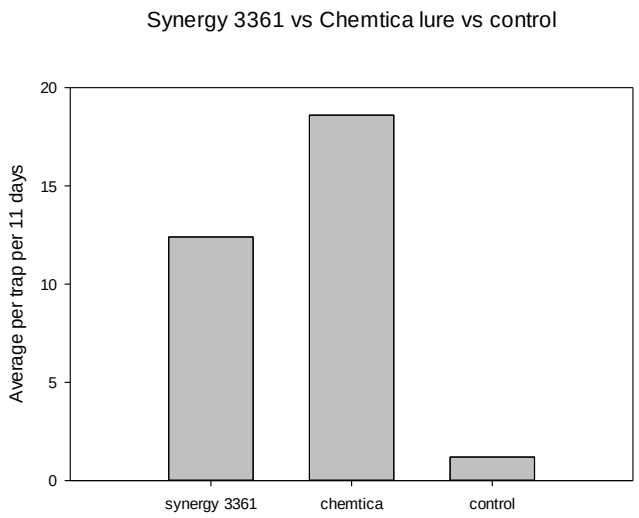


Figure 13. Trap catch of two bottle trap designs compared to 12 funnel trap, all traps were equipped with Chemitca lures.

The relative effectiveness of traps constructed from soda bottles was determined by comparing the trapcatch of a 12-funnel trap with two different bottle trap designs, the flat bottle and the half bottle. Clearly the 12-funnel trap is more efficient in capturing beetles than the bottle trap designs. The latter two did not differ much.

Experiment 14



Comparison of the trapcatch using 12-funnel trap of quercivorol lures produced by either the synergy company (Synergy 3361) or by the Chemtica company.

This experiment tests the lures produced by two different companies Chemtica and Synergy Semiochemicals against each other and compares the two lures with traps without lures. Both lures work well.

Experiment 15

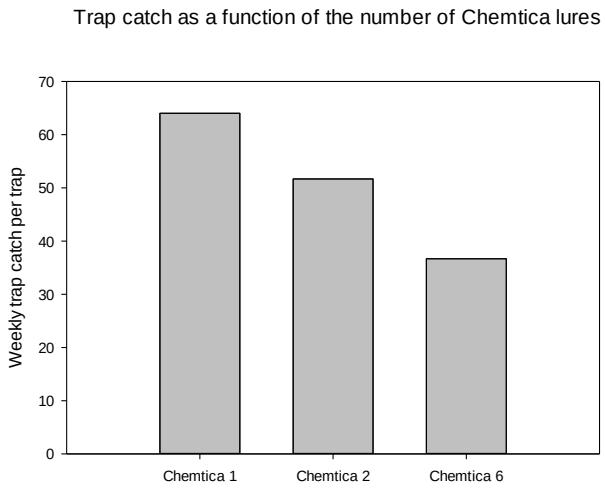


Figure 15 Influence of number of Chemtica lures (6 vs 2 vs 1) on trap catch of 12 funnel trap.

The number of lures determines the local concentration of the attractant. In this experiment we determine if the number of lures attached to a trap influences the number of beetles caught per trap.

The results of this experiment show that higher number of lures reduce the number of beetles caught in the trap.

Experiment 16

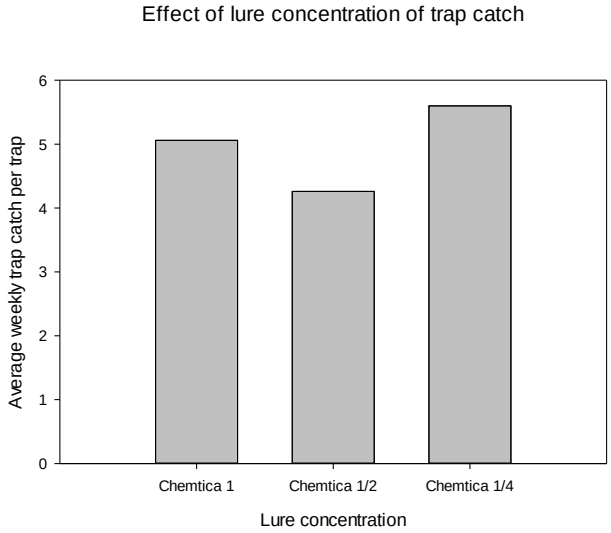


Figure 16 influence of release rate of Chemtica lure on the trap catch using 12-funnel traps, release rate was either full, half or quarter strength. Tests were done inside an avocado orchard.

Experiment 17

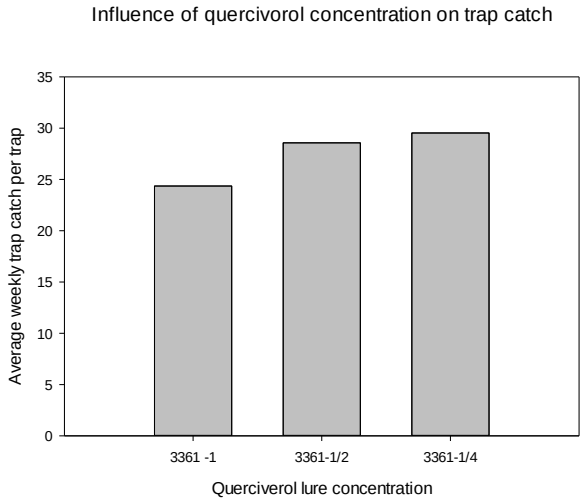


Figure 17 influence of release rate of Chemtica lure on the trap catch using 12-funnel traps, release rate was either full, half or quarter strength. Tests were done inside an avocado orchard.

Experiment 18

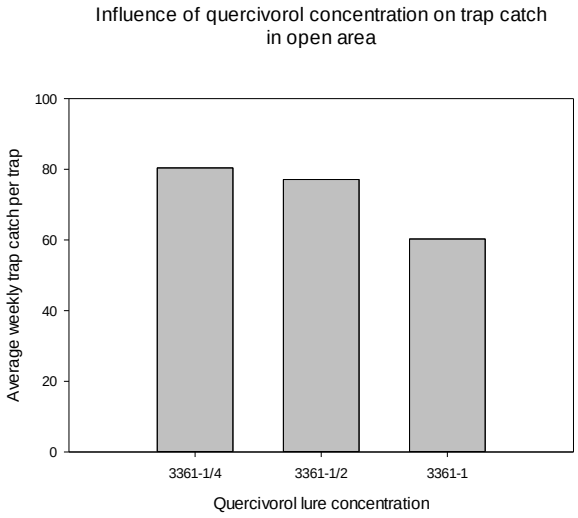


Figure 17 influence of release rate of Chemtica lure on the trap catch using 12-funnel traps, release rate was either full, half or quarter strength. Tests were done inside an open area near a riparian forest infested with PSHB.

In the experiments comparing the lure concentration there did not appear to be much difference in the number of beetles caught by traps equipped with full, half or quarter dosages of quercivorol, for either producer. Initially experiments were done inside avocado groves, where no differences were found, assuming that in open space there might be a difference a third experiment was done to test this and there again no difference was found between full dosage, and reduced dosage.

Experiment 19

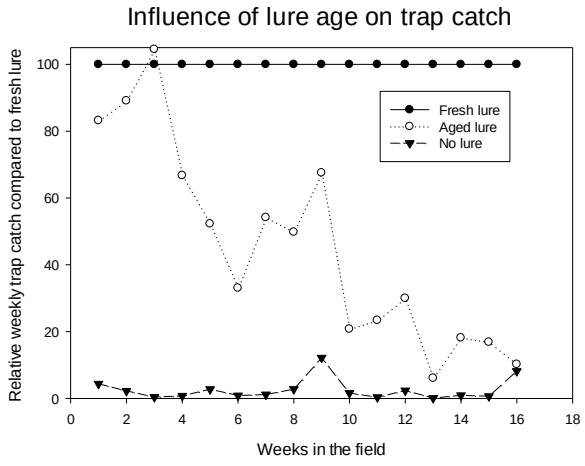


Figure 19. Results of trapping experiment to determine the duration of activity of the Chemtica lure, by comparing aged lured with fresh lures and control traps without lures.

On week 1 lures were hung on the traps that remained on these traps for the duration of the experiment (aged lures) and their effectiveness was compared with the trap catch on traps equipped with fresh lures each week (fresh lures).

From previous experiments it was clear that the full dosage lures remained active for a long time. In this experiment we determined if there was a reduction in ability to capture beetles with the aging of the lures. Each week a new lure was hung on traps and we compared the trap catch of the fresh lure with a lure that had remained on the traps from the beginning of the experiment. Clearly over time the lures degrade in their ability to attract beetles relative to fresh lures, and after about 16 weeks in the field the aged lure has lost its ability to attract beetles.

Experiment 20

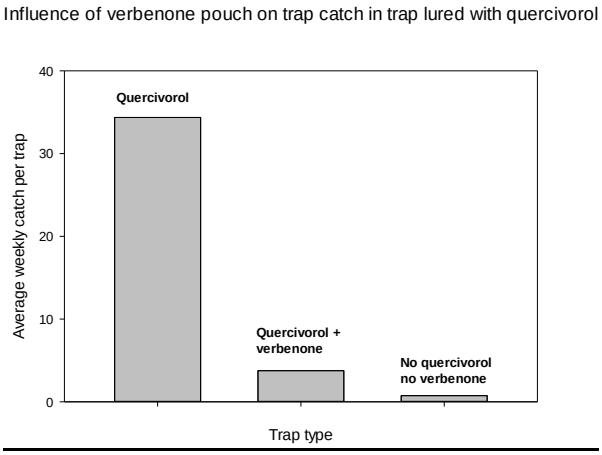


Figure 20. The influence of the anti-aggregation pheromone Verbenone on the trap catch of 12 funnel traps equipped with quercivorol and verbenone, quercivorol alone or control traps without lures. Quercivorol used was from Chemtica.

Some ambrosia beetles are deterred by the chemical verbenone, a known antiaggregation pheromone for some bark beetles. A clear reduction in number of beetles caught in traps equipped with both quercivorol and verbenone was detected compared to traps equipped with quercivorol only.

Yearly flight activity

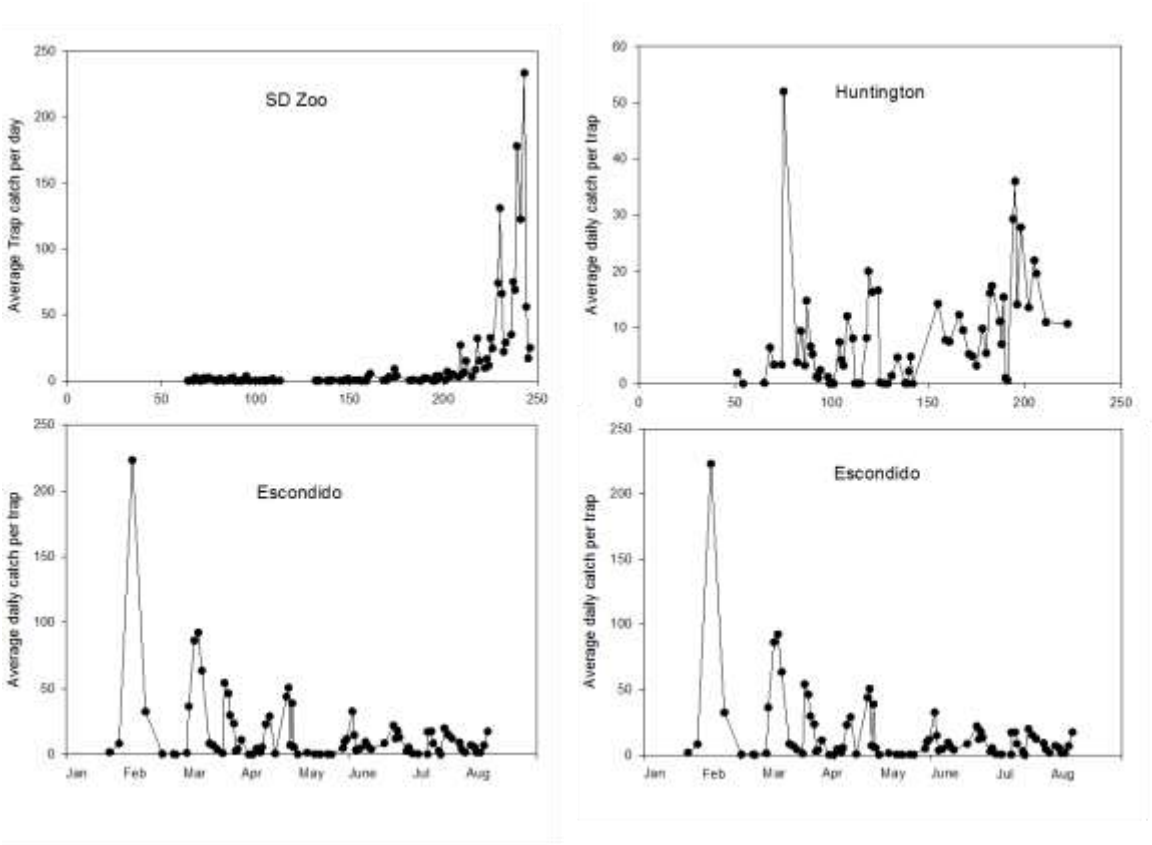


Figure 21. Average daily trap catch in funnel traps equipped with quercivorol in Escondido, Sand Diego Zoo and Huntington Library and gardens during part of the year 2015

Discussion

Our work has identified lures that are attractive to the KSHB and PSHB found in southern California and can be produced at a scale so that they are available on a consistent basis. Of the different quercivorol lures both the 3361 (Synergy) and the lure produced by (Chemtica) attracted beetles at a similar rate. In subsequent experiments it was shown that the lures remain active for a long time up to 16 weeks, but that over time the attractiveness of the lures declines compared to a fresh lure. In addition, we showed that the amount of quercivorol released by the lure can be reduced to ¼ of the current release rate without substantial loss of the number of beetles attracted by the lure for at least the first 3 weeks. There appears to be a negative correlation between the number of lures attached to a trap and the number of beetles caught by the trap. A trap containing 6 lures caught fewer beetles than a trap with 2 lures, and similarly a trap with a single lure catches more beetles than a trap with 2 lures. Going from traps equipped with lures with full release rate to half a release rate to one quarter release rate does not make a significant difference in beetle catch.

The funnel traps with 12 funnels were more efficient in capturing beetles than those with three funnels or with vanes. However, the three funnel and vane traps are cheaper to acquire ($26) than the 12-funnel trap. Finally, traps constructed from soda bottles at a cost of less than $1 caught approximately 40% of the beetles that a 12 funnel trap would catch.

To optimize the trap catch of the PSHB and KSHB the use of ethanol in the trapping cup should be avoided, because it deters to some extend the beetles. Ethanol in the trapping cup is also very attractive to some other invasive ambrosia beetles such as *Xyleborinus saxeseni*. This species a bit smaller than KSHB and PSHB, but separating them from each other can be a time-consuming activity. In order to be able to use the dna of the captured beetles to identify as either KSHB or PSHB the DNA of the captured beetles needs to be preserved. DNA generally breaks down faster in the presence of water and higher temperatures, agents that dry out the specimens will also preserve their dna. Consequently, we tested different preservatives in the collection cup to determine their relative effectiveness, it appeared that the chemical solution DESS caught more individuals in the traps than the other methods, certainly more than keeping the cups dry. In dry cups the beetles stay alive and eventually die and their DNA is preserved in the drying out process. However, since there is no fluid in the cup they also have a better change to escape. To test if we could improve the trap catch using dry cups we varied the opening to the cup by adding a small funnel to the lower funnel on the 12-funnel trap, to inhibit the beetles flight out of the cup. Results showed that this did not influence the number of beetles retained in the cup.

Different colors were tested using vane traps, ultimately the silver colored traps appeared to catch the most beetles. To determine if treating the vanes on the vane traps with Teflon would improve the trap catch we compared black vane traps with and without Teflon treatment and found no differences in trap catch.

Yearly flight patterns of the beetles.

Each year new beetle infestations were generally discovered avocado groves in fall. It seemed like the beetles were flying the most in the later part of the summer. This pattern was particularly obvious in traps placed close to the San Diego zoo in 2015 (Fig 21). In the month of September as many as 600 beetles per day were caught in a trap. In other places such as the Huntington Library and Gardens and in some avocado groves the yearly pattern was not quite as distinct. One hypothesis is that during the latter part of the summer host trees suffer from water deficits and become less suitable hosts for the beetle's ability to grow fungi in the tree. Consequently, beetles occurring in riparian areas of the wild lands, where the water becomes harder to come by at that time of the year, will start to fly away and land in places with hosts that are well watered, such as avocado groves. During the early part of the season, from February to June, the beetles flight dynamics are largely driven by afternoon temperatures. Beetles will fly if the afternoon temperatures are higher than 20C. Since that only happens during heat waves in the winter, this can result in substantial flights in the early part of the year. Beetle populations can build up inside the trees in winter because the beetles will continue to develop as long as the winter temperatures remain over 15C, thus providing the population that can fly out when the afternoon temperatures reach 20C. In summer the afternoon temperatures are almost always suitable for flight and no strong flight peak is observable, simply because the population ready to fly does not build up as it does in winter. In December and January the afternoon temperatures rarely go over 20C and consequently the beetles rarely fly during those months.

Conclusions

Effective lures to capture beetles are available and the dosage of the quercivorol chemical in those lures can be reduced.
12-funnel funnel traps are more efficient in capturing beetles than vane traps or bottle traps
Treating vane traps with Teflon does not improve their trapping efficiency
Silver colored vanes appear to capture more beetles than vanes of other colors
Verbenone on quercivorol lured traps substantially reduces the trap catch of the traps

Yearly flight pattern of the beetles is such that in winter and spring (Feb-May) large flights may occur after the afternoon temperatures reach 20C and higher
In summer large flights are rare and the afternoon temperatures are practically always suitable for flight
In the later part of the summer September, October and parts of November, large flights may occur when water stressed trees are present in the land scape.

Summary

- Effective lures that are consistently available have been developed
- Chemicals that may deter beetles from attacking trees have been identified
- Methods to capture beetles using traps have been optimized
- Yearly patterns of beetle flight have been detected and interpreted

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