Chapter 4

# Manipulation of Insect Behavior with Specialized Pheromone and Lure Application Technology (SPLAT®)

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SPLAT® (Specialized Pheromone and Lure Application Technology) emulsion is a unique controlled-release technology that can be adapted to dispense and protect a wide variety of compounds from degradation, including semiochemicals, pesticides, and phagostimulants, in diverse environments. ISCA Technologies, Inc., in collaboration with colleagues in academia, government, and industry, has been developing SPLAT®-based insect control products for close to a decade. This chapter provides an overview of SPLAT® technology and existing commercial formulations and describes ongoing efforts


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to develop new SPLAT® mating disruption, attract-and-kill, and repellent products for pest control in agricultural and forest environments.

## Introduction

ISCA Technologies, Inc. (Riverside, CA U.S.A.) acquired SPLAT® (Specialized Pheromone and Lure Application Technology) in 2004. SPLAT® is a chemical controlled-release emulsion technology that has been used to dispense compounds to control a variety of insect pests. SPLAT® formulations have been commercialized both domestically and internationally. This chapter begins with a technical description of how SPLAT® functions and a discussion of what sets it apart from other controlled-release formulations used in semiochemical-based insect control. This is followed by three sections, each focusing on one of three semiochemical-based insect control techniques: Mating disruption, attract-and-kill, and repellents. Each section provides an introduction to the technique and summary of existing commercial SPLAT® products for insect control using that technique, and follows with one or more case studies of new SPLAT® formulations being developed to control agricultural or forestry pests using the technique being discussed.

## Specialized Pheromone and Lure Application Technology (SPLAT®)

### Description and Attributes

Although most semiochemical controlled-release formulations have taken the form of devices, such as aerosol dispensers (Puffer®, Suterra, LLC), polyethylene tubes (Isomate®, Shin-Etsu Chemical Co., Ltd.), and laminated polymers (Disrupt®, Hercon Environmental), ISCA has taken an alternative approach and commercialized a chemical formulation in the form of a controlled-release emulsion, SPLAT® (Specialized Pheromone and Lure Application Technology). Although adapting SPLAT® to release new compounds can pose technical challenges, the versatility of this flowable formulation provides many advantages. SPLAT® emulsions can be created to hold a range of semiochemical concentrations and additives to create a formulation that releases the optimal rate of semiochemical for the desired amount of time, while protecting active ingredients from environmental, chemical, and biological degradation. In addition, the rheological properties of SPLAT® can be adjusted to create emulsions with a wide range of physical properties. This has enabled SPLAT® products to be dispensed using a variety of manual and mechanical application techniques (Figure 1), allowing easy application to virtually any substrate or plot size. In addition, SPLAT®, unlike most other semiochemical dispensers, is not restricted to a particular point source size. Any amount of SPLAT®, large or small, can constitute a point source. This provides yet another way to optimize the amount of volatile insect control compound released per point source for optimal efficacy. The versatility of SPLAT® has made the technology adaptable to virtually any semiochemical-based insect control application. Additional advantages of SPLAT® include the biodegradability of its inert ingredients and low manufacturing cost, which decrease environmental impacts and enable commercialization of affordable semiochemical-based control products.



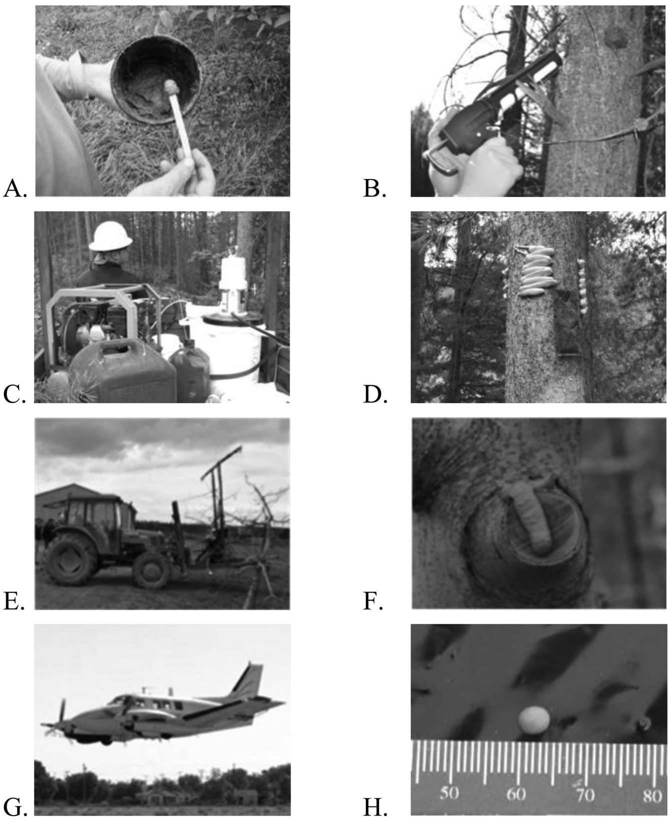


*Figure 1. Application of SPLAT® using a variety of methods. A. SPLAT® applied with stick, B. SPLAT® Verb Repel applied with caulking gun, C. SPLAT® Verb Repel applied with John Deere Gator®-mounted mechanical applicator, D. SPLAT® Verb Repel dollop from application depicted in C, E. SPLAT® applied with tractor-mounted mechanical applicator, F. SPLAT® dollop resulting from application depicted in E, G. SPLAT® GM applied with airplane-mounted mechanical applicator, H. SPLAT® GM dollop resulting from application depicted in G.*

## Application

SPLAT® formulations typically have a paste or cream-like consistency (Figure 1). SPLAT® is a non-Newtonian, shear-thinning, thixotropic fluid, which means that SPLAT® viscosity decreases when the emulsion is placed under stress, such as when it is stirred or pumped, but increases again when the stress is removed. This property is advantageous in that the less viscous SPLAT® can easily be manipulated (e.g., stirred or pumped), but quickly thickens upon application to a surface, aiding in product adhesion. A wide variety of manual and mechanical applicators can be used to apply SPLAT®. The most basic SPLAT® applicator can be a stick, spatula, or knife. More advanced manual applicators include syringes, grease guns and caulking guns (Figure 1 A, B). Indeed, SPLAT® formulations are regularly sold loaded into standard caulking tubes and applied with off the shelf caulking guns. In addition, numerous mechanical applicators have been adapted or created specifically to apply SPLAT® with a variety of motorized vehicles, including tractors, all-terrain vehicles, and even motorcycles (Figure 1 C-F) (*1–3*). SPLAT® has also been sprayed from motorized backpack sprayers and applied aerially (Figure 1 G, H).

## Controlled-Release Technology

The aqueous component of the SPLAT® emulsion gives the product its liquid character, allowing it to flow. The non-aqueous component of the emulsion is the controlled-release device. It comprises the active ingredients (e.g., semiochemical compounds or pesticides) and the additives that will protect these and fine-tune their release rates from the dispenser. Upon application, the aqueous component of SPLAT® evaporates from the dispenser within 3 hours, leaving the rainfast, non-aqueous component firmly affixed to the substrate, where it will release the active ingredients until all available molecules are dispensed (Figure 2). The longevity of the dispenser depends on the manner in which the particular SPLAT® formulation was created, its composition, how it was applied, as well as the environmental conditions to which it is exposed following application (*4*). SPLAT® products are typically formulated to release semiochemicals for 2 weeks to 6 months.

SPLAT® is a "matrix-type" or "monolithic" diffusion-controlled release device. Diffusion-controlled release devices are ones where the diffusion of the active ingredient through the device controls its release rate. Monolithic dispensers are diffusion-controlled release devices where the active ingredient is dispersed or dissolved in a matrix. If the active ingredient is dispersed in the matrix, it must dissociate from the other molecules in its crystal cell and solubilize into the matrix before release can occur. If it is dissolved in the matrix, this first step is bypassed (*4*). In the majority of cases, we expect hydrophobic arthropod pheromones to be dissolved in the SPLAT® matrix when the product is applied. The movement of the active ingredient dissolved within the matrix occurs by diffusion and follows Fick's First Law (*5*), which states that molecules move down their concentration gradients at a rate that is directly proportional to their concentration gradient. Movement of the molecule within the matrix occurs in one of two ways. If the molecule is very small compared to the size of the amorphous spaces in the matrix, it diffuses through the matrix by moving from one such space to another. If it is very large compared to the size of those spaces, then segments of the molecules comprising the matrix will have to be rearranged for diffusion of the active ingredient to occur. Crystalline regions in the matrix are virtually impermeable to molecules of the active ingredient. Upon reaching the surface of the matrix, the active ingredient is released into the environment. Whether the release rate of the active ingredient to the environment is zero or first order depends on the partition coefficient of the active ingredient between the matrix and the environment. If the active ingredient readily partitions to the environment, then its rate of release will be diffusion-controlled and first order. If, however, partitioning of the active ingredient to the environment is relatively slow, then its partition coefficient will determine its release rate from the matrix and the device will exhibit zero order release kinetics. The partitioning of the active ingredient to the environment is a function of the solubility of the active ingredient in the matrix; compounds more soluble in the matrix partition to the environment more slowly (*4*). SPLAT® emulsions in a field environment typically exhibit first-order release rates (*6*).

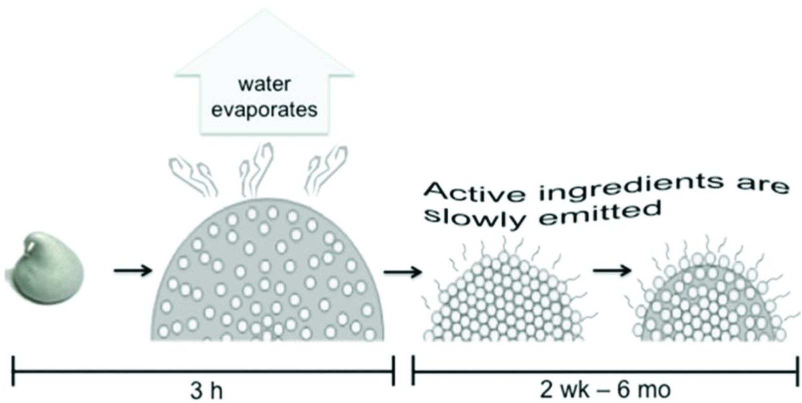


*Figure 2. Following application, the SPLAT® emulsion dries and becomes rainfast within 3 hours, then releases active ingredients at a controlled rate for 2 weeks to 6 months.*

## Formulations

SPLAT® formulations have been developed to release a variety of compounds, including sex pheromones, kairomones, attractants, repellents, phagostimulants, and insecticides. Several SPLAT® mating disruption and attract-and-kill formulations are commercially available. SPLAT® repellent formulations will also soon become available commercially. Development of novel SPLAT® products is an active area of research. Efficacy trials conducted in the development of several of the newest SPLAT® mating disruption, attract-and-kill, and repellent formulations are described in the sections below.


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# SPLAT® Mating Disruption Formulations

## Introduction

Mating disruption consists of dispensing a synthetic form of one or more components of the natural pheromone blend of an insect, or biologically-equivalent compounds, where host plant(s) are present. The presence of the species' sex pheromones in the environment delays or prevents mating of the insect (*7*), reducing fecundity and subsequent populations. Although using semiochemicals to disrupt communication among insects, rather than using these compounds as they are naturally used in insect communication (e.g., to attract or repel insects), was a relatively revolutionary idea when the first insect sex pheromones were identified 50 years ago, the use of semiochemicals for mating disruption was suggested and tested early on by several scientists (*8–13*). It has proven to be a powerful technique for insect control and has become the most commonly-used semiochemical-based insect control method (*14*, *15*).

The mechanisms by which mating disruption works have been discussed in several publications (*14–21*). Currently accepted mechanisms of mating disruption comprise: 1) competitive attraction (also known as "false trail following"), 2) camouflage, 3) desensitization, which includes both adaptation and habituation, and 4) sensory imbalance. Recent laboratory investigations have demonstrated that responses of females of some moth species to their own sex pheromone alters their behavior in ways that may also enhance mating disruption (*22–24*). Combinations of these mechanisms often function together in a mating disruption system and the mechanisms involved in mating disruption differ depending on both the species being controlled and the pheromone formulation used. Mating disruption research historically focused heavily on determining the efficacy of the technique for various insect species in field trials, with few studies to determine the mechanism by which mating disruption worked for the species and formulation being investigated (*14*, *16–18*). However, there has been an increasing effort by researchers in recent years not only to continue to prove the efficacy of the technique for new formulations and additional insect pest species, but also to provide evidence for the mechanisms by which mating disruption occurs for the species and formulations being investigated (e.g., (*15*, *19*, *20*, *25–27*)).

One must also be aware of the constraints of the technique to use it most effectively. Gut et al. (*14*) have provided a thorough analysis of these constraints. The success of mating disruption for a particular pest is impacted by biological and ecological factors (e.g., pest's host specificity, dispersal capacity, and population density), male response to pheromone (e.g., whether males are susceptible to adaptation or habituation), chemical characteristics of the pheromone (e.g., evaporation rate and propensity to adhere to surfaces), and the physical environment (e.g., effects of environmental conditions, such as heat and wind, plot size and shape, and site topography) (*14*). Taking these factors into account, researchers can choose the mating disruption formulations and application techniques best adapted to the target insect and treatment location.


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It is also important to keep in mind that for some pests, constraints may be too great for an economically-viable and successful mating disruption program to be designed and in these cases, alternative control techniques will need to be implemented (*14*, *17*).

The earliest SPLAT® formulations were created for mating disruption, principally of lepidopteran pests (*6*, *28–30*). Current SPLAT® mating disruption formulations include products for control of both lepidopteran and coleopteran pests (*2*, *6*, *27*, *31*, *32*). SPLAT® formulations are developed in close partnership with experts in academia, government, and industry, and often can become the first semiochemical-based control products for a pest species (e.g., SPLAT® EC, SPLAT® Tuta). SPLAT® mating disruption formulations have been commercialized for pests amenable to control using this technique and efforts have been made to determine the mechanisms by which mating disruption using some SPLAT® formulations functions (*26*, *27*). ISCA currently sells 10 SPLAT® mating disruption formulations worldwide (Table I) (*31*). Additional formulations are currently under development. The latest formulation, soon to be commercialized, SPLAT® EC for control of the carob moth, *Ectomyelois ceratoniae*, is discussed below.



### Carob Moth, *Ectomyelois ceratoniae*

*Ectomyelois ceratoniae* (Lepidoptera: Pyralidae), the carob moth, is a widely distributed polyphagous pest that infests numerous fruit and nut crops, including dates, pomegranates, citrus, walnuts, figs, and pistachio, as well as stored nuts and seeds (*33–37*). The carob moth was first detected in the United States in 1982 in the Coachella Valley of California, the principal date-growing region of the country, and is now a major pest in this crop, with infestation levels ranging from 10% to 40% (*38*). There is also concern that the carob moth may eventually infest the Central Valley of California, a major growing region for several crops known to be susceptible to this pest (*39*). Control of the carob moth currently relies on frequent prophylactic sprays of malathion dust. There is an urgent need for safer alternative control methods.

SPLAT® EC was formulated for controlled release of (*Z,E*)-7,9,11-dodecatrienyl formate, a parapheromone (pheromone mimic) of the major component of the carob moth pheromone, (*Z,E*)-9,11,13-tetradecatrienal. Baker *et al.* (*40*, *41*) identified the sex pheromone of the carob moth to be an 8:1:1 blend of (*Z,E*)-9,11,13-tetradecatrienal, (*Z,E*)-9,11-tetradecadienal, and (*Z*)-9-tetradecenal, with the major component, (*Z,E*)-9,11,13-tetradecatrienal, primarily responsible for the attractiveness of the pheromone blend. They noted that synthetic blends of the carob moth pheromone were inferior to gland extracts in eliciting male responses, especially in field trapping studies, and postulated that this result was due to the decomposition of the highly labile triene major component of the pheromone. Todd *et al.* (*42*) synthesized (*Z,E*)-7,9,11-dodecatrienyl formate, a more stable analogue of (*Z,E*)-9,11,13-tetradecatrienal, and demonstrated that it effectively mimicked the major component of the carob moth pheromone both at the cellular and behavioral levels, and that it was equally or more effective than the synthetic blend of the natural pheromone components in field trapping studies.

**Table I. Commercial SPLAT® mating disruption products**

| *Product* | *Pest* | *Availability* |
|---|---|---|
| SPLAT® OFM[a,b] | *Grapholita molesta* | U.S. & international |
| SPLAT® Cydia[a] | *Cydia pomonella* | U.S. & international |
| SPLAT® LBAM[a] | *Epiphyas postvittana* | U.S. & international |
| SPLAT® GM[a] | *Lymantria dispar* | U.S. & international |
| SPLAT® Tuta[a] | *Tuta absoluta* | U.S. & international |
| SPLAT® CLM[a] | *Phyllocnistis citrella* | U.S. & international |
| SPLAT® EC[a] | Ectomyelois ceratoniae | U.S.[c] & international |
| SPLAT® OFM/PFM[a] | *G. molesta/ Carposina sasakii* | International |
| SPLAT® PBW[a] | *Pectinophora gossypiella* | International |
| SPLAT® GRAFO/ BONA[b] | *G. molesta/ Bonagota salubricola* | Brazil |

[a] Commercialized by ISCA Technologies, Inc., U.S.A. [b] Commercialized by ISCA Tecnologias Ltda., Brazil. [c] EPA registration pending.

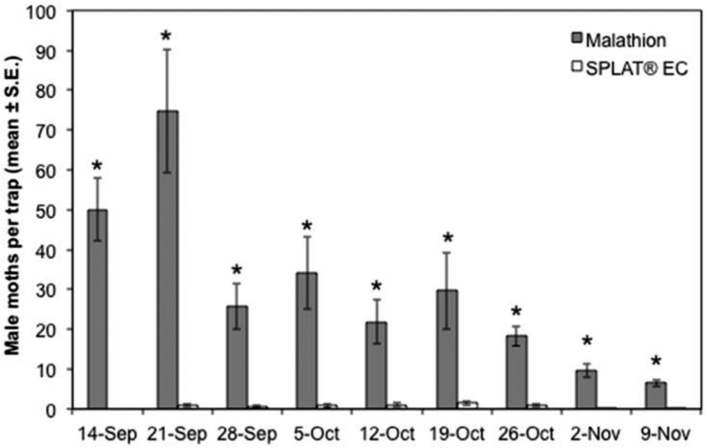


Field trials of SPLAT® EC were conducted in two date gardens in the Coachella Valley of California. At both locations, the experiment was set up in a randomized complete block design with 1.6-ha plots, each containing 196 palms of the variety 'Deglet Noor'. There were three replicates at the first location and two at the second. Three treatments were tested at the first location: SPLAT® EC, 5% malathion dust (Gowan, Yuma, AZ), and a non-treated control. Two treatments were evaluated at the second location: SPLAT® EC and 5% malathion dust. SPLAT® EC was applied as two 2.5-g dollops per tree, one placed at the top of the tree, where date bunches are located, and the second placed on the trunk, *ca.* 1.5 m up from the ground, for a total of *ca.* 610 g SPLAT® EC per ha. A single application of SPLAT® EC was made at the beginning of the trial. Malathion dust was applied *ca.* every 2 weeks throughout the trial for a total of 4 applications.

The SPLAT® EC and malathion-treated plots were evaluated with male captures in carob moth parapheromone-baited traps. One parapheromone-baited trap was placed in the center of each plot and male moths in each trap were counted weekly. Moth capture data were analyzed by repeated measures ANOVA on square root-transformed data (PROC GLM, SAS Institute 2003). All plots were also evaluated by performing fruit damage evaluations at harvest. For this assessment, carob moth infestation was determined by collecting all of the fruit from the largest date bunch present on the 16 palms located in the center of each plot. The selected bunch was removed from the palm, the fruit were mixed, and *ca.* 200 dates were collected randomly. Of these, 100 were selected randomly and evaluated for carob moth infestation by examining the fruit for moth webbing at the calyx end of the date. Fruit infestation data were analyzed with a one-way ANOVA (PROC GLM, SAS Institute 2003). Mean fruit infestation for the three treatments at location 1 were separated with Tukey's test (PROC GLM, SAS Institute 2003).



A.




B.

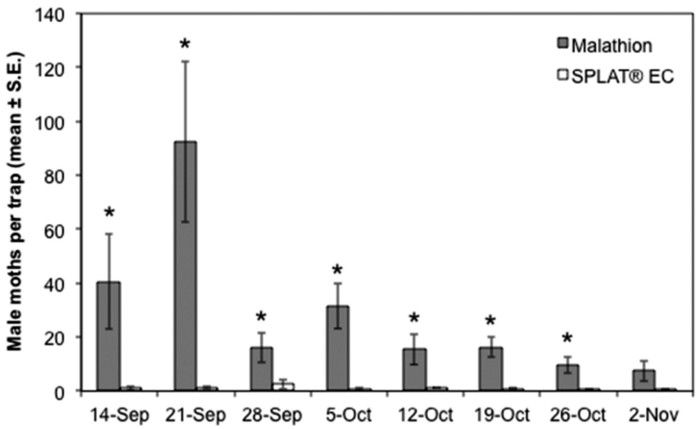



*Figure 3. Field efficacy of SPLAT® EC for carob moth control in dates. Male moth captures in parapheromone-baited traps at A. location 1 and B. location 2. Malathion treatments labeled with an asterisk were significantly different from SPLAT® EC on that sampling day (ANOVA, $P \geq 0.05$).*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Male moth captures in the SPLAT® EC-treated plots were lower than in the malathion-treated plots at both locations. At location 1, the differences in moth captures in SPLAT® EC- and malathion-treated plots were statistically significant throughout the trial (Figure 3). At location 2, moth captures in the SPLAT® EC- and malathion-treated plots were significantly different on all sampling dates, except November 2, when the difference was nearly significant ($P = 0.057$). The extremely low numbers of male moths captured in parapheromone-baited traps in the SPLAT® EC-treated plots demonstrated that mating disruption was nearly complete from the one-time SPLAT® EC application through harvest.

Whereas moth capture data were collected only in SPLAT® EC- and malathion-treated plots, fruit infestation data were collected in all plots, including the non-treated control plots at location 1. Carob moth infestation at location 1 was equivalent in SPLAT® EC and malathion-treated plots and significantly lower in these treatments compared to the non-treated control (Table II). Results at location 2 were similar, with no significant difference in fruit infestation in the SPLAT® EC- and malathion-treated plots. The results were economically-important because the cost of the single SPLAT® EC application (material and application) was equivalent to the total cost for all malathion dust treatments. In addition, the SPLAT® EC treatment provided the same level of carob moth control as malathion dust without negative human health and environmental impacts. Furthermore, unlike malathion dust, which is applied directly to the dates and dries out the fruit, SPLAT® EC is not applied to the fruit, yielding dates with hire water content for a superior quality product. ISCA synthesizes (*Z,E*)-7,9,11-dodecatrienyl formate in-house and is currently the only manufacturer of carob moth parapheromone lures and mating disruption products worldwide.

**Table II. Field efficacy of SPLAT® EC for carob moth control in dates. Values are mean ± S.E. of fruit infestation at harvest. Means ± S.E. followed by the same letter within rows are not significantly different (location 1: Tukey, $P \geq 0.05$; location 2: ANOVA, $P \geq 0.05$).**

| | *Treatment* | | |
|---|---|---|---|
| *Location* | *Pheromone* | *Malathion* | *Non-treated* |
| 1 | 8.1 ± 0.6 a | 10.3 ± 1.1 a | 14.8 ± 1.3 b |
| 2 | 4.1 ± 0.6 a | 4.0 ± 0.7 a | n/a |

## Conclusions

SPLAT® formulations were initially developed for insect mating disruption and several have had commercial success (Table I). Developing new SPLAT® mating disruption formulations remains a focal point of ISCA's product development efforts.

## SPLAT® Attract-And-Kill Formulations

### Introduction

The attract-and-kill strategy is also referred to as "lure-and-kill" and "attracticide", as well as by other terms (e.g., male annihilation, lure-and-sterilize, lure-and-infect, bait spray), depending on the type of attract-and-kill strategy being used (*43*). Broadly, attract-and-kill consists of attracting males, females, or both sexes of a pest species to an insect control agent (e.g., insecticide, sterilant, or insect pathogen). Upon contact, the insect is either killed (immediately or after a delay) or sublethal effects of the control agent diminish the pest population by reducing the insect's fertility or ability to mate (*43*). The insect attractant can be a chemical attractant, a visual cue, an acoustic cue, or a combination of these. Crude baits (e.g., food lures) are also used in attract-and-kill devices and entire plants (e.g., trap crops) have been used as attractants for this technique as well (*15*, *43*, *44*). Since SPLAT® is a chemical controlled-release technology, we will focus on a discussion of attract-and-kill devices that use chemical attractants.

Attract-and-kill technology, although it has shown promise, has historically not been investigated or developed as intensively as mating disruption. Although the technique has proven effective against some species of Coleoptera, Lepidoptera, and Diptera, research efforts have been disproportionately aimed at developing the technique to manage tephritid fruit flies, which are difficult or impossible to control using other methods (*14*, *15*, *43*, *44*). However, there has been interest in recent years in developing attract-and-kill products and strategies for a wider variety of pests, including both established and emerging pest species (e.g., (*45–49*)).

Unlike mating disruption, which can control insect populations *via* a variety of mechanisms, there is only one way that attract-and-kill can achieve insect control. Pest insects (ideally both sexes) must be lured to a control agent that exerts its affect on the individual following contact. This requires the synthetic attractant to be more effective than natural attractants in the environment to successfully out-compete these. Furthermore, the attractant must not only effectively attract the target insect from a distance, but also cause the insect to contact the formulation. Thus, attractants used in attract-and-kill formulations must be highly effective for the technique to work, versus those used in mating disruption, which do not need to meet such high efficacy standards, since source contact is not necessary for mating disruption to work. An excellent attractant and appropriate insect control agent are indispensible ingredients of an effective attract-and-kill product (*43*).

Although attract-and-kill shares some of the same constraints as mating disruption, it is generally believed to be a more robust pest control technique (*43*). Just as mating disruption occurring by competitive attraction, the effectiveness of attract-and-kill is also reduced at high pest densities or when too few lures are applied to compete with natural attractants (*15*, *43*). It also must be adapted to the biology and ecology of the pest (e.g., by optimizing formulation placement and timing of application). Unlike mating disruption, however, it is less sensitive to environmental factors, such as site topography and plot size, and can successfully be used in situations where mating disruption is likely to fail. Although attract-and-kill products most often contain insecticides, which make them less environmentally-friendly and potentially more of a concern to the public than mating disruption products, they offer several advantages over conventional insecticides. These include the use of smaller amounts of insecticide and the option to apply the product away from the harvestable crop, increasing both worker and consumer safety. Attract-and-kill products can also be used to lure and control pests out of areas that cannot be treated with conventional sprays. Depending on the attractant used, attract-and-kill products may be very selective (i.e., if insect sex pheromones are used). However, attractants with broad effects, such as plant kairomones, should be tested to determine their impact on non-target organisms (*15*, *43*). Another important difference between mating disruption and attract-and-kill products for manufacturers is that unlike mating disruption products, these insecticide-containing formulations cannot take advantage of legislation which has simplified and reduced the cost of EPA registration of arthropod pheromone-based insect control products that do not contain insecticides (*50*).

The SPLAT® matrix is not only well-adapted for dispensing and protecting semiochemicals from degradation, but it has proven to effectively dispense and protect insecticide active ingredients as well. ISCA currently has four attract-and-kill formulations available internationally: Hook™ ME, Hook™ CL, and Hook™ ME+CL for control of *Bactrocera* sp. fruit flies and Hook™ RPW, for control of the red palm weevil, *Rhynchophorus ferrugineus* (Coleoptera: Curculionidae). Several additional SPLAT®-based attract-and-kill formulations are currently being developed. Two of these are discussed below: Hook™ FAW for control of the fall armyworm, *Spodoptera frugiperda* and Hook™ Tuta, for control of the tomato leafminer, *Tuta absoluta*. Development of STATIC™ Spinosad ME for control of *Bactrocera* sp. fruit flies, which has been commercialized by Dow AgroSciences (Indianapolis, IN) is also discussed.

## Fall Armyworm, *Spodoptera frugiperda*

*Spodoptera frugiperda* (Lepidoptera: Noctuidae), the fall armyworm, is native to the tropical regions of the Americas. Adults can migrate great distances, which can result in infestations as far north as Canada, although they are not able to survive the cold winters in regions north of southern Florida and Texas. Fall armyworm larvae are highly polyphagous. Although they prefer to feed on grasses, the fall armyworm has also been reported to feed on numerous other agricultural and non-agricultural plants. Larvae primarily damage plants and reduce yields through extensive defoliation of the host. Although this occurs more rarely, they are also capable of cutting plant stalks and occasionally feed directly on seeds and fruits of their hosts. Although natural enemies can significantly reduce fall armyworm populations in regions where it overwinters and some crops genetically modified to express *Bacillus thuringensis* insecticidal proteins can effectively control fall armyworms, nonetheless, large amounts of insecticides are often used to control this insect pest (*51–54*). A SPLAT®-based formulation, Hook™ FAW, containing the fall armyworm pheromone and an insecticide, is being developed as an attract-and-kill product for this pest and has been tested on field populations in Brazil.

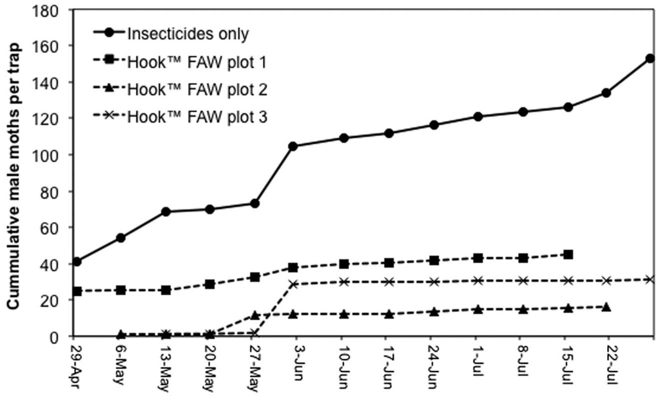


*Figure 4. Field efficacy of HOOK™ FAW for fall armyworm control in corn. Grower-standard insecticide sprays were applied in all plots.*

**Table III. Field efficacy of Hook™ FAW for control of fall armyworm in corn. Assessment of plant damage. Values are mean ± S.E of average plant damage determined at all sampling times in each plot. Grower-standard insecticide sprays were applied in all plots. Means ± S.E. followed by the same letter within columns are not significantly different (Tukey, $P \geq 0.05$).**

| *Plot* | *Davis scale rating* | *% damage* | *n* |
|---|---|---|---|
| Hook™ FAW plot 1 | 5.4 ± 0.52 ab | 54.8±12.01 ab | 4 |
| Hook™ FAW plot 2 | 4.1±0.46 bc | 50.5±5.25 b | 5 |
| Hook™ FAW plot 3 | 2.9±0.33 c | 50.7±4.51 b | 7 |
| Insecticides only | 6.9±0.35 a | 84.8±7.10 a | 4 |

A preliminary study of Hook™ FAW was conducted in large corn plots in Mogi Mirim, Sao Paulo, Brazil. Hook™ FAW was applied to three 50-ha plots. A single 27-ha plot served as a control. Hook™ FAW was hand-applied using a metering device on the upper leaves of the plants, close to the stalks, at a rate of 500 g/ha when plants had three to four leaves. A second Hook™ FAW application was made at a rate of 1 kg/ha 3 weeks later. Grower-standard insecticide sprays were applied in all plots. Hook™ FAW efficacy was evaluated with three pheromone lure-baited traps per plot. In addition, fall armyworm plant damage was quantified weekly by evaluating 25 plants at each of three sampling points in each plot using the Davis scale (*55*) and by quantifying the percentage of each plant damaged. Plant damage evaluations were conducted weekly starting the week after Hook™ FAW was applied, until the plants reached the tassel stage. Moth captures per trap were reduced in plots treated with Hook™ FAW versus plots only treated with insecticides (Figure 4). Plant damage was also significantly reduced in all plots treated with Hook™ FAW versus plots only treated with insecticides, except for plot 1 (Table III). Further development of the Hook™ FAW formulation is on-going.

## Tomato Leafminer, *Tuta absoluta*

*Tuta absoluta* (Lepidoptera: Gelechiidae) is known by a variety of common names, including: Tomato leafminer, tomato borer and South American tomato pinworm. Larvae feed on solanaceous crops and show a preference for tomatoes. They can feed on all above-ground tissues of tomato plants, including the leaves, stems, and fruit, during all life stages of the plant. Tomato leafminers have a high reproductive potential, with up to 12 generations per year, and each female capable of laying up to 260 eggs in her lifetime. The tomato leafminer does not have an obligate diapause and can overwinter at the egg, pupil, or adult stage, which contributes to this pest's persistence and potential to develop large populations. If left untreated, tomato leafminer damage can cause 100% crop loss (*56–59*).

The tomato leafminer is native to South America, but since 2006, has spread to North Africa, Southern and Western Europe, and the Middle East (*59–61*). Control of the tomato leafminer has historically relied heavily on the use of insecticides, with up to 36 applications of insecticides per season (4 to 6 applications per week) used to control it. Due to its short generation time and high reproductive potential, the tomato leafminer is highly likely to develop insecticide resistance and indeed, in endemic regions, use of new insecticides to control the tomato leafminer has inevitably been followed by reports of resistance to these insecticides (*57*, *58*). It is highly likely that the tomato leafminer will be introduced to other tomato-growing regions of the world, including the United States. The tomato leafminer poses a major economic threat to worldwide tomato production (*59*, *61*).

A tomato leafminer attract-and-kill SPLAT® formulation, Hook™ Tuta, was created that contained the tomato leafminer pheromone and an insecticide. This formulation was tested in staked tomato plots at two locations in the municipality of Caçador, Santa Catarina, Brazil. Plant spacing in both locations was 1.5 m x 0.54 m. At the private farm, plots were 937 $m^2$ and each contained *ca.* 1325 tomato plants, while at the Epagri Experiment Station, plots were 212 $m^2$ and each contained *ca.* 260 tomato plants. At both locations, the experimental design was randomized complete block with four replicates. Two treatments were tested: Hook™ Tuta + insecticides and insecticides only. Hook™ Tuta was applied as 0.5-g dollops at a rate of 1.5 kg/ha. Grower-standard insecticide sprays were applied to both Hook™ Tuta and insecticides only plots. Adult tomato leafminer populations were monitored weekly with pheromone traps. In addition, four plants were randomly marked in each plot and fruit damage was assessed for 25 fruits on each plant 59 days post-treatment (100 total fruits per plot). Although fruit injury was similarly low in plots treated with Hook™ Tuta and plots treated with insecticides alone (Table IV), mean captures of male moths in pheromone-baited traps in plots treated with Hook™ Tuta vs. plots treated with insecticides alone over the duration of the study were reduced by 85% on the private farm and 78% at the Epagri Experiment Station (Figure 5).

**Table IV. Field efficacy of Hook™ Tuta for control of the tomato leafminer in staked tomatoes. Values are mean ± S.E. of percent tomato fruit damaged. Grower-standard insecticide sprays were applied in all plots.**

| | *Treatment* | |
|---|---|---|
| *Location* | *Hook™ Tuta* | *Insecticides only* |
| Private farm | 3.5 ± 0.6 | 3.2 ± 0.5 |
| Epagri Experiment Station | 3.8 ± 0.8 | 3.0 ± 0.7 |



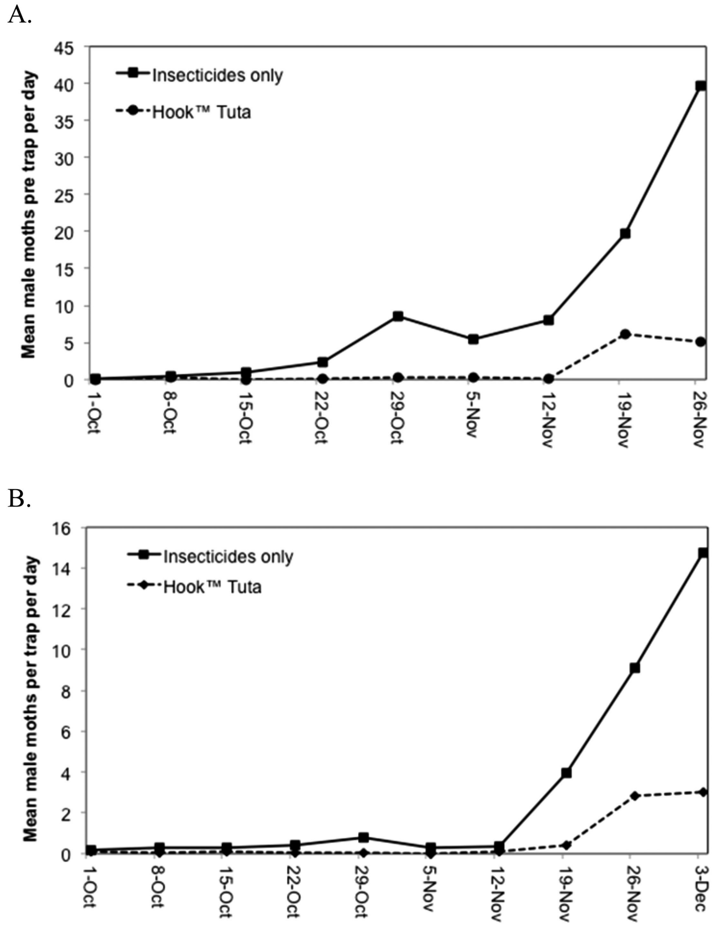


*Figure 5. Moths captured in pheromone-baited traps in field efficacy trials of Hook™ Tuta for control of the tomato leafminer in staked tomatoes. A. private farm, B. Epagri Experiment Station. Grower-standard insecticide sprays were applied in all plots.*


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The tomato leafminer's high level of impact on tomato production in regions where it is established, the high likelihood that it will continue to spread to other tomato-growing regions of the world, and the difficulty of controlling the tomato leafminer with insecticides, not to mention the environmental and human health impacts of relying solely on conventional insecticides to control this pest, highlight the necessity for alternative tomato leafminer control options, such as Hook™ Tuta. ISCA is currently the only United States registrant of the tomato leafminer pheromone and also holds an EPA registration for SPLAT® Tuta, the only EPA-registered tomato leafminer mating disruption product. When it becomes commercially-available, Hook™ Tuta will provide an additional control option for growers battling the tomato leafminer in endemic and newly-infested regions.



## Fruit Flies, *Bactrocera* sp.

The tephritid family of fruit flies (Diptera: Tephritidae) includes numerous agricultural pests of great economic importance. Significant efforts are made to monitor and control fruit flies in countries where these pests are established, as well as in regions where the risk of introduction and establishment of tephritid fruit flies is high (*62*, *63*). Several members of the genus *Bactrocera,* including *Bactrocera dorsalis* (oriental fruit fly), *Bactrocera cucurbitae* (melon fruit fly), and *Bactrocera tryoni* (Queensland fruit fly), are of special concern because of their wide host ranges (>100 known host plants), which include numerous agricultural crops, and their capacity to cause 100% crop loss (*62*, *63*). The availability of compounds that are highly attractive to males of most *Bactrocera* species has enabled the use of attract-and-kill (often referred to as male annihilation technique - MAT) to control these fruit flies. Methyl eugenol (ME) (*64*) or cue-lure (CL) (*65*) are attractive to over 90% of males of species in the subfamily Dacinae (*66*), which comprises the genera *Bactrocera* and *Dacus*. Although attract-and-kill has been effective, it has so far relied on the use of organophosphate-based products that pose risks to worker, food, and environmental safety. In addition, most attract-and-kill control efforts have used lures placed in traps that require high cost and labor inputs to set up and maintain.

SPLAT® MAT ME and SPLAT® MAT CL, containing the reduced-risk insecticide spinosad (Dow AgroSciences) and the male fruit fly attractants methyl eugenol (ME) (*64*) or cue-lure (CL) (*65*), were created as alternative management tools to replace organophosphate-based MAT products for controlling *Bactrocera* fruit flies in area-wide fruit fly management programs. In collaboration with ISCA, Vargas and colleagues conducted a series of laboratory and field studies testing the efficacy of SPLAT® MAT ME and SPLAT® MAT CL versus organophosphate-based standards against the oriental fruit fly and the melon fruit fly in Hawaii (*67–69*) and the oriental fruit fly and the Queensland fruit fly in Tahiti (*70*). These studies demonstrated that SPLAT® MAT was as effective or superior to current MAT technologies for extended periods of time (up to 16 weeks), even though the toxicity of spinosad to the target species sometimes decreased below that of the conventional pesticides present in the standard products as the study progressed. In addition to matching the efficacy of current formulations, the SPLAT® formulations also provided benefits in terms of ease of application, versus current MAT techniques, which often involve applications of solid or liquid MAT formulations in traps, requiring more time and cost to apply and service than the SPLAT® products, not to mention the inability to mechanize the application of trap-based products, which greatly limits their utility for fruit fly control efforts over large areas. Weathered SPLAT® formulations also had improved longevity versus Min-U-gel (Floridin Co., Quincy, FL), a flowable organophosphate-based MAT product known to have limited field life in high temperature and high rainfall environments, such as tropical regions where Dacinae species are important agricultural pests (*63*, *71–74*). SPLAT® MAT ME is currently sold as STATIC™ Spinosad ME by Dow AgroSciences for control of *Bactrocera* fruit flies.



### Conclusions

SPLAT® attract-and-kill formulations have proven to be effective and can provide an alternative control option for pests that may offer advantages over mating disruption, mass trapping, and the use of conventional insecticide sprays. Two SPLAT®-based attract-and-kill products are currently commercially-available, STATIC™ Spinosad ME is available in the United States and CIDA GRAFO/BONA is sold for control of *Grapholita molesta*, the oriental fruit moth, and *Bonagota salubricola,* the Brazilian leafminer, by ISCA Tecnologias Ltda. in Brazil. ISCA intends to continue to increase its attract-and-kill product portfolio both domestically and internationally.

## SPLAT® Repellent Formulations

### Introduction

We define repellents as compounds that deter or inhibit insects from finding, feeding on, or ovipositing on an attractive host substrate. Although a number of semiochemicals with repellent effects against agricultural and forest pests have already been identified, to date, they have only played a very minor role in the control of these insects. This is due to a combination of factors, including the availability of cheap and effective control alternatives for some insect pests, the lack of adequate formulations for delivery, and substantial regulatory obstacles for registering new repellent products (*15*, *75*, *76*). Although the use of repellents alone or in combination with attractants as part of a push-pull strategy has been shown to be effective in agriculture and forest systems, it requires a greater understanding of insect behavior and ecology than conventional or even other semiochemical-based alternatives, such as mating disruption and attract-and-kill (*15*, *76*). Although SPLAT® is well-adapted for delivering volatile insect repellents, the cost of bringing these technologies to market for commercial agricultural or forestry use is often prohibitive as a result of small market size and the high cost of registering products. Repellent chemicals are often best adapted for control of a limited number of insect species, in a limited number of crops, and only for growers willing and able to adopt these new techniques, which makes these a niche product (*15*, *76*). Although the EPA has reduced data requirements, costs, and registration time for biopesticides, which generally pose lower human and environmental risks than conventional chemicals, registration of new biopesticides is still a costly and time-consuming process, especially for plant kairomones, most of which are not exempt from EPA registration and not eligible for pheromone regulatory relief (*15*, *50*, *75*, *76*). Even though the obstacles to commercialization of insect repellents are high, there are, nonetheless, situations where commercialization of insect repellents is warranted. These include cases, such as the ones described below, where repellents are effective and have the potential to provide a cost-effective alternative or significant enhancement to conventional control tactics or available semiochemical control-based alternatives (*15*). ISCA and collaborators are actively developing SPLAT® repellent formulations against several important pests, including the mountain pine beetle, *Dendroctonus ponderosae* (SPLAT® Verb Repel) and the Asian citrus psyllid, *Diaphorina citri* (SPLAT® ACP Repel). Field trials with both of these formulations have been successful and are summarized below.



## Mountain Pine Beetle, *Dendroctonus ponderosae*

*Dendroctonus ponderosae* (Coleoptera: Curculionidae), the mountain pine beetle, is a bark beetle native to western North America that colonizes several pine species, most notably lodgepole pine, ponderosa pine, sugar pine, limber pine, western white pine, and whitebark pine. Girdling of phloem tissues by colonizing adults and developing larvae kills the host tree. The extensive and severe outbreaks that have occurred in recent years indicate that the mountain pine beetle is one of the foremost threats to western North American forests, and will remain such in the future (*77*, *78*). Extensive levels of tree mortality associated with mountain pine beetle outbreaks may result in replacement of host trees by other tree species and plant associations, with subsequent impacts on timber and fiber production, fuels and fire behavior, water quality and quantity, fish and wildlife populations, aesthetics, recreation, grazing capacity, real estate values, biodiversity, carbon storage, threatened and endangered species, and cultural resources, among others.

Like many bark beetles, the mountain pine beetle uses a complex system of semiochemical communication in host location, selection and colonization, and mating behaviors (*79*). Mountain pine beetles infest the lower and mid-tree bole in a behavioral sequence facilitated by aggregation pheromones and host kairomones. During the latter stages of tree colonization, increasing amounts of verbenone are produced by the autoxidation of α-pinene to *trans*- and *cis*-verbenol and then to verbenone (4,6,6-trimethylbicyclo[3.1.1]hept-3-en-2-one) (*80*) by intestinal and gallery-inhabiting microbes from both beetle sexes (*81*, *82*). Verbenone is considered an anti-aggregation pheromone component and believed to reduce intra- and interspecific competition by altering adult behavior to minimize overcrowding within the host tree and to provide cues as to host suitability (*83*–*85*).

Verbenone has been registered for management of mountain pine beetles as an alternative to standard techniques that rely on the use of conventional insecticides or tree removals to suppress populations and protect susceptible hosts. Three formulations are currently registered, and include pouches (several registrants) and the Disrupt Micro-Flake® VBN and Disrupt Bio-Flake® VBN formulations (Hercon® Environmental, Emigsville, PA). Pouches are most commonly used and are stapled at maximum reach (*ca.* 2 m height) to individual trees prior to mountain pine beetle flight in spring, and applied in a grid pattern to achieve uniform coverage when stand protection is the objective. Although pouch formulations have been reasonably effective in reducing mountain pine beetle attacks in lodgepole pine stands (*79*), treatment failures are not uncommon, and indicate a need for improved formulations and more effective means of dispersing verbenone in forests (*86*). A pilot study of the initial SPLAT® Verb Repel prototype was conducted on the Bridger-Teton National Forest in western Wyoming. Twenty-one randomly-selected, individual lodgepole pine trees were treated with SPLAT® Verb Repel, with an additional 30 trees randomly-selected as non-treated controls. All trees in the study were confirmed uninfested by mountain pine beetles prior to treatment. Either *ca.* 32 g (15 trees) or *ca.* 39 g (6 trees) of (−)-verbenone was applied per tree in four equivalently-sized SPLAT® Verb Repel dollops with a pneumatic, John Deere Gator®-mounted mechanical application system (Figure 1 C, D). SPLAT® Verb Repel was applied in mid-July, a few weeks after the initiation of mountain pine beetle flight in the area. Each SPLAT® Verb Repel-treated and non-treated tree was baited with one mountain pine beetle tree bait (Contech Inc., Delta, BC, Canada) affixed at *ca.* 2.4 m height on the north side of the tree to challenge trees used in the study with sufficient bark beetle pressure to assess treatment efficacy. The baits were removed from all trees *ca.* 30 days later, at which time, the integrity of SPLAT® Verb Repel dollops was visually inspected. Attack densities were assessed in mid-September. Visual signs of attack (boring dust and pitch tubes) were recorded for each of the treated and non-treated trees. The following June, the presence (dead) or absence (live) of crown fade was recorded for each experimental tree to assess levels of tree mortality.

Only two attacks (pitch tubes) were observed on one of the SPLAT® Verb Repel-treated trees in September and all SPLAT® Verb Repel-treated trees were alive the following June. By contrast, 28 of the 30 non-treated trees were attacked by September and only 2 remained alive the following June (Table V). Although treatment efficacy was excellent, the SPLAT® Verb Repel dollops did not adequately adhere to the tree boles. Both the SPLAT® Verb Repel formulation and application methodology were modified to address this issue in a subsequent study (see below).

A second field study was initiated in the same area last year to determine the effectiveness of the improved SPLAT® Repel formulation and application method. Thirty randomly-selected, individual lodgepole pines were treated with SPLAT® Verb Repel using a caulking gun (Figure 1B), with an additional 30 trees randomly-selected as non-treated controls. All trees in the study were confirmed uninfested by mountain pine beetles prior to treatment. Four dollops of SPLAT® Verb Repel (7 g of (−)-verbenone per tree) were applied at *ca.* 3-m height on the tree bole. All experimental trees were baited with one mountain pine beetle tree bait (Contech Inc.) on the northern aspect at *ca.* 2.4-m height for 113 days. In October, visual signs of mountain pine beetle attack were recorded for each treated and non-treated tree. In addition, trees within an 11 m radius of each SPLAT® Verb Repel-treated and non-treated tree were inspected for signs of mountain pine beetle attack. Whereas 28 non-treated trees and an additional 61 trees within an 11 m radius of the non-treated trees were mass attacked by mountain pine beetle, no SPLAT® Verb Repel-treated trees or surrounding trees were mass attacked (Table VI). Crown fade will be used to assess tree mortality in summer 2013.

**Table V. Effectiveness of SPLAT® Verb Repel in protecting individual lodgepole pines from mountain pine beetle attack. Values are numbers of trees of 21 SPLAT® Verb Repel-treated or 30 non-treated trees.**

| | *End of season evaluation* | | | | *Next season evaluation* | |
|---|---|---|---|---|---|---|
| *Treatment* | *No. not attacked* | *No. minor attacked[a]* | *No. strip attacked[b]* | *No. mass attacked[c]* | *No. alive* | *No. dead* |
| SPLAT® Verb Repel | 20 | 1 | 0 | 0 | 21 | 0 |
| Non-treated | 2 | 0 | 3 | 25 | 2 | 28 |

[a] Two mountain pine beetle attacks on the tree bole. [b] One face of the tree bole attacked. [c] Entire circumference of the tree bole attacked.

**Table VI. Effectiveness of SPLAT® Verb Repel in protecting individual and neighboring lodgepole pines from mountain pine beetle attack[a]**

| | *Treated tree* | | *Trees within 11 m radius of treated tree* |
|---|---|---|---|
| *Treatment* | *No. not attacked* | *No. mass attacked[b]* | *No. mass attacked[b]* |
| Verb Repel | 30 | 0 | 0 |
| Non-treated | 2 | 28 | 61 |

[a] N=30 per treatment. [b] Entire circumference of bole attacked.

An evaluation of SPLAT® Verb Repel for protecting 0.4-ha plots of lodgepole pine from mountain pine beetle infestation is on-going on the Caribou-Targhee National Forest in southeastern Idaho. The following treatments are being evaluated: Non-treated control, verbenone pouches (Contech Inc.), and SPLAT® Verb Repel. Preliminary results indicate that SPLAT® Verb Repel is more effective at preventing mountain pine beetle mass attacks within small plots than the verbenone pouch.

## Asian Citrus Psyllid, *Diaphorina citri*

*Diaphorina citri*, the Asian citrus psyllid, vectors *Candidatus liberibacter* species that are the causative agents of huanglongbing (or citrus greening) disease, the most devastating disease of citrus worldwide (*87*). Although research to identify Asian citrus psyllid pheromone attractants is on-going, attractant pheromone-based control technologies, such as mating disruption or attract-and-kill are not currently available (*87–91*). Guava, interplanted with citrus, has been reported to lead to reduced Asian citrus psyllid populations (*92*, *93*), an affect that has been attributed to guava leaf volatiles (*94*). Stelinski and colleagues identified dimethyl disulfide (DMDS), a compound isolated from crushed guava leaves, as a potent repellent to the Asian citrus psyllid (*95*, *96*). Development of a SPLAT® ACP Repel formulation containing this compound has yielded promising results.

A field trial was conducted with the first SPLAT® ACP Repel prototype in a 200-ha abandoned orchard of mature, *ca.* 18-year old sweet orange trees (var. "Valencia") planted at *ca.* 284 trees per ha and heavily infested with Asian citrus psyllids. Plots were square and contained 35 trees (5 x 7 trees). SPLAT® ACP Repel applied at a rate of 50 g per tree was compared to an non-treated control. Asian citrus psyllid populations in each plot were quantified at 3, 7, 11, 14, and 21 days post-treatment by counting the number of Asian citrus psyllids in 10 trees in each plot. The experimental design was randomized complete block and there were five replicates per treatment. Asian citrus psyllid populations in the SPLAT® ACP Repel plots were reduced by at least 50% for the duration of the 3-week study (Figure 6).

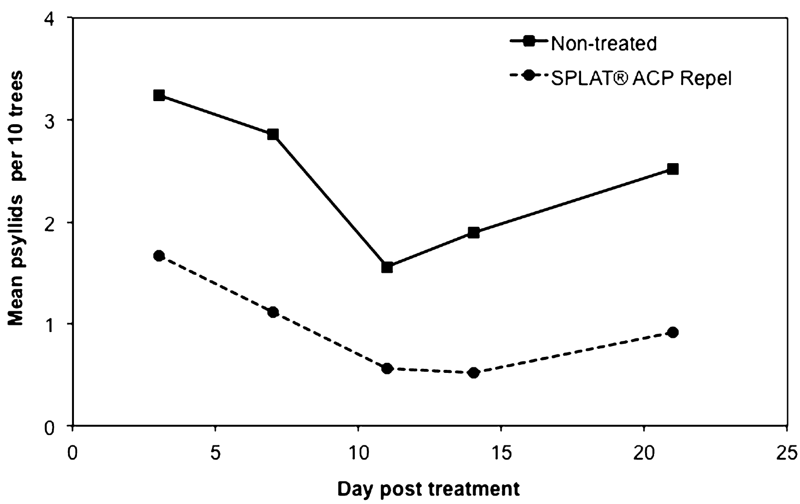


*Figure 6. Field efficacy of the first SPLAT® ACP Repel prototype formulation for control of the Asian citrus psyllid in small citrus plots. Psyllid counts in trees.*


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Following this initial field trial, four modified SPLAT® ACP Repel formulations were created in an effort to increase the effective longevity of the formulation and to reduce the release rate of the highly volatile active ingredient. These formulations were field-tested in mature sweet orange trees (var. "Valencia"). The trees were 12 years old, planted on a 3 x 6 m spacing, and averaged 4 m in height. Each plot consisted of 20 trees. SPLAT® ACP Repel was applied at a rate of 6 kg/ha. This amounted to applying six 5-g SPLAT® ACP Repel dollops per tree. The efficacy of the prototype SPLAT® ACP Repel formulations was compared to that of a non-treated control and each treatment was replicated four times. Four yellow sticky card traps were used to assess population densities of Asian citrus psyllids in each plot. SPLAT® ACP Repel #4 provided approximately 75% repellency of Asian citrus psyllids through the five week duration of the trial (Figure 7). ISCA will continue to work with collaborators to develop this technology for use in Asian citrus psyllid integrated pest management programs.

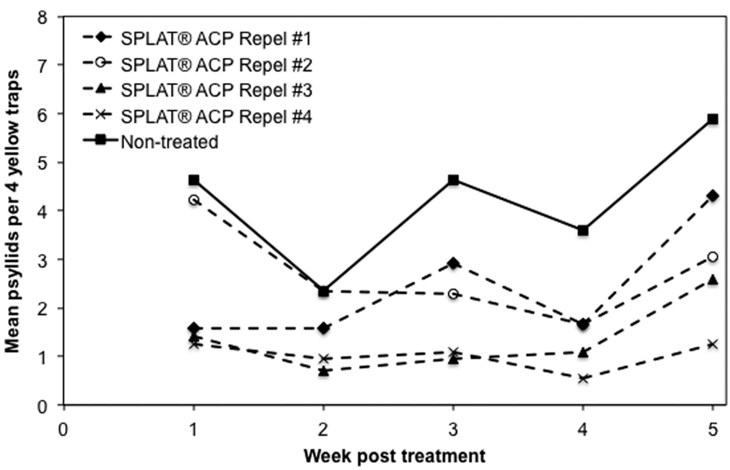


*Figure 7. Field efficacy of four modified prototype SPLAT® ACP Repel formulations in small citrus plots. Psyllid captures in yellow sticky card traps.*

## Conclusions

Although to date, few semiochemical-based insect repellents have been commercialized for use in agriculture and forestry, we believe these products can be valuable tools for some integrated pest management programs and should be pursued in cases where existing control methods have not proven adequate. Repellent semiochemicals, when formulated in appropriate dispensing systems, can play an important role in effective control programs (*76*). SPLAT® will be useful in this regard.


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## Conclusions and Future Directions

SPLAT® is unique among commercial semiochemical dispensers in providing a matrix that is capable of dispensing a wide variety of compounds and can be applied using a virtually unlimited number of manual and mechanical techniques. The versatility of SPLAT® makes it adaptable for use in any semiochemical-based insect control program, regardless of semiochemical, crop, or plot size. SPLAT® mating disruption and attract-and-kill formulations have been developed for important agricultural and forestry pests both domestically and internationally. Several of the existing SPLAT® mating disruption formulations have also been certified for use in organic crop production. Repellent formulations are currently being developed for important agriculture and forestry pests, as well as attractant formulations for beneficial insects. ISCA will continue to work with collaborators to test the limits of the SPLAT® matrix for dispensing insect behavior-modifying compounds in a variety of environments as it participates in research to create new semiochemical-based insect control tools.



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