Chapter 15

Development of Specialized Pheromone and Lure Application Technologies (SPLAT®) for Management of Coleopteran Pests in Agricultural and Forest Systems

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Since ISCA Technologies' market introduction of Specialized Pheromone and Lure Application Technology (SPLAT®) in 2004, it has been implemented for effective pest control through a variety of techniques, including mating disruption, mass trapping, attract and kill, and repellency. The majority of SPLAT®-based pest control products have targeted lepidopteran (e.g., SPLAT® OFM, for oriental fruit moth, *Grapholita molesta*; SPLAT® GM for gypsy moth, *Lymantria dispar*; SPLAT® EC, for carob moth, *Ectomyelois ceratoniae*) and dipteran pests (e.g., SPLAT® MAT Spinosad ME for

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tephritid fruit flies, Tephritidae). This chapter will describe a relatively new family of SPLAT® formulations designed to attract or repel coleopteran pests. Following a brief description of the characteristics of SPLAT® and its advantages over other forms of semiochemical-based pest control, each section will begin with an introduction to each product's method of action, specifically mating disruption, attract and kill, and repellency, and will then summarize current research on the application of each technique to combat a specific coleopteran pest: oriental beetle (OrB), *Anomala orientalis* (SPLAT® OrB & SPLAT® OrB A&K), red palm weevil (RPW), *Rhynchophorus ferrugineus* (Hook™ RPW), and mountain pine beetle (MPB), *Dendroctonus ponderosae* (SPLAT® Verb).

Specialized Pheromone and Lure Application Technology (SPLAT®)

Semiochemical methods of pest control have numerous advantages over the use of conventional insecticides, both in terms of efficacy and environmental sustainability. Semiochemicals, such as sex pheromones used by insects to locate a mate, and host plant kairomones, which serve as important cues to help insects navigate their environment, are naturally occurring, often species-specific substances. Artificial introduction of such substances into a field environment often allows the grower to elicit a specific response from a specific insect pest, leaving desirable nontarget organisms, like natural enemies and pollinators, often unaffected. Behavioral manipulation by means of semiochemical applications has been shown to involve less risk of the target species developing resistance to the active ingredient (AI), a common shortcoming among conventional insecticides (*1*). Furthermore, unlike conventional insecticides, which are often applied as cover sprays over the entire crop, semiochemicals tend to be deployed in discrete point sources. This reduces the likelihood of nontarget organisms coming into contact with the compound, and enables growers to avoid contamination of food crops.

Despite these advantages, it has proven difficult to develop and commercialize effective, economically-viable semiochemical-based pest control technologies. The primary obstacle to wide-spread adoption of these technologies is the high cost of semiochemicals, which are generally more expensive than conventional pesticides (*2–4*). In many cases, however, it is not the intrinsic cost of the semiochemical itself that makes the end product economically unfeasible, but rather the method by which it must be deployed in the field. Past and current semiochemical formulations have typically been developed as devices, which by their very nature require manual application. This can lead to prohibitively high application costs, especially in large and/or heavily infested fields, requiring high application densities. These costs can be further compounded by the physical limitations of the active semiochemical (i.e., chemical instability/volatility, sensitivity to light or heat), as well as by the mechanism of behavior manipulation in which it is employed. Mating disruption formulations, particularly those that work by camouflage (defined later in this chapter), generally require large quantities of pheromone to be deployed in order to be effective. In addition, many semiochemical products have demonstrated inconsistent capability to maintain the release rate and period necessary to achieve effective pest control. For example, an early bubblecap formulation developed for management of MPB infestations failed to release its AI, verbenone – the same AI contained in ISCA's MPB repellent product, SPLAT® Verb, to be discussed later in this chapter – at levels sufficient to compete with pheromones naturally emitted during an infestation, and therefore failed to adequately repel the pest (*5*).

These general shortcomings in semiochemical formulations make them prone to failure under high pest pressure. In these situations, successful application of these products often requires treatments of conventional insecticides prior to deployment, in order to knock down the pest population in the field to sufficiently low numbers for the semiochemical AI to exert the desired influence over the pest's behavior. This is a particularly severe hindrance to the acceptance of semiochemical technology as a viable method of pest control, since the health and environmental hazards associated with conventional insecticides represent, from the viewpoint of stakeholders, researchers, and the general public, some of the most persuasive justifications for developing such technologies in the first place.

Mindful of these prior deficiencies, ISCA Technologies has developed an innovative and versatile alternative: Specialized Pheromone & Lure Application Technology (SPLAT®). Rather than a single device, SPLAT® is an amorphous, flowable controlled-release emulsion, with chemical and physical properties that may be adjusted by small changes in composition, in processing, or in application method to suit a wide variety of pest management techniques, while minimizing application costs. It is a shear-thinning, thixotropic, non-Newtonian fluid that enters a liquid state when agitated, such as by stirring, but quickly solidifies when the source of agitation is removed. This property allows for easy manual or mechanical application in the field, enabling the user to customize point source size and distribution to the requirements of each field, while still ensuring that the product will persist in the field for a sufficient period to achieve effective pest control. SPLAT® formulations typically become rainfast within 3 hours following application, and can provide continuous controlled release of semiochemical AIs for intervals ranging from 2 weeks to 6 months. The rate of release of the incorporated AIs into the field is determined by the AI molecules' rate of diffusion through the SPLAT® matrix, which occurs in accordance with Fick's First Law of Diffusion (*6*), stating that molecules move from regions of high concentration to regions of low concentration at a rate that is directly proportional to their concentration gradient. This rate may be precisely controlled or adjusted by subtle changes in the formulation's characteristics and application method. Because of its controlled-release capacity, SPLAT® formulations typically use smaller amounts of AIs than other semiochemical products, reducing application costs and environmental impact.

This product's unique adaptability makes SPLAT® formulations amenable to a virtually unlimited range of application methods. In previous applications, SPLAT® products have been applied by hand, using such simple tools as a spatula or knife to protect relatively small numbers of high-value plants; delivered through metered syringes and caulking guns; sprayed mechanically via modified applicator vehicles, including tractors, all-terrain vehicles, and motorcycles; loaded into tanks of airplanes and sprayed over hundreds of thousands of hectares of forests; and even fired in pellets from paintball guns. This technology boasts additional advantages in terms of reduced environmental impact. SPLAT® is biodegradable, and all of its inert ingredients have been certified as food safe by the US Environmental Protection Agency (US EPA). A number of commercial SPLAT® formulations have even been granted organic status, including SPLAT® GM Organic for mating disruption of the gypsy moth and SPLAT® EC Organic for the carob moth, *Ectomyelois ceratoniae*.

While SPLAT® formulations have been developed for control of dozens of insect pests, including multiple representatives of the orders Lepidoptera, Diptera, and Hemiptera, this chapter focuses solely on those designed to target coleopteran pests, specifically: SPLAT® OrB for mating disruption and attract and kill of OrB in blueberries, *Vaccinium* spp.; Hook™ RPW for attract and kill of the RPW in date palms, *Phoenix dactylifera*; and SPLAT® Verb, a repellent for MPB in lodgepole pine, *Pinus contorta*.

SPLAT® Technologies for Control of the Oriental Beetle, *Anomala orientalis*

Mating Disruption

Mating disruption, currently the most commonly employed method of semiochemical-based pest control, is a technique of insect behavior manipulation that seeks to reduce the reproductive success of the target pest, thereby diminishing its population and impact on crops over time. In order to deter individuals from locating a mate, this technique requires synthetic sex pheromones, or chemically and biologically similar analogs, to be dispensed into the environment at levels that disrupt males' abilities to detect and respond to the natural pheromone plume emitted by a calling female.

In addition to reducing pest populations by interfering with mate finding, and consequently reducing the number of successful mating events, the artificial introduction of sex pheromones required to achieve mating disruption may also result in loss of fitness among females of the target species, as a consequence of delayed mating. Females have only a limited amount of time in which to reproduce and to select an appropriate site for oviposition. Recent studies have shown that a delay in mating, such as this would be caused by a decrease in males' abilities to locate females due to the presence of synthetic pheromone, can negatively impact females' levels of reproductive fitness by decreasing fecundity (number of eggs carried by the female), fertility (capability of eggs to produce viable offspring), and pre-oviposition period (*7*).

A number of mechanisms by which mating disruption can occur have been proposed and investigated. These may operate separately or in combination, depending on the target pest and the pheromone used to control it. The most widely accepted of these mechanisms are described below.

1. **Competitive attraction**, also referred to as "false trail following" occurs when males orient and respond to synthetic pheromone plumes produced by semiochemical dispensers, rather than the natural plume emitted by a calling female. This mechanism of mating disruption is population density dependent, and decreases in efficacy as the population of the pest increases.
2. **Camouflage**, unlike competitive attraction, is density independent, and requires that the environment be saturated with synthetic pheromone to the extent that mate-seeking males are incapable of homing in on females.
3. **Desensitization** takes place when males' responses to the female sex pheromone are significantly diminished due to over-exposure, and may occur either through adaptation of the males' olfactory receptor neurons, or by habituation of the insects' central nervous system (*7*, *8*).
4. **Sensory imbalance** achieves mating disruption by impeding the male's ability to recognize the female sex pheromone within the treated environment, thereby preventing him from responding to it.

Understanding which of these mechanisms operates predominately in a given scenario may help to determine how to deploy a pheromone formulation in such a way as to achieve the highest level of mating disruption, and consequently, the largest reduction of pest population and damage to host plants (*9*). Unfortunately, the majority of past research has been primarily aimed at evaluating the quality and reliability of control achieved by mating disruption techniques for various insect species, rather than identifying the precise mechanism by which they function.

In the development and testing of SPLAT® OrB, a mating disruption formulation for the oriental beetle, ISCA Technologies and its collaborator, Dr. Cesar Rodriguez-Saona, Assistant Professor of Entomology at Rutgers University, endeavored not only to verify the product's efficacy against the target pest, but also to determine how its application disrupted the insect's reproduction.

SPLAT® OrB for Mating Disruption

Native to Asia, OrB was introduced to the United States from Japan in the early 20th century. It was first detected in Connecticut in 1920, following a shipment of infested nursery stock (*10*, *11*), and has since expanded its range through the northeastern United States, from Maine west to Ohio, and south to South Carolina. It is a major pest of several plant varieties throughout this region, including soft fruits such as blueberries, raspberries, **Rubus** spp., and cranberries, **Vaccinium** spp., as well as ornamental nursery stock and turfgrass. Of particular concern is the threat posed by OrB to the blueberry industry of New Jersey, a crop recently valued at approximately $82 million (*12*, *13*). As with other susceptible crops, the damage inflicted by OrB results exclusively from the feeding of the larvae on the roots of the plant (adults reportedly do not feed). Infested blueberry bushes produce fewer fruits, have smaller leaves and generally reduced vigor, and tend to have shorter life spans than uninfested bushes (*14*). In severe infestations, OrB grubs can virtually destroy the entire root system of the plant, leading to its death (*14*).

SPLAT® OrB was developed in order to provide blueberry growers with an alternative OrB control method to standard treatments, which currently consist of soil applications of the neonicotinoid insecticide, imidacloprid. Growers have expressed a number of concerns regarding the use of this insect neurotoxin, including its high cost of application; the possibility of OrB developing resistance to its mode of action; and the harmful effects it may exert on beneficial nontarget organisms, for example, the OrB natural enemy, *Tiphia vernalis* (*15*), and the honeybee, *Apis mellifera,* a pollinator necessary to ensure proper fruit set in blueberry crops (*16*). These concerns have generated increasing interest in the investigation of mating disruption as a tool to manage OrB, an opportunity made possible in the early 1990s by the identification and successful synthesis of the sex pheromone for this species, a 9:1 blend of (*Z*)-7-tetradecen-2-one and (*E*)-7-tetradecen-2-one (*17–19*). SPLAT® OrB relies solely on the major component, (*Z*)-7-tetradecen-2-one, for its behavior-modifying capabilities, in light of the findings of Facundo et al. (*20*), which demonstrated that this compound alone was equally attractive to OrB males as the 9:1 blend composing the natural pheromone.

SPLAT® OrB was tested in 2008-2009 in three commercial blueberry farms, located in Atlantic Co., New Jersey, compared against a plastic "bubble" dispenser formulation using the same AI [(*Z*)-7-tetradecen-2-one], for efficacy of mating disruption in OrB. The two treatments were applied at two corresponding rates in the field: 2.5 g AI/ha and 5 g AI/ha. Plastic pheromone dispensers were hung directly from blueberry plants, at a height of approximately 20 cm above the ground. SPLAT® OrB was applied in 0.5-g dollops (ca. 3.5 cm long, 1.5 cm wide, 2 mm thick), each containing 5 mg AI, deposited on wooden stakes (15 x 1.5 cm plant labels; Gemplers, Bellville WI) using a plastic spatula. The stakes were inserted into the soil, each close to the base of a blueberry bush, so that the SPLAT® OrB dollop was positioned 5-10 cm above the ground. This placement was intended to enable maximum impact on OrB mating, which has been shown to occur at the surface of the soil (*21*). Both pheromone treatments were applied throughout a 1-ha plot, along an evenly-spaced grid between blueberry bushes. Each treatment was replicated three times, with treatments within each blueberry farm assigned to plots in a randomized complete block design. Plots were separated from one another by a minimum distance of 50 m. Both pheromone-treated and untreated (control) plots (equivalent in size) were treated with fungicides and insecticides according to the growers' standard program. None, however, were treated with imidacloprid.

Treatment efficacy for both SPLAT® OrB and plastic pheromone dispensers were evaluated based on the following factors:

1. Trap shutdown was measured by the number of OrB caught in pheromone-baited traps. Three traps were placed in each plot, and monitored on a weekly basis for the duration of the study.
2. Female mating status was evaluated by placing 5 screened cages in each plot, each containing a virgin female OrB. These cages were designed to permit entry by OrB males, allowing them access to the caged female, but preventing escape from the cage by either sex. Cages remained in the

field for 3 nights, and were then retrieved to evaluate OrB mating success, via egg laying by caged females. Cages were deployed 3 times over an interval of 2 weeks, on June 20 and 24, 2009, and on July 1, 2009.

3. Grub density (number of OrB larvae per blueberry bush) was evaluated using 5 potted 2- to 3-year-old bushes, placed in each plot. One virgin female was tethered in each pot (n = 75 virgin females), replaced every week for a period of 4 weeks. Larval density for each potted blueberry plant was ascertained through destructive sampling of root systems.

A disruption index (DI) was calculated for this experiment according to the following equation: (average OrB capture/trap in control plots) - (average OrB capture/trap in treatment plots) / (average OrB capture/trap in control plots) x 100. Data on OrB trap captures, female mating status (percentage of mated OrB females retrieved from cages), and grub density in potted blueberry bushes, as well as calculated DI values were subjected to analysis of variance (ANOVA) (Minitab 13.32; Minitab Inc., State College, PA). Trap capture and grub density data were log-transformed for normality when required. Female mating status data and DI values were arcsine square root-transformed. Means for all data were separated using Tukey tests ($\alpha = 0.5$).

Field observations were conducted in one of the blueberry farms to ascertain whether male OrB approached pheromone point sources in the field (Figure 1), a pattern which would be useful in determining the mechanism by which SPLAT® OrB disrupted mating in OrB. Observations were made for a 30-minute period in each treatment plot every 4 h, starting at 8:00 and ending at 20:00 (all observations were carried out on July 3, 2009). Ten SPLAT® OrB-treated stakes, chosen at random, were observed per plot for 3-5 minutes. Similar observations were carried out in random locations in control plots as well. The number of OrB detected within 1 m of each observation point was recorded. Data for OrB attraction to pheromone point sources were log-transformed (x + 0.1), analyzed using ANOVA, and then subjected to Tukey tests.

In June-July 2008, a mark-release-recapture study was conducted in a blueberry field at Rutgers Blueberry/Cranberry Center, to ascertain the distance at which OrB males respond to both artificial and natural pheromone sources. Four sources were evaluated: virgin OrB females, SPLAT® OrB (0.5-g dollop containing 5 mg AI), plastic dispensers (containing 0.1-g AI), and rubber septa (300 µg AI). SPLAT® OrB dollops, plastic dispensers, and rubber septa were placed inside individual Japanese beetle traps, while virgin females were placed in wire cages attached to the trap. Male OrB to be used for this study were trapped out of the field and taken to the laboratory to be marked according to their intended release site. Markings were made using enamel paints (The Testor Corp., Rockford IL) on the pronotum and/or left or right elytra. Laboratory observations indicated no effects of the paints on beetle behavior. Marked OrB were released at the following distances from each treatment: 3.75, 7.5, 15, 30, and 60 m, on 3 different days in 3 consecutive weeks (240 OrB released in total). Resulting data were arcsine transformed and analyzed via ANOVA. Means were separated via Tukey test.

Figure 1. Male OrB approaching a dollop of SPLAT®OrB in a blueberry field. The beetle walking towards, and then visiting, a dollop of SPLAT®OrB (inset, arrow points to the beetle).

In order to determine the effect of SPLAT® OrB application density on beetle attraction to pheromone sources, an additional study was conducted at the Atlantic Co., NJ blueberry farms evaluating OrB trap captures in plots treated with 4 different densities of SPLAT® OrB dollops: 0 dollops (0 g AI)/ha, 25 dollops (0.125 g AI)/ha, 250 dollops (1.25 g AI)/ha, and 500 dollops (2.5 g AI)/ha. All SPLAT® OrB dollops were applied to wooden stakes and deployed in the field as previously detailed. Data for the 3 pheromone-baited traps placed in each plot (20 m between traps) was summed and log-transformed. This was followed by ANOVA analysis and Tukey test ($\alpha = 0.05$). DI values were arcsine square root-transformed prior to ANOVA analysis and Tukey test. The data from this study were also plotted in order to test the predictions put forward by Miller et al. (*8*) for the competitive attraction mechanism of mating disruption.

Trap captures in pheromone-treated plots (both hand-applied plastic dispensers and SPLAT® OrB dollops) were found to be significantly lower compared to those in untreated plots ($F = 31.8$; df = 4,8; $P < 0.001$). The higher treatment rate of 5 g AI/ha produced disruption index (DI) values 6-9% higher than the lower treatment rate of 2.5 g AI/ha. Fewer caged females were found to have mated ($F = 14.9$; df = 4,8; $P = 0.001$), and a smaller number of males were found in cages that were placed in pheromone treatment plots ($F = 22.5$; df = 4,8; $P < 0.001$). Grub density was also reduced in SPLAT® OrB-treated plots at both application densities, as well as in plots treated with the higher density of plastic dispensers ($P \leq 0.05$). Field observations conducted in SPLAT® OrB and plastic dispenser plots revealed that beetles did approach pheromone sources in the field, and did so at all hours of the day at which observations were made – a finding of particular significance, given the fact that these insects normally exhibit cryptic behaviors. In contrast, observers stationed at random locations within control plots reported no OrB sightings for the duration of the study. The results of the mark-release-recapture study carried out at the Rutgers Blueberry/Cranberry Center further confirmed OrB attraction to point sources. Both SPLAT® OrB and plastic pheromone dispensers were found to exert a stronger level of attraction in OrB than rubber septa and virgin females, and were capable of drawing the beetles to the traps from distances of more than 60 m. Fewer marked OrB were recovered as the distance between the release site and the pheromone source increased.

The SPLAT® OrB density study showed that the higher application rates for this formulation (250 dollops/ha and 500 dollops/ha) resulted in larger reductions in OrB trap captures, and DI values approximately 10% higher than were seen in the lower density plots (25 dollops/ha), suggesting that the higher application rate is required to achieve the most effective mating disruption against this pest. In addition, the data produced by the density study were found to conform to Miller et al.'s (*8*) predictions for competitive attraction (Figure 2). OrB trap capture data (un-transformed), when plotted against SPLAT® OrB dollop density, yielded a graph that descends concavely as density increases, asymptotically approaching zero. This suggests that the ability of males to successfully detect and locate a pheromone source decreases as the density of the pheromone sources increases – a strong indication that competitive attraction is taking place, especially taking into account the field observations conducted in the pheromone-treated plots, which visibly confirmed OrB attraction to point sources. Furthermore, when the data was plotted as a Miller-Gut plot [1/OrB male trap capture (y-axis) vs. point source density (x-axis) and as a Miller-de Lame plot [OrB male trap capture (y-axis) vs. (point source density x OrB male trap capture) (x-axis)], it produced straight lines, the former with a positive, and the later with a negative slope, also in accordance with predictions for the competitive attraction mechanism for mating disruption.

Conclusions

SPLAT® OrB applied at low densities in the field was equally as effective at disrupting mating in the OrB as plastic pheromone dispensers, but proved more effective at reducing grub density in potted blueberry plants than plastic dispensers at the lower application rate. SPLAT® OrB is amenable – indeed, is designed for – mechanized application, and would therefore represent a significant advantage over hand-applied dispensers in terms of labor costs, since the amount of pheromone applied (and its associated cost) is the same for both formulations. However, due to restrictions on the experimental use of the OrB sex pheromone [a ketone, not exempt from the tolerance requirements established by the US EPA for use on fruit crops (*12*, *22–24*)], current formulations must be retrievable from the field, effectively ruling out mechanical application for the present. Should the US EPA approve registration of SPLAT® OrB, thereby releasing it from the limitations of an experimental use product, this formulation may prove to be a valuable OrB management tool for growers of blueberries and other susceptible crops.

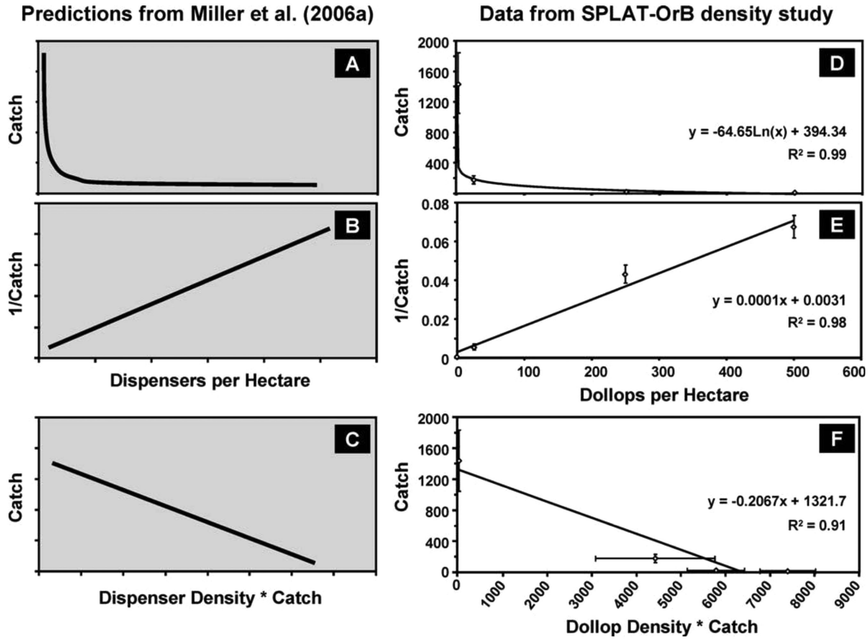

Figure 2. SPLAT® OrB promotes mating disruption through the mechanism of competitive attraction. Predictions for competitive attraction as described in Miller et al. (8): untransformed number of trap captures against dispenser density (A); Miller-Gut plot (B); Miller-de Lame plot (C). These plots are compared with data from our SPLAT® OrB density study: plot of untransformed oriental beetle male captures per trap versus SPLAT® OrB dollop density (D), Miller-Gut plot for oriental beetle data (E), and Miller-de Lame plot for oriental beetle data (F). Data are means ± SE, and equations for best-fit curve and lines are presented in graphs DEF. (Reproduced with permission from reference (12). Copyright 2010 The Entomological Society of America.)

A mechanical applicator for SPLAT® OrB in blueberries has been developed and tested (Figure 3). Precise applications of dollops to the soil at the base of blueberry plants can be adjusted by changing the speed at which the user drives the vehicle through the field.

Field observations in pheromone-treated plots, as well as the results of the mark-release-recapture and SPLAT® OrB density studies all point to competitive attraction as a key mechanism in OrB mating disruption. Beetles were found to perceive and approach pheromone sources, and to be preferentially attracted to SPLAT® OrB over virgin females. These findings not only indicate SPLAT® OrB's potential as an effective mating disruption product, but also suggest an alternative means of control for this pest: the incorporation of an insecticide into SPLAT® OrB, to create a novel attract-and-kill formulation.

Figure 3. The mechanical applicator mounted on a 'gator' in a blueberry field. The applicator has two adjustable arms that protrude from the back of the gator towards the ground that contain valves that open and close periodically, at pre-set intervals and pre-set opening times, allowing the SPLAT® OrB under pressure in the pipes, to exit and fall onto the ground. By setting the SPLAT®OrB pressure, the time interval between openings, and the time that the valve is opened, the user is able to precisely deposit dollops at regular intervals simply by adjusting the speed of the vehicle. Inset shows a gray dollop of SPLAT® OrB near a US $0.10 coin, for scale.

Attract and Kill

While attract and kill (A&K) is at its heart a simpler, more straightforward technique of insect pest management than mating disruption, which, as previously illustrated, can occur by a variety of different mechanisms, it can be deceptively difficult to implement effectively in a field environment. At its most basic level, this technique can be defined as follows: an attractant, which may be a crude bait or semiochemical, or even a visual or auditory cue, lures the target insect (males, females, or both) to a point source either containing or directly adjacent to a toxicant. This control agent is generally an insecticide, but sterilants (as in lure and sterilize) and insect pathogens (as in lure and infect) have also been used. Upon contact or ingestion of the toxicant at or within the point source, depending on its mechanism of action, the target insect either dies or suffers detrimental effects that, over time, result in reduced pest populations. In some cases, the toxicant does not necessarily have to kill the insect in order to effect population suppression, as long as its sublethal effects sufficiently impair the insect's reproductive capacity (*25*). For example, one study found that sublethal exposure to an A&K formulation for codling moth, *Cydia pomonella*, caused males of the species to undergo autotomy (self-amputation) of the thoracic limbs, significantly impeding their physical ability to mate and reducing their general fitness (*26*, *27*).

In order to successfully manage a pest population, an effective A&K system calls for a highly-optimized product, in terms of the attractant and the insect control agent to be used. The lure must be a strong enough attractant to draw the target insect directly to the point source and induce contact, or in the case of some stomach-poison insecticides, to induce feeding. The insecticide, too, must be highly potent, and as fast acting as possible, ensuring that all or nearly all of the insects that are attracted to the point source are either killed or incapacitated shortly after contact/ingestion, before they have the opportunity to mate. In a review on the potential of A&K strategies for insect pest management, El-Sayed et al. (*27*) put forth 3 crucial events that must take place in order for any A&K formulation to achieve effective control. First, the insect must successfully locate and contact the point source, which should ideally be more attractive to the target insect than any natural attractant source present in the field. Second, the attracted insect must then ingest an adequate quantity, or spend a sufficient period of time in contact with the insecticide, or other toxicant, to achieve the desired detrimental effect. Third, the resulting level of mortality/debilitation must be such that it has a significant negative effect on the pest population in treated areas (*27*).

In addition to the high standards of efficacy demanded for successful application, there are a number of other drawbacks to the implementation of A&K methods of pest control. The addition of an insecticide into a semiochemical formulation, such as SPLAT® OrB, creates complications regarding product efficacy, as well as public opinion and acceptance of the product. Interactions between the toxicant and the attractant within the formulation may negatively impact is effectiveness, most obviously if the former component is an insecticide that is repellent to the target species (*27*). The general public may be more reticent to accept A&K control techniques than those that function by mating disruption, out of concern that the toxicants they employ may harm the environment or nontarget species (*28*).

However, A&K formulations differ from traditional applications of chemical insecticides on several critical points. Developers of A&K products almost invariably select low-risk insecticides, those that have demonstrated minimal hazards to mammals and other non-target species, as well as to the environment, and deploy them in miniscule amounts compared to conventional cover sprays (*25*, *29*, *30*). Also, the application of A&K products in lieu of conventional insecticides eliminates the danger of spray drift (*25*). In many cases, as with the use of sex pheromones as lures, the attractant is species-specific, further reducing the risk of exposure to non-targets. While the efficacy of A&K declines as pest population density increases, as does mating disruption (*29*, *31–34*), the former technique has been shown to be less subject to the limitations of environmental factors, such as the topography and size of the treatment plot (*27*).

The results of the Rodriguez-Saona et al. (*12*) trial of SPLAT® OrB for mating disruption, described above, identified this product as having a high potential for use as an A&K formulation for OrB. During these studies, the beetles were observed to approach SPLAT® OrB dollops in the field at all times of the day, and to preferentially pursue odor plumes produced by this treatment over those produced by virgin female OrB. As a result, ISCA scientists designed SPLAT® OrB A&K, a SPLAT® formulation combining the same AI used in the mating disruption product (the major component of the OrB sex pheromone) with the reduced-risk contact insecticide, cypermethrin. This product was tested by the same research team as the 2010 mating disruption study, to determine 1) whether SPLAT® OrB A&K was capable of delivering the same efficacy and reliability of pest control as its mating disruption counterpart, 2) whether efficacy of either formulation increased with higher point source density, and 3) the field longevity of each formulation. Their work is described in the following section.

SPLAT® OrB for Attract and Kill

A field study evaluating the efficacy of SPLAT® OrB and SPLAT® OrB A&K for control of the OrB was conducted by Dr. Rodriguez-Saona and his research team in 2011, in two commercial blueberry farms in Atlantic Co., New Jersey (Atlantic Blueberry Co., 7201 Weymouth Road #A, Hammonton; and Bellbay Farms, 131 Main Road, Hammonton). Four pheromone treatments were applied over 1-ha experimental plots, separated from one another by a minimum distance of 90 m.

The following treatments were assigned to plots within blocks according to a randomized complete block design:

1. SPLAT® OrB [1% (*Z*)-7-tetradecen-2-one] applied as 250 1-g dollops
2. SPLAT® OrB [1% (*Z*)-7-tetradecen-2-one] applied as 500 1-g dollops
3. SPLAT® OrB A&K [1% (*Z*)-7-tetradecen-2-one + 2% cypermethrin] applied as 250 1-g dollops
4. SPLAT® OrB A&K [1% (*Z*)-7-tetradecen-2-one + 2% cypermethrin] applied as 500 1-g dollops
5. Untreated control

In accordance with the EPA's restrictions regarding the experimental use of unregistered pest control products, all SPLAT® OrB formulations were applied to wooden stakes (the same as were used in the 2010 mating disruption study), using a caulking gun (Newborn X-lite, Newborn Brothers Co., Inc., Jessup MD) calibrated to dispense a 1-g dollop of material. SPLAT® OrB-treated stakes were inserted into the ground along an evenly spaced grid between blueberry plants. This method of application ensured that the treatments could be easily removed at the conclusion of the study (per EPA requirements). All treatments were replicated

3 times (1 replicate/block). The application of pheromone treatments commenced on June 3, 2011, with SPLAT® OrB for mating disruption, and ended 3 days later on June 6, 2011, with SPLAT® OrB A&K. No applications of imidacloprid were made to any of the treatment or control plots, though all were treated with other insecticides and fungicides according to the growers' standard program.

The efficacy of the 4 SPLAT® OrB treatments was evaluated based on the following criteria:

1. Trap shutdown was measured by the number of OrB males captured in three Japanese beetle traps (Trécé, Salinas, CA) baited with 300 μg (Z)-7-tetradecen-2-one (ISCA Technologies, Riverside, CA) placed in each plot. Traps were deployed on June 10, 2011 and were monitored weekly. Pheromone lures were replaced at 3 week intervals. Trap capture data was log transformed ($\log_{10}$ + 0.01) and subjected to ANOVA (GLM Univariate; IBM SPSS v19, Statistics Base) and Tukey test. A disruption index (DI) was also calculated, using the formula, [(Average OrB captured/trap in untreated plots + 0.01) – (Average OrB captured/trap in SPLAT®OrB-treated plots + 0.01) / (Average OrB captured/trap in untreated plots + 0.01) x 100]. The resulting DI values were arcsine square root-transformed before undergoing ANOVA and Tukey test.
2. Grub density was measured by tethering virgin OrB females to potted 2- to 3-year-old blueberry bushes, as described in the 2010 mating disruption study. Four of these potted bushes were placed near the center of each plot at the end of June 2011, and were removed in mid-July 2011. After their removal from the field, the plants were stored in a greenhouse until the following November, allowing any OrB larvae present within the plots to attain a sufficient size as to be easily detectable before being counted.
3. Behavioral observations were conducted to verify that OrB approached the pheromone point sources in the field. Observations were conducted in the treatment plots on July 19, 2011 at three times of the day, 800, 1200, and 2000. Ten SPLAT® OrB point sources, selected at random, were visually monitored in each plot for a period of 3-5 minutes. Similar observations were also carried out at randomly chosen locations within the untreated plots. As in the 2010 mating disruption study, observers stationed in both the treatment and control plots recorded the number of OrB detected within 1 m of the observation point.

A laboratory study was also performed on the residual activity of SPLAT®, evaluating the following treatments: 1) SPLAT® OrB [1% (*Z*)-7-tetradecen-2-one], 2) SPLAT® OrB A&K [1% (*Z*)-7-tetradecen-2-one + 2% cypermethrin], and 3) SPLAT® Blank (no AI) as a control. One fresh, unaged 1-g dollop of SPLAT® was applied at the center of a plastic petri dish (95 mm in diameter, 15 mm in height; Fisher Scientific, Pittsburgh, PA). A total of 20 dishes were prepared for each treatment, with each dish representing one replicate. Beginning at Day 0, one OrB male was placed in each treatment dish with the SPLAT® dollop, and the lid of the petri dish was placed on loosely to allow for adequate airflow. Mortality among OrB due to contact with the formulation was recorded at 2, 12, 24 and 48 h of exposure. The procedure was repeated with all 3 SPLAT® formulations, aged in a 25°C incubator for 15, 21, or 30 d. For the 30-d aged dollops, 16 rather than 20 replicates were made. Cotton balls soaked with water were included in the treatment dishes along with the SPLAT® dollops for the 21- and 30-d aged samples, following abnormally high mortality for the 15-d assay, presumably due to lack of moisture. The significance of the relationship between SPLAT® formulation and OrB mortality was compared for each sample-age category, via a Chi-square goodness of fit test with a Bonferroni correction for multiple pairwise comparisons. OrB trap captures were significantly lower in SPLAT® OrB treatment plots than in control plots ($F = 178.5$, 4 df, $P < 0.0001$) in all cases, irrespective of formulation or point source density. There was no significant difference in trap captures of OrB between plots treated with SPLAT® OrB and those treated with SPLAT® OrB A&K. Beetle captures in all treatment plots remained at or close to zero, with consistently high DI values (87.8–89.5%) throughout the entire study. No significant differences were observed in DI values between SPLAT® treatments. The equivalently high levels of efficacy achieved by the low (250 dollops per ha) and high application density (500 dollops per ha) in both formulations (Figure 4) suggest that OrB could potentially be managed below threshold levels in blueberry fields using the more cost-effective lower application rate.

As also occurred in the previous mating disruption study, OrB were observed near point sources during all observation periods in treatment plots, whereas none were seen in the control plots for the length of the study. Both living and dead beetles were seen, and most were observed close to point sources near plot borders as opposed to the interior of the treated field. These findings provide strong evidence that SPLAT® OrB A&K maintains the same level of attraction as the mating disruption formulation, in spite of the addition of cypermethrin, a compound which, according to the results of the residual activity study, exerts a fairly reliable degree of mortality in the target insect. A period of 48 h of exposure to SPLAT® OrB A&K resulted in 100% mortality of OrB, regardless of the age of the material. In contrast, the final mortality rates for beetles exposed to SPLAT® OrB (without insecticide) were not significantly different from those of OrB exposed to a blank sample of SPLAT®. Exposure to SPLAT® OrB A&K resulted in significantly higher levels of final mortality than SPLAT® OrB or SPLAT® (blank), in all cases except those assayed on Day 15, when, as previously stated, OrB exposed to the control treatment suffered an abnormally high level of mortality.

While sample age did not appear to affect OrB mortality rate at 48 h of exposure to SPLAT® OrB A&K, it does appear to have impacted the amount of time required for mortality to reach 100%. Plots of OrB mortality over time suggest that as the age of the material increases, so too does the length of time required to achieve mortality in exposed beetles.

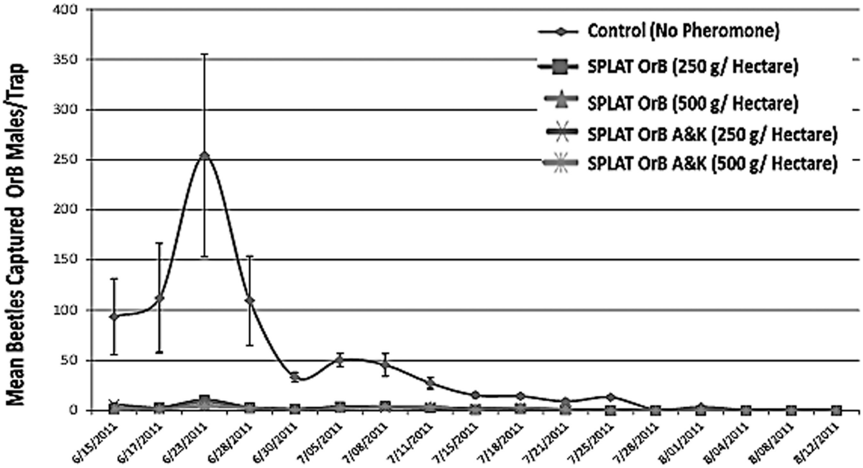

Figure 4. Trap catches of male beetles in pheromone traps in OrB pheromone-treated and control fields. Mean (± SE) number of male oriental beetles captured in pheromone-baited traps on each observation date. Three traps were located in each plot; data represent the average from nine traps per treatment. SPLAT® OrB (1% (Z)-7-tetradecen-2-one) applications were made on June 3 and SPLAT® OrB A&K (1% (Z)-7-tetradecen-2-one + 2% Cypermethrin) applications were made on June 6, 2011. Traps were installed on June 10 and lures were replaced every three weeks.

Conclusions

Treatments of SPLAT® OrB and SPLAT® OrB A&K resulted in equivalent reductions in OrB trap capture in treated blueberry fields. While it remains unclear from these results whether control by SPLAT® OrB A&K occurs through attract and kill or by mating disruption, the data generated by this study are encouraging. SPLAT® OrB A&K retained the necessary degree of attraction to lure OrB towards the point source in the field, and the results of the residual activity study strongly indicate that this formulation's insecticidal component, cypermethrin, functions as an adequate killing agent against this pest species.

ISCA Technologies and its collaborators are continuing research on the optimization of the SPLAT® OrB attract and kill formulation. Dr. Rodriguez-Saona and his research team are currently engaged in a study to evaluate the frequency that OrB approach, locate and contact SPLAT® dollops in the field. Experiments are also being conducted on the use of alternative varieties of insecticide in SPLAT® OrB, to improve the formulation's field efficacy and longevity.

Hook™ RPW: Attract and Kill for the Red Palm Weevil, *Rhynchophorus ferrugineus*

The RPW is an internal tissue borer known to prey on 40 palm species around the world (*35*, *36*). Originally reported as a pest of coconut palm in India in the early twentieth century (*37*), RPW has spread rapidly across the globe since the 1980s, presumably through transport of infested plant material, and is now present on every continent except Antarctica. It is considered a major pest of date palm throughout the Middle East and Mediterranean regions, and has been reported in half the date-producing countries of the world (*38*).

The weevil's activity as an internal tissue feeder makes its presence in a field notoriously difficult to detect. The most obvious symptoms of infestation, such as the presence of chewed plant material (frass), tunnels bored through the tree trunk or frond petiole base, and the fermented odor and gnawing sounds produced by feeding larvae (*39*, *40*), only become evident after the weevils infesting the tree have already completed at least one life cycle. Because infested trees can only be saved if treated early, through the application of stem-injected insecticides, a number of early detection systems have been developed since RPW attained global pest status, including endoscope technology (*41*); auditory equipment to detect and amplify the sound of RPW grubs feeding on internal plant tissues (*42*, *43*); and the use of sniffer dogs trained to identify and respond to the characteristic fermented odor of an infestation (*44*). Late-stage infested trees may contain multiple generations of the pest, resulting in such extensive damage to the plant that it is not uncommon for the trunk of the tree to break off at the point of the infestation, or even topple over. Palms that have reached this severe level of infestation must be removed from the field and destroyed.

Ferrugineol (4-methyl-5-nonanol), an aggregation pheromone produced by male RPW, was first identified and synthesized in the early 1990s (*45*), and has since proven a valuable tool for the management of this destructive insect. Though the pheromone is attractive to both sexes, traps baited with ferrugineol tend to capture significantly more females than males, with the average reported ratio of captured RPW being 2:1, female to male (*46–51*). Furthermore, RPW females captured in these traps tend to be young, gravid, and/or fertile individuals, indicating excellent potential for RPW population suppression through application of this semiochemical (*52*, *53*).

Ferrugineol has traditionally been deployed among susceptible host plants in food-baited pheromone traps (FBPTs), either for monitoring purposes or mass trapping in order to reduce populations. FBPTs typically take the form of bucket traps, partially buried in the ground among palm trees, to facilitate entry by the weevils and to prevent their escape. Traps are commonly baited with sugar-rich foods (dates are commonly used) and a sufficient quantity of water to keep the bait material from drying out, in addition to a ferrugineol lure. The presence of the food bait is thought to synergize the attractive effect of the pheromone. Insecticide solutions are usually included as well, in order to ensure that captured weevils do not return to the field. One study conducted in Costa Rica found that 90% of test weevils (in this case, the South American palm weevil, *Rhynchophorus palmarum*, a close relative of RPW) placed in similar FBPTs escaped in 24 h or less when no toxicant was included (*54*). While these FBPTs have been employed successfully to manage RPW in several countries (*38*), they require frequent servicing (i.e., regular replacement of food baits and water) in order to maintain efficacy. Faleiro (*40*) reported that the efficiency of FBPTs diminished appreciably if food baits were not replaced with fresh material every 1 to 2 weeks. This requirement makes FBPTs a costly and labor-intensive method of RPW management, especially in cases of severe infestations, which demand that traps be placed in the field at higher densities (*55*).

Hook™ RPW, an attract-and-kill formulation that combines the aggregation pheromone ferrugineol with the insecticide cypermethrin, was designed as a lower maintenance, more cost-effective strategy to control RPW populations. With this product, ISCA Technologies sought to develop a more attractive, longer-lasting lure than any formulation currently available – one that would function in the absence of food baits, rendering the maintenance associated with FBPTs unnecessary, and when coupled with a potent, fast-acting insecticide, might enable users to do away with the trap altogether, instead applying the product directly to palm trees.

Dr. H.A.F. El-Shafie and Dr. J.R. Faleiro, of the Date Palm Research Centre of Excellence, King Faisal University, Saudi Arabia, et al. (*55*) carried out field trials in an RPW-infested date plantation, located in Al-Guaibah, Al-Hassa, comparing the efficacy of Hook™ RPW to that of the standard mass-trapping program currently implemented to manage the pest in that region. The date plantation selected for this study was planted with date palms of the "Khalas" cultivar; palms comprising the study sites were approximately 10 years in age, 3 m in height, and stood at a density of 150 trees per ha. The test plantation was being treated in accordance with a standard mass trapping program, at a density of one FBPT per ha, at the time the study was conducted.

Hook™ RPW was applied in 100 3-g dollops over a 0.4-ha treatment plot, containing roughly 60 date palms (250 dollops per ha). Although this formulation was intended for direct application to the surface of palm trees, for this study each Hook™ RPW dollop was deposited at the bottom of its own individual bucket trap, to allow accurate measurement of the number of RPW successfully attracted to and killed by the formulation. Each Hook™ RPW trap consisted of a 5-L bucket with 8 3-cm openings immediately below the rim, evenly spaced around the circumference of the bucket, to allow RPW to enter the trap. Traps were evenly spaced along 4 100-m rows, each with 25 traps positioned 4 m apart. Hook™ RPW-treated rows were separated by a distance of 10 m (equivalent to 5 rows of planted date palm trees). The number of RPW caught and killed in Hook™ RPW-treated plots was compared to RPW captures resulting from a standard FBPT program, represented in this study by 2 FBPTs positioned 60 m from either edge of the Hook™ RPW-treated plot. Each FBPT contained 200 g of dates in 1 L of water spiked with 0.01% Carbofuran 10G, along with a ferrugineol lure (Ferrolure + 700 mg), in a 5-L bucket trap with 4 openings; the food bait was renewed on a weekly basis. RPW trap catches for both the Hook™ RPW- and FBPT-treated plots were recorded every week for 13 weeks. This period of time was intended to correspond with the period of peak weevil activity at this location, as identified by El-Garhy (*56*), from March 29 to June 28, 2011.

To compensate for the discrepancy in trap placement density between treatments (Hook™ RPW vs. FBPTs), data on the total number of RPW caught per week in treatment plots was converted to number of RPW caught per ha per week. The resulting data were subjected to a non-parametric Mann-Whitney test to ascertain the impact of treatment on rate of weevil capture. All statistical tests were performed at $\alpha = 0.05$.

In order to evaluate any potential bait-lure synergy attending the application of Hook™ RPW, an additional field study was conducted using the following treatments:

1. Hook™ RPW, applied as a 3-g dollop in a bucket trap without food bait, in the same manner as in the Hook™ RPW/FBPT comparison study.
2. Traditional FBPT, containing the same components as those deployed in the comparison study.
3. Hook™ RPW with food bait, consisting of a traditional FBPT (same as treatment 2), with the addition of a 3-g dollop of Hook™ RPW hung from the lid of the bucket trap in a plastic container.

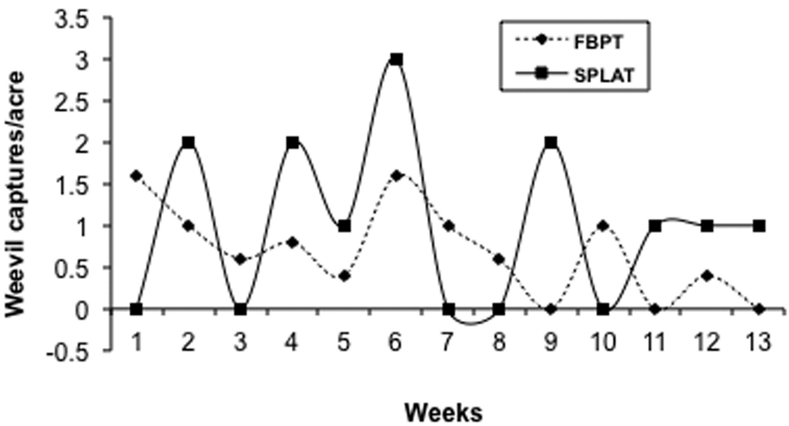

Figure 5. Weekly capture of red palm weevil per 0.4 ha (acre) over 13 weeks of the period of peak weevil activity Al-Guaibah, Al-Hassa, Saudi Arabia in 5-L bucket trap with 4 openings baited with Hook™ RPW, applied as 3-g dollops once at day zero, compared to number of RPW caught and killed in standard FBPT traps with a ferrugineol lure (Ferrolure + 700 mg) and a standard food lure. The food lure of the FBPT traps contained 200 g of dates in 1 L of water spiked with 0.01% Carbofuran 10G, renewed every week throughout the 13 weeks of the experiment.

The experimental site for the bait-lure synergy study was divided into 7 blocks, and a segment of each block assigned to each of the 3 treatments, resulting in 7 replicates per treatment. Treatments within the same block were separated by a distance of 15 m, and blocks were spaced 25 m from one another. Treatments were rotated to different positions within their block each week, to correct for positional bias, and RPW captures were recorded at the same interval for a period of 3 weeks. Data on mean number of RPW captured per trap per week were subjected to a non-parametric Kruskal-Wallis test.

Analysis of trap capture data (Figure 5) revealed no significant difference in trapping efficacy between traps baited with Hook™ RPW (mean = 2.5 RPW per ha per week) and traditional FBPTs (mean = 3.5 RPW per ha per week), suggesting that the Hook™ formulation is capable of delivering the same quality of RPW control as FBPTs, without the need for the companion inputs of food baits, water, or additional insecticide required with the use of currently-available ferrugineol lures. These findings are further supported by the results of the bait-lure synergy study, which indicated that Hook™ RPW sustained the same level of attraction to RPW regardless of the presence or absence of food baits. Data on mean ranks of RPW caught per week per trap, produced by the Kruskal-Wallis test, revealed no significant difference in trapping efficacy among traps baited with Hook™ RPW alone and FBPTs with and without Hook™ RPW. This SPLAT®-based formulation also demonstrated excellent field longevity, maintaining efficacy for the full 3-month test period despite the hot, dry conditions of Saudi Arabia.

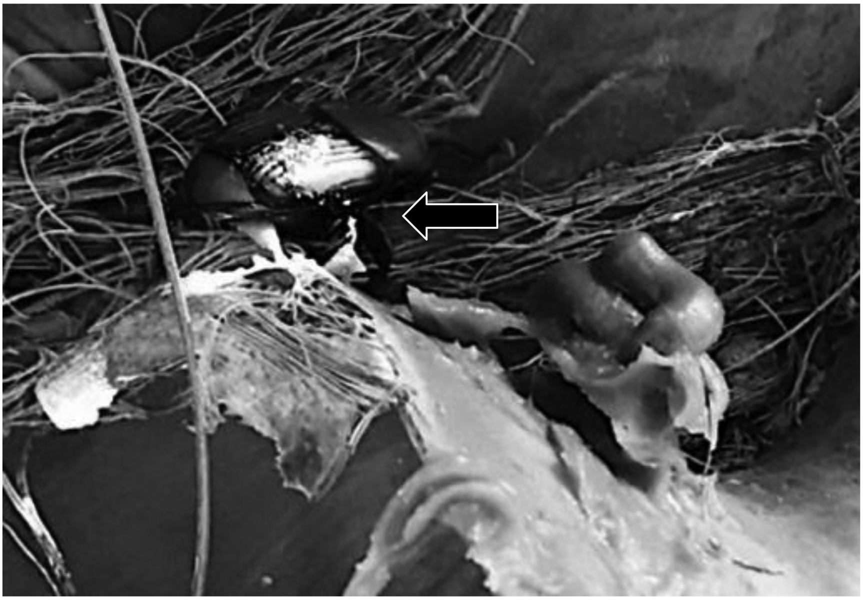

Figure 6. Red palm weevil (RPW) visiting a Hook™ RPW dollop (dollop bottom right). While contacting the Hook™ RPW dollop, beetles receive a transfer of material (arrow points to a white blob attached to the elytra and leg). Field and laboratory observations indicate that the weevils receive lethal doses of insecticide when contacting Hook™ RPW point sources. They are found dead or dying near the dollop, usually on the ground. Direct observations indicate that after a few minutes of contact with Hook™ RPW, the beetles become sedentary, dying within a few hours.

Conclusions

The results of this study verified that Hook™ RPW exerts an equivalent level of RPW attraction to that achieved by currently employed food-baited pheromone traps. Ongoing and future research on this formulation will focus on its application directly in the field, without the use of a trap. In order to make such an application feasible without posing any danger of inadvertently triggering infestations in treated palms, the killing efficacy of the insecticidal component of Hook™ RPW must be confirmed. To this end, ISCA Technologies has engaged the cooperation of a number of research partners for additional field testing and bioassays in Europe, South East Asia and the Middle East. Preliminary testing has produced persuasive evidence of Hook™ RPW's attract-and-kill capacity (Figure 6).

SPLAT® Verb: A Repellent for Mountain Pine Beetle, *Dendroctonus ponderosae*

Repellents

For purposes of this chapter, we define an insect repellent as any compound that manipulates an insect's behavior in such a way as to prevent its locating, feeding, or ovipositing on an otherwise appropriate substrate. Such compounds may exert repellent effects through a variety of mechanisms, and include antifeedants, oviposition deterrents, and other inhibitors. Repellents may be used alone, to repel insects from a host or environment the user desires to protect, or in tandem with an attractant component deployed as part of a "push-pull" strategy intended to intensify the effect of the repellent (*57*). By means of such a coordinated system, an insect may be diverted away from its preferred host or habitat to a location where its presence can do less harm; where it can be trapped and subsequently removed from the field; or where it can be eliminated through the application of insecticides, with less risk of contamination to the protected crop or harmful effects to other organisms.

While a number of repellents have been developed for control of hematophagous insects (*58*), chiefly for pests of medical or veterinary importance (*57*, *59*), few have been developed for agricultural or forest pests despite a growing body of research demonstrating their potential use in these sectors. Repellent formulations using semiochemicals face the same obstacles to commercialization as other semiochemical pest management techniques. They must compete with relatively effective, comparatively inexpensive insecticides that are usually available to manage the target pest (*58*), and unlike such insecticides, they work effectively only against a limited number of insects for a few select host plants. Semiochemical repellents also require the user to have a thorough understanding of the target pest's behavior and its place within its ecosystem compared to conventional pesticide use, or even other forms of semiochemical pest control, like mating disruption or attract and kill (*4*, *57*). The niche market that repellent products occupy, coupled with the large investment of time and resources required to secure product registration, often make the research and development of these products a costly venture, especially for small businesses.

However, repellents can be of great value in the management of forest pests, where the massive, largely inaccessible areas that characterize the susceptible "crop" make applications of conventional insecticides impractical or socially unacceptable at any appreciable scale. ISCA Technologies is currently expanding its involvement in the forestry sector. This commenced with the launch of SPLAT® GM for gypsy moth mating disruption – since adopted by the USDA Forest Service's Slow the Spread Program – and with the introduction of SPLAT® Verb. This product is designed to protect susceptible pines from attack by MPB, an aggressive species of bark beetle responsible for devastating losses in forests throughout much of western North America. SPLAT® Verb has shown a consistently high degree of efficacy during all field tests in areas with moderate to heavy MPB population pressure. This research, conducted in collaboration with teams from the USDA Forest Service led by Dr. Christopher J. Fettig of the Pacific Southwest Research Station and Mr. A. Steven Munson of Forest Health Protection, is summarized below.

SPLAT® Verb for Mountain Pine Beetle

Unlike most bark beetles, MPB is a primary tree killer of several tree species, including lodgepole pine, ponderosa pine, *P. ponderosa*, whitebark pine, *P. albicaulis*, and sugar pine, *P. lambertiana*. MPB utilize the phloem tissues of their host trees as a food source, a place of shelter, and a nursery in which to establish and rear their brood. In order to gain access to these tissues, the attacking beetles must first overcome the tree's formidable physical and chemical defenses. Pines and other conifers have evolved to respond to an attack by boring insects by flushing large amounts of oleoresin, in some cases containing insecticidal and antimicrobial components, to the site of the attack to enmire or drown the attacking insects, as well as to seal any wounds caused by their activity (*60*, *61*).

The MPB's method of overcoming this defense mechanism is through mass attack behavior. Like other aggressive (host-killing) bark beetles, this species has evolved a highly sophisticated and complex system of chemical communication that synchronizes mass attack on a susceptible host tree, consisting of a number of aggregation pheromones (*62*, *63*), such as *cis*- and *trans*-verbenol, and *exo*-brevicomin (*64–66*), and host-produced kairomones, such as α-pinene and myrcene (*67*). This system enables recruitment of an overwhelming number of beetles (hundreds to thousands, depending on the species, size, and vigor of the host) to attack the tree within a limited time frame (*61*, *68–73*). If MPB recruitment reaches this threshold, the tree's defensive responses are effectively disabled. Beetle-killed trees are easily identifiable within 1 year of a successful mass attack by the appearance of fading crowns.

Outbreaks of MPB have now impacted >8.9 millon ha in the western United States, appearing to have peaked in 2009 (*74*). These mortality events are part of the ecology of western forests and influence many processes, but the economic and social implications are significant. An outbreak in British Columbia, Canada has affected >18 million ha, resulting in the most extensive MPB outbreak in recorded history (*75*). Since it began in 2000, an estimated 723 million m^3 of timber, corresponding to roughly half this region's marketable pine, has been killed (*75*). This drastic increase in MPB's impact on western forests has largely been attributed to recent warming trends (*76*) and an abundance of susceptible hosts (*77*). Warmer temperatures favor MPB outbreaks by facilitating the survival of overwintering larvae and by increasing the frequency and severity of drought stress in host trees, limiting their production of resin and increasing their susceptibility to attack (*60*, *78–81*). Forest management practices have also been suggested as contributing factors, as years of fire suppression efforts and reductions in levels of harvesting have resulted in overly-dense forests of mature trees – optimal conditions for an MPB outbreak (*82–84*).

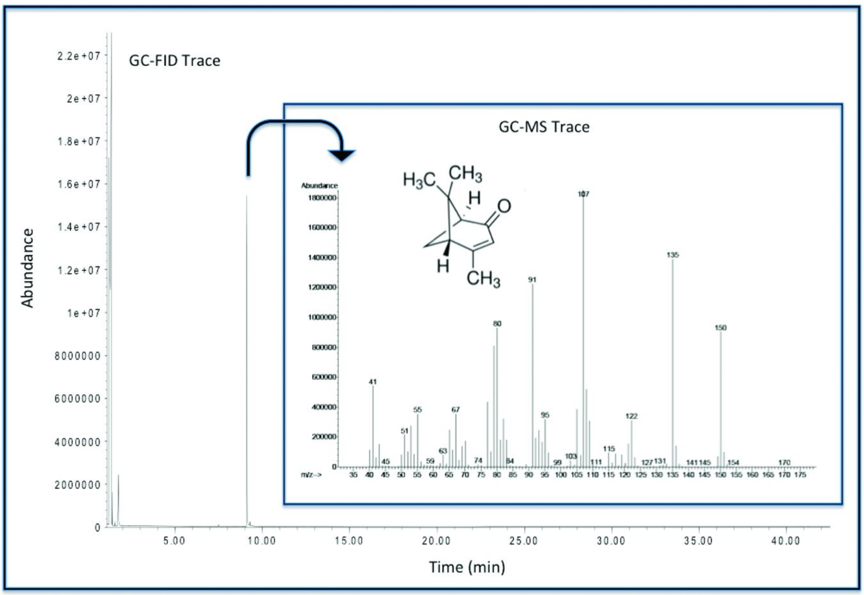

Figure 7. A representative analysis using gas chromatograph (GC) and the inset showing the mass-spectrometrometer (GC-MS) run of the solvent extraction of a single SPLAT® Verb dollop, aged for a month under direct sun exposure in Riverside, California. The trace shows a single large peak, characterized as verbenone (inset).

Previous methods of control of this widespread pest, such as forest thinning to reduce stand density, sanitation treatments to remove infested hosts (*82*, *84–87*), and preventative insecticide treatments – the carbamate carbaryl is among the most commonly used (*88*) – are often limited to small spatial scales. Insecticide treatments in particular are costly and labor-intensive to implement. Repellent applications of the principal anti-aggregation pheromone of MPB, verbenone (4,5,5-trimethylbicyclo [3.1.1] hept-3-en-2-one), have long been viewed as a promising alternative to protect individual trees, stands or small landscapes of susceptible hosts. Produced during the latter stages of mass attack, this pheromone indicates to approaching MPB that the tree currently undergoing colonization is already overcrowded, and is therefore an undesirable host. The anti-aggregative behavior induced by verbenone is thought to contribute to survival and health of MPB brood by preventing overcrowding within the host tree (*89*). Verbenone may also be an indicator of host tissue quality and its quantity a function of microbial degradation. Verbenone is a natural, safe compound, approved by the US EPA as a management tool for MPB (and other bark beetles species), and by the US Food and Drug Administration (FDA) as a food additive (*90*).

Though verbenone has shown potential as an MPB repellent in several studies (*91–96*), it has yet to be widely adopted by stakeholders in the forestry sector, largely due to the inconsistent efficacy of available formulations, particularly if deployed in areas under heavy MPB population pressure. Most have taken the form of devices, which as previously discussed, entails certain limitations of range and flexibility of application, and many have demonstrated inconsistent capacity to sustain adequate release at rates necessary to impart desired behaviors. The performance of these repellents in the field may have been further compromised by the propensity of verbenone to photoisomerize to chrysanthenone, a chemical that exerts no effect on MPB behavior (*97*). SPLAT® Verb was designed to correct for these deficiencies (Figure 7), to release biologically-active quantities of verbenone long enough to cover the entire MPB flight period in most locations (8-12 weeks), through its UV-protected, rainfast flowable matrix.

Field studies of SPLAT® impregnated with verbenone began in the summer of 2011 and are ongoing. A pilot study to assess the capacity of a prototype of SPLAT® Verb to protect individual lodgepole pines from MPB attack commenced in mid-July 2011 on the Grey's River Ranger District, Bridger-Teton National Forest, Wyoming. Twenty-one lodgepole pines, selected at random, were treated with SPLAT® Verb applied in 4 large dollops spaced evenly around the circumference of the tree trunk at a height of ca. 4 m. The SPLAT® material was deposited onto the bark through the spray nozzle of a Graco 15:1 automotive-style grease pump, fitted with a 5-m hose. Fifteen of the SPLAT® Verb-treated trees received approximately 533 g of the formulation (ca. 32 g verbenone), while the remaining 6 trees received approximately 650 g of SPLAT® (ca. 39 g verbenone). Thirty additional lodgepole pines were also randomly selected to serve as untreated baited controls. One MPB tree bait (Contech Inc., Delta, BC, Canada) was attached to each treated and untreated tree, at a height of ca. 2.4 m from the ground on the north side of the tree, to ensure adequate MPB pressure. Tree baits were attached immediately following SPLAT® application, and were removed ca. 1 month later. Treatment efficacy was assessed by recording tree mortality rates due to MPB attack between treated and untreated trees, as evidenced by crown fade, 1 year after treatment. About 93% of untreated baited controls (28 out of 30) died from MPB attack, while no mortality was observed in SPLAT® Verb-treated trees.

Figure 8. Manual application of SPLAT® Verb for individual tree protection using a caulking gun. Arrow points to a SPLAT® Verb dollop containing ca. 1.75 g of verbenone; an individual tree receives four dollops.

Encouraged by these results, a second field study was conducted in the same area the following year, with an enhanced prototype of SPLAT® Verb and an application procedure adjusted to correct for deficiencies identified during the 2011 study. The optimized SPLAT® Verb formulation (10% AI) was applied to 30 randomly-selected lodgepole pines at a reduced application rate of 7 g of verbenone per tree (i.e., a load comparable with commercial pouch formulations) (Figure 8). The material was deposited in 4 evenly-spaced, evenly-sized dollops per tree using a caulking gun (Newborn XLite) at a height of ca. 2.5 m. As in the 2011 study, all treated trees were baited with an MPB tree bait (Contech Inc.), which was left in place for the first 113 d of the study. Thirty lodgepole pines were also randomly selected and baited to serve as untreated controls. All experiment trees were inspected for signs of MPB infestation before treatment; any trees displaying such signs were excluded from the study. Treatments and tree baits were applied prior to the beginning of MPB flight in the area. No SPLAT® Verb-treated trees showed signs of MPB attack, while 28 of 30 untreated baited controls (93.3%) died. An interesting pattern was observed concerning the frequency of MPB attacks in the vicinity of treated and control trees. Two trees growing within an 11-m radius of SPLAT® Verb-treated trees experienced MPB mass attack, compared to an additional 61 mass-attacked trees within the same distance of untreated trees. These data indicate that not only does SPLAT® Verb effectively prevent mass attack on the tree to which it is applied, it also casts a protective "halo effect" of repellency over trees growing within at least 11 m of treated trees.

The efficacy of the enhanced prototype of SPLAT® Verb has also been examined on 0.4-ha plots in the Montpelier Ranger District, Caribou-Targhee National Forest, Idaho. SPLAT® Verb, applied at a cumulative rate of 875 g verbenone per ha at a height of ca. 2 m on tree boles in appropriate dollop sizes to attain adequate coverage over the experimental plot, was examined against untreated control plots and plots treated with a verbenone pouch formulation (Contech Inc., 7 g AI). Verbenone pouches were stapled to the tree bole at the same height as SPLAT® Verb dollops along a 9.1 by 9.1-m grid (125 U per ha). A single MPB tree bait (Contech Inc.) was affixed to 1 tree in the center of each plot. Each treatment was replicated 5 times. All treatments were applied before peak MPB flight. Tree mortality, based on crown fade, was determined in July 2013. Analyses are ongoing.

Conclusions

SPLAT® Verb has demonstrated excellent performance as a repellent for MPB in all field studies. As a result, ISCA Technologies began commercialization of SPLAT® Verb in 2012, and has since obtained unconditional registration with the US EPA (Reg. No. 80286-20), as well as state registrations in Colorado, Idaho, Montana, Nevada, Oregon, Washington and Wyoming. Registration is pending in California.

ISCA Technologies and its collaborators are continuing research and development of SPLAT® Verb. Dr. Fettig and his research team are currently evaluating SPLAT® Verb as an MPB repellent for protection of individual ponderosa pines, a host species in which verbenone has generally been regarded as ineffective (*5*, *96*, *98*). Preliminary results indicate similar levels of efficacy can be expected in ponderosa pine as have been observed for lodgepole pine. For example, in one study 89.7% of trees (26 out of 29) were mass attacked by MPB in the untreated baited control compared to 14.3% (4 out of 28 trees) in the SPLAT® Verb-treated plots. In 2014, experiments are planned to determine optimal (minimum) release rates and distribution patterns for applying SPLAT® Verb to individual trees and small-scale stands. In addition, based on research by Fettig et al. (*99–101*), ISCA Technologies is developing an alternative verbenone formulation, SPLAT® Verb Plus, which combines a 3-component blend of non-host angiosperm volatiles (NAVs) with verbenone to increase its repellent effect. Our primary goal for SPLAT® Verb Plus is to create an effective deterrent to mass attack by a species of bark beetle closely related to MPB, the western pine beetle, *Dendroctonus brevicomis*, which has been identified as a primary tree killer of ponderosa pine and has proven difficult to manage with verbenone alone (*102–106*). However, the addition of NAVs to SPLAT® Verb may also improve its potency against MPB (*100*), in addition to other bark beetle species that respond to verbenone.

Conclusions and Future Directions

Effective pest management often requires a multifaceted, integrated approach that (a) combines management strategies, such as monitoring and trapping programs, mating disruption and other behavioral-manipulation tactics, as well as the conscientious and selective use of insecticides; (b) considers the behavior and ecology of the target pest, nontarget organisms and the host(s); and (c) addresses the welfare of workers, user groups and consumers, as well as the natural environment. The versatility and adaptability of SPLAT® technology can complement such a strategy, in terms of "filling in the gaps" of existing pest management programs. To that end, SPLAT® formulations have been successfully applied using a wide range of tactics, including sex pheromones (e.g., SPLAT® OrB), reduced-risk insecticides (e.g., SPLAT® OrB A&K, Hook™ RPW), and repellents (e.g., SPLAT® Verb), against an equally varied array of insect species, including members of the orders Lepidoptera, Diptera, Hemiptera, and as outlined in this chapter, Coleoptera. Development of new SPLAT® formulations for agricultural, forest and medical/veterinary pests is one of ISCA's most active areas of research. Our organization will continue to engage the cooperation of expert researchers and stakeholders, both within the United States and abroad, to explore and evaluate new opportunities for the implementation of SPLAT® technology in semiochemical-based pest control applications.

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