CHAPTER 16

Use of Repellents Formulated in Specialized Pheromone and Lure Application Technology for Effective Insect Pest Management

Agenor Mafra-Neto, Christopher J. Fettig, A. Steven Munson, and Lukasz L. Stelinski

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INTRODUCTION

An insect repellent is a chemical compound or blend of compounds that deters insect activity on or near otherwise attractive substrates. Although repellents have played a key role in the

control of hematophagous insects and a large number of repellent semiochemicals, including some pheromones have already been characterized for agricultural and forestry insect pests, they are not widely commercialized in agriculture or forestry.[1–3] The vast majority of repellents are labile semiochemicals that quickly vanish once applied in the field, as conventional slow-release formulation technologies are often difficult to apply and/or inefficient in controlling the emission rate of the active ingredient. 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 forestry systems, the effective application of such compounds requires the user to have a more comprehensive knowledge of insect behavior than the use of conventional insecticides, which are not only simpler to use but typically less costly.[1,4]

The specificity of semiochemical repellents, which are often best adapted for a limited number of insect species in a restricted number of crop or forest systems, presents another challenge to their successful implementation on a large scale, as do the intricacies of the U.S. Environmental Protection Agency's (EPA's) registration process for new biopesticides, which can be very costly and time consuming.[1,2,5] This small-sized specific market, combined with the high cost of product development and registration, hampers the commercialization of repellent technologies, especially for minor crops or tree species. Despite the many impediments to commercialization of insect repellents, there are some situations where their use in agriculture and forestry is desirable and warranted. ISCA Technologies (Riverside, California), together with collaborators from academic, government, and private sectors, is actively developing novel repellent formulations against several important pest species. Here, we describe two case studies utilizing ISCA Technologies' controlled-release matrix SPLAT® (Specialized Pheromone & Lure Application Technology): SPLAT Verb, a repellent for the mountain pine beetle, *Dendroctonus ponderosae* Hopkins, a pest of lodgepole, *Pinus contorta* Douglas ex Loud., and other pines; and SPLAT ACP Repel, a repellent for the Asian citrus psyllid (ACP), *Diaphorina citri* Kuwayama, a serious pest of citrus plants.

Insects inhabit a complex, constantly shifting, olfactory landscape, comprising a plethora of different volatiles emanating from the biotic and abiotic environment. Olfaction is often considered the prevalent sense that mediates insect behavioral sequences resulting in host selection.[6,7] Many insects possess sophisticated olfactory systems equipped with numerous olfactory receptor proteins[8] expressed on the dendritic membranes of sensory neurons housed in olfactory sensilla. The recognition of a host plant by insects is believed to be based on either specific olfactory receptors/olfactory sensory neurons that detect specific odorants released from a specific plant or combinations of olfactory receptors/olfactory sensory neurons that together detect specific ratios of general odorants in a blend.[9] Olfactory receptors represent the molecular basis for the specificity of olfactory sensory neurons.[10] Many different herbivorous insects have olfactory sensory neurons/olfactory receptors tuned to the components of commonly occurring green leaf volatile alcohols and aldehydes, which are major constituents of green plants.[11–13]

Phytophagous insects may have the ability to discriminate between hosts and nonhosts, as well as between hosts of different quality.[11,14] For example, herbivore-induced plant volatiles are important signals for an ovipositing female, allowing her to judge the quality of the host plant before laying eggs, which is a crucial decision for the survival and development of her offspring. Volatiles released from nonhost plants are also important cues that may warn insects to avoid nonhost or less preferred host plants, or enable them to select the right habitat and a suitable host plant. For example, nonhost volatiles may modulate host-selection behaviors of bark beetles by reducing their attraction to pheromones or host kairomones, or by enhancing the effect of antiaggregation pheromones.[3,15] The large number of attractive and nonattractive volatiles released by plants, combined with differing combinations, constitutes a major challenge for herbivorous insects attempting to identify their host plants in a complex olfactory landscape.

Specialized Pheromone and Lure Application Technology

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), semipermeable plastic membranes (BeetleBlock™, Synergy Semiochemicals), and laminated polymers (Disrupt®, Hercon Environmental), ISCA Technologies has taken an alternative approach: the controlled-release emulsion SPLAT, which is a unique technology that can be adapted to dispense a wide variety of compounds, including semiochemicals, pesticides, and phagostimulants, while protecting them from degradation across a broad range of diverse environments. Although adapting SPLAT to release new compounds can pose major technical challenges, the versatility of this flowable formulation provides many compensatory benefits. SPLAT emulsions can be designed to hold a vast array of semiochemical concentrations and additives to create a formulation that releases the optimal rate of a given semiochemical for a desired period, while shielding 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, allowing the use of a variety of manual and mechanized application techniques (Figure 16.1 and Figure 16.2). Unlike most other semiochemical dispensers, SPLAT is not restricted to a particular point source size. Any amount of SPLAT constitutes a point source, providing yet another way to optimize application rates and coverage in the field. Furthermore, the biodegradability and low manufacturing cost of SPLAT significantly decrease environmental impacts and enable commercialization of more affordable semiochemical-based control products.

Figure 16.1 **(See color insert.)** SPLAT Verb for protecting individual *Pinus contorta* from *Dendroctonus ponderosae* attack was applied with mechanical application equipment housed in the bed of a John Deere Gator (left, center). The same system can be adapted to a helicopter, airplane, tractor, or pickup truck. *Pinus contorta* baited with a *Dendroctonus ponderosae* tree bait (brown pouch) following application of SPLAT Verb during a pilot study (right).

Figure 16.2 Mechanical application of SPLAT. Here, SPLAT is dispensed from a 57-L drum using a pneumatic pump supplied by an air tank (alternatively, a gas-powered air compressor may be used). This basic model can be replicated in any number of ways depending on the equipment available and field characteristics.

MOUNTAIN PINE BEETLE, *DENDROCTONUS PONDEROSAE*

Dendroctonus ponderosae is native to forests of western North America and is one of a few species of bark beetles that behave as true predators in that host colonization typically results in mortality of the host tree.[16] The extent of tree mortality resulting from *Dendroctonus ponderosae* may be limited to small spatial scales (e.g., individual trees or small groups of trees at endemic population levels) or may affect entire landscapes. During the early stages of an outbreak, the beetles typically target trees already under stress from other sources,[17] such as mechanical injury, drought stress, other insects, root disease, and/or old age. *Dendroctonus ponderosae* is the most damaging insect pest of *Pinus contorta* in western North America,[18] and outbreaks appear to be increasing in response to climate change[19–21] and existing forest conditions. In the western United States, stands in age classes of 60–120 years, and with densities >400 stem/ha, tend to be highly susceptible to *Dendroctonus ponderosae*.[22]

In the past decade, we have witnessed unprecedented levels of tree mortality attributed to *Dendroctonus ponderosae* outbreaks across much of western North America. An ongoing outbreak in British Columbia, the largest outbreak ever documented, affected 11 million hectares in 2007[23] and has since grown to encompass >17.5 million hectares.[24] In the United States, the outbreak impacted >9 million hectares.[25] Although *Dendroctonus ponderosae* is an important part of the ecology of these forests, extensive levels of tree mortality resulting from outbreaks may have undesirable impacts, for example, negatively affecting aesthetics, fire risk and severity, recreation, timber production, and real estate values, among many other factors.

Management of *Dendroctonus ponderosae*

During *Dendroctonus ponderosae* outbreaks, federal, state, and industrial landowners conduct more frequent aerial detection surveys.[26] Infestations can easily be spotted from low-flying aircraft as polygons of red and fading trees. If *Dendroctonus ponderosae* populations are deemed sufficient to warrant control, a continuous program of ground surveillance and aerial surveys is often initiated. A number of risk and hazard rating systems are available to predict the susceptibility of a given stand to *Dendroctonus ponderosae*,[27–31] and chemical-,[32] silviculture-,[22] and semiochemical-based control methods[4] have been developed.

Semiochemical Modulation of Dendroctonus ponderosae *Mass Attacks*

Like many bark beetles, *Dendroctonus ponderosae* uses a complex system of chemical communication during host location, host selection, and host colonization.[33,34] The initiation of gallery formation induces females to produce an aggregation pheromone composed of *trans*-verbenol and *cis*-verbenol.[35,36] At the same time, the host tree releases α-pinene,[37] myrcene, and terpinolene[34,38,39] in response to the attack, which increases the attractiveness of the target tree.[34,36,37,40] The aggregation pheromone recruits other pioneering females to the target tree and induces them to bore into the bark.[41,42] *exo*-Brevicomin is produced by both sexes and appears to be attractive at low concentrations and inhibitory at higher concentrations.[43,44] *cis*-Verbenol, produced by female *Dendroctonus ponderosae,* has been shown to increase the attraction of conspecific females to *exo*-brevicomin, but its effect is less than that of *trans*-verbenol.[36]

Successful host colonization depends on recruiting a minimum number of *Dendroctonus ponderosae*[33,45] to attack the tree and overcome its defenses,[46] a process that is completed within 2–3 weeks. As the abundance of colonizing male *Dendroctonus ponderosae* increases, levels of male-secreted *exo*-brevicomin and frontalin increase[34,47–51] while concentrations of the aggregation pheromones *trans*- and *cis*-verbenol and host monoterpenes decline.[40] This reduces the attractiveness of the target tree and commences latter stages of tree colonization. The secretion of 2-phenylethanol by males[44] and the release of 1-octen-3-ol by females[52] may further reduce attraction to the target tree.

Autoxidation of α-pinene to *trans*- and *cis*-verbenol and then to verbenone,[53] primarily by intestinal and gallery-inhabiting microbes within both sexes of *Dendroctonus ponderosae*,[54,55] inhibits additional *Dendroctonus ponderosae* from infesting the target tree. This inhibition is necessary for reproduction, because limiting the number of infesting beetles increases the likelihood of brood survival.[56] Although the attractant *trans*-verbenol is still being secreted by the beetles in the infested tree, the attracted beetles are repelled from the focus tree by verbenone[57–61] and *exo*-brevicomin.[43] These newly arriving *Dendroctonus ponderosae* then reorient to adjacent trees, where the cycle of colonization may be repeated.[62]

Pheromone-Based Strategies to Manage Dendroctonus ponderosae

Pheromone-based strategies examined for the management of *Dendroctonus ponderosae* involve aggregation pheromones deployed in trap-out, trap-tree, or concentration approaches,[63–65] and the use of antiaggregants to interrupt colonization of hosts.[4,65–75] Semiochemically driven push and push–pull strategies have been proposed[65,76,77] and assayed,[77] but the push-only strategy is regarded to be preferable given that it is much less expensive and simpler to implement than push–pull.[77]

Verbenone

Verbenone is produced in vivo by some insects and is also found in a variety of plants including a wide range of angiosperms.[78–85] It has been approved by the Food and Drug Administration as a food additive[86] and is a constituent of strawberry, raspberry, dill, rosemary, and spearmint flavor mixtures used in the food industry.[87–89] Because verbenone is naturally occurring and can be effective for reducing levels of tree mortality attributed to *Dendroctonus ponderosae* in several tree species,[90] it is currently approved by the U.S. EPA as a biopesticide for use in forestry. Formulations currently registered include pouches (several registrants), Disrupt Micro-Flake® VBN, Disrupt Bio-Flake® VBN, and Disrupt Bio-Dispenser BB (Hercon® Environmental).

Although verbenone has been studied extensively with the goal of protecting individual trees and stands from mortality attributed to *Dendroctonus ponderosae*,[90] results have been inconsistent, a shortcoming that has negated the widespread adoption of verbenone as a management tool for *Dendroctonus ponderosae*. In some cases, it is likely that consistent and acceptable efficacy was not achieved because of shortcomings in the formulation of verbenone. For example, a polyolefin bead formulation was shown to release inadequate levels of verbenone for an inadequate length of time,[91] resulting in inconsistent field results.[92] In another example, a bubblecap formulation was ineffective in protecting individual *Pinus contorta*, likely due to insufficient release of verbenone.[93] Today, although most pouch formulations have been demonstrated to release adequate levels of verbenone for adequate periods,[94,95] they are applied at relatively low densities (typically, <124 U/ha), which limits coverage. It is possible that a high density of long-lasting point sources would provide better dispersal of verbenone and would better simulate the natural release of verbenone in a forest stand, thus ensuring greater efficacy than that achieved by larger dispensers, such as pouches.[96] Furthermore, pouches are manually applied to the bole of each tree. They are not amenable to mechanization of application and require retrieval from the field after treatment, making them difficult to adopt in large-scale programs due to the associated labor costs.

SPLAT Verb

An ideal formulation of verbenone for management of *Dendroctonus ponderosae* should (1) provide monolithic reservoir-type release at adequate rates (>50 mg/day) for an adequate period (~3 months in *Pinus contorta*); (2) allow for the application of a relatively high density of point sources per unit area (>375 ha); (3) allow for application by manual or mechanized means using conventional

equipment; and (4) be fully biodegradable (within ~12 months), allowing the formulation to be left in the field. Additional desirable attributes include (1) ease of flow from the storage reservoir to the point of application (this would ensure that it could be manually or mechanically applied by ground or aerial equipment); (2) good adhesion as applications must remain fixed to the intended target for several months regardless of weather conditions (it must be rainfast and ultraviolet [UV] protected); (3) eco-friendly; and (4) unobtrusive (the formulation should be almost invisible to the general public). As mentioned earlier, ISCA Technologies has developed a SPLAT formulation that addresses these desirable characteristics. SPLAT is inexpensive, flowable, rainfast, and UV protected and adheres to most surfaces, sticking particularly well to pine bark (C. J. Fettig, A.S. Munson, and A. Mafra-Neto, et al. unpublished data). It can be stored in regular containers and can be cleaned easily using nothing more than soapy water. Applications of SPLAT to plants have no phytotoxic effects. Point sources eventually fall to the ground and biodegrade without consequence. Most importantly, the release rate of most active ingredients from SPLAT is surprisingly constant, decreasing slowly over a period of weeks to months depending on the formulation. The amorphous and flowable quality of this highly adaptable product allows for easy transition from small-scale manual applications (single trees) to large-scale mechanized applications (campgrounds, stands, etc.).

Chemical Stability of SPLAT Verb under Field Conditions

SPLAT Verb was designed to release verbenone over a sustained period (8–24 weeks, depending on dollop size) at levels that disrupt the aggregation behavior of *Dendroctonus ponderosae*, thereby preventing or reducing its number of attacks and subsequent levels of tree mortality. A weakness of some other formulations of verbenone is that under direct sunlight verbenone may be photoisomerized to chrysanthenone,[97] a compound with no known effects on bark beetle behavior. Although conventional UV-reflecting pouch release devices contain UV stabilizers that scavenge UV-generated radicals, Fettig et al.[94] indicated that trace amounts of filifolone, a thermal- or photorearrangement product of (+)-chrysanthenone,[98] were present in pouches deployed for several weeks in ponderosa pine, *Pinus ponderosa* Dougl. ex Laws., forests in California.[94] Conversely, data from rigorous analytical chemistry of SPLAT Verb dollops aged up to 12 months in *Pinus contorta* forests in Idaho and Wyoming indicate that this product protects verbenone extremely well. That is, it was only after the dollops were aged for >12 months in the field that we detected the first traces of chrysanthenone (A. Mafra-Neto, C. J. Fettig, and A. S. Munson, et al. unpublished data), and this was found in only one sample. In addition to assays in forests, we also aged dollops of SPLAT Verb in Riverside, California, which has a warm and sunny climate. Our results indicate that even in Riverside isomerization of verbenone to chrysanthenone did not occur in the SPLAT Verb formulation and therefore would likely not represent a substantial concern in areas where SPLAT Verb is likely to be most commonly used (e.g., the Rocky Mountains). It is important to note that these and similar analyses do not address changes in the chemical stability of verbenone once released into the active airspace, which may also influence levels of inhibition, but over which we have no control (Figure 16.3).

Fieldwork

Protecting Individual Pinus contorta *from* Dendroctonus ponderosae

Study 1

A pilot study was conducted in 2011 on the Bridger-Teton National Forest, Wyoming. Trees treated with SPLAT Verb and untreated controls (n = 21 SPLAT Verb, due to limited quantities available; n = 30 untreated control) were confirmed to be uninfested by *Dendroctonus ponderosae* prior to treatment. Four large dollops of SPLAT Verb were applied at approximately 3 m in height

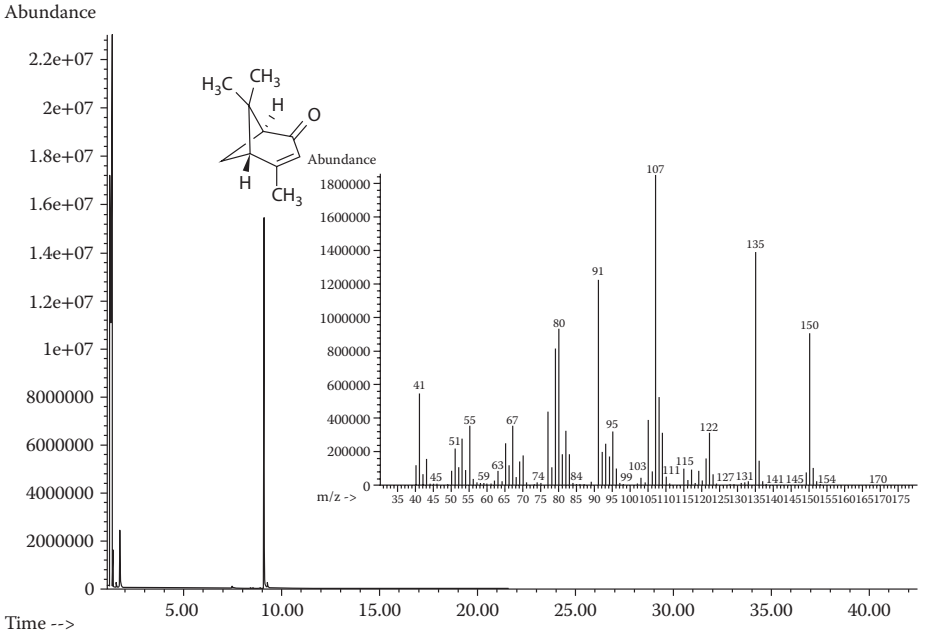

Figure 16.3 A representative analysis using gas chromatograph and mass spectrometer of the solvent extraction of a single SPLAT Verb dollop that was aged for 1 month under direct sun exposure in Riverside, California. The trace shows a single large peak, characterized as verbenone.

on the tree bole, using a Graco 15:1 automotive-style pneumatic-grease pump (Graco, Minneapolis, Minnesota) with a 5-m hose attached to a spray nozzle. The pump was powered by a portable gasoline-driven air compressor. All equipment was housed in the bed of a John Deere Gator™ (Figure 16.1). A total of 15 trees received approximately 32 g of (–)-verbenone per tree (~533 g of SPLAT Verb), and the remaining 6 trees received approximately 39 g of verbenone per tree (~650 g of SPLAT Verb). We recognized that these rates are higher than operationally applied for individual tree protection (typically, one or two 7-g verbenone pouches are used), but timing precluded us from determining application rates until after treatments were implemented. Treatments were applied in mid-July (13.9°C–25.6°C, 33%–65% relative humidity, winds <5 km/h) approximately 7–10 days after the initiation of *Dendroctonus ponderosae* flight in the area. A 10-minute rainfall occurred immediately after application. All SPLAT Verb–treated and –untreated control trees were baited with one *Dendroctonus ponderosae* tree bait (Contech, Delta, British Columbia, Canada) positioned at a northern aspect approximately 2.4 m in height on the tree bole. Untreated control trees were baited before those treated with SPLAT Verb, an important caveat but one we feel had little impact given that only limited flight (~3 weeks) occurred before the baiting of SPLAT Verb–treated trees. Baits were removed from all experimental trees approximately 30 days after baiting, at which time all trees treated with SPLAT Verb were visually evaluated for dollop integrity. Preliminary assessments of tree health were performed in September 2011 by visually examining trees for signs of *Dendroctonus ponderosae* attack. Final evaluations were based on the presence (dead) or absence (alive) of crown fade in June 2012.

Only one SPLAT Verb–treated tree showed signs of *Dendroctonus ponderosae* attack, whereas 83.3% of untreated control trees suffered mass attack (boring dust and/or pitch tubes encircling the tree bole) by *Dendroctonus ponderosae* at levels high enough to suggest that mortality was imminent. Evaluations of tree mortality in the following year indicated that SPLAT Verb provided 100% tree protection, whereas only 6.7% of the untreated control trees survived (Table 16.1, Figure 16.4).

Table 16.1 Preliminary Study of the Effectiveness of SPLAT Verb for Protecting Individual *Pinus contorta* from *Dendroctonus ponderosae* Attack, Bridger-Teton National Forest

Treatment	*N*	Percentage of Trees Alive
SPLAT Verb	21	100.0
Untreated control	30	6.7

Figure 16.4 (See color insert.) Crown fade (yellow-brown needles) in untreated control trees used in SPLAT Verb pilot study. Only 6.7% of the untreated control trees were without signs of crown fade at the time when final evaluations were made (June of the field season following treatment).

Study 2

Based on the preliminary results obtained from the pilot study (Study 1), a second study was initiated in the same area to confirm the effectiveness of SPLAT Verb for the protection of individual *Pinus contorta*. A total of 30 randomly selected *Pinus contorta* were treated with SPLAT Verb using a caulking gun (Newborn XLite, Newborn Brothers Co., Inc., Jessup, Maryland) (Figure 16.5). Four dollops [total 7 g of (–)-verbenone per tree] were applied at approximately 3-m. An additional 30 Pinus contorta were randomly selected as untreated controls. All experimental trees were confirmed to be uninfested by *Dendroctonus ponderosae* prior to treatment and baited with one Dendroctonus ponderosae tree bait (Contech) on the northern aspect at approximately 2.4-m height for 113 days.

Preliminary assessments of tree health were performed in September 2012 by visually examining the trees for signs of *Dendroctonus ponderosae* attack (as in Study 1). None of the SPLAT Verb–treated trees showed signs of mass attack (Table 16.2), whereas 93% of untreated control trees suffered mass attack. We also evaluated all host trees within a 11-m radius of each experimental tree for signs of attack (Table 16.2). None of the trees surrounding SPLAT Verb–treated trees suffered mass attack by *Dendroctonus ponderosae*, whereas 61 trees surrounding untreated controls exhibited mass attack, suggesting that the zone of inhibition surrounding each SPLAT Verb–treated tree was at least 11 m. This represents a much larger area than that previously reported for other

Figure 16.5 Manual application of SPLAT Verb using a caulking gun. Each treated tree received 7 g of verbenone.

Table 16.2 Effectiveness of SPLAT Verb for Protecting Individual Neighboring *Pinus contorta* from *Dendroctonus ponderosae* Attack, Bridger-Teton National Forest

Treatment	Treated Trees	Trees Within 11-m Radius of Treated Tree	
	Not attacked (%)	Mass attacked[a] (%)	Number mass attacked[a]
SPLAT Verb	100	0	0
Nontreated	6.7	93.3	61

Note: Trees were evaluated 3 months after treatment for signs of attack.
[a] Entire circumference of bole attacked.

formulations of verbenone. For example, one study found that verbenone bubblecaps inhibited *Dendroctonus ponderosae* attraction to baited multiple-funnel traps at a distance <4 m in *Pinus contorta* forests in British Columbia.[99] Similarly, Fettig et al.[94] evaluated a 5-g verbenone pouch and reported similar results for the western pine beetle, *Dendroctonus brevicomis* LeConte, a closely related species, in *Pinus ponderosa* forests in California. No significant differences were observed among *Dendroctonus brevicomis* captures at 0.5, 1, or 2 m from the pouch, but significantly more *Dendroctonus brevicomis* were collected at 4 and 9 m.[94]

Protecting 0.4 ha Plots of Pinus contorta *from* Dendroctonus ponderosae

Although demonstrating the efficacy of SPLAT Verb for the protection of individual trees is important, verbenone is most typically applied to protect small-scale areas (campgrounds). Accordingly, we evaluated SPLAT Verb for small-scale stand protection using 0.4-ha experimental plots on the Caribou-Targhee National Forest, Idaho. Treatments included (1) untreated control, (2) (–)-verbenone pouch (7-g pouch, Contech), and (3) SPLAT Verb (7 g of (–)-verbenone). Verbenone pouches were stapled in an approximately 9.1 × 9.1 m grid (125 U/ha) to the north side of the nearest tree at a height of approximately 2 m. SPLAT Verb was also applied to the north side of trees at a height of approximately 2 m in suitable dollop sizes to achieve adequate coverage and cumulative application rates of 875 g/ha of (–)-verbenone. A complete census of each plot of trees was conducted prior to the treatment interval. Any trees attacked by *Dendroctonus ponderosae* were excluded from

TABLE 16.3 Effectiveness of Verbenone Treatments for Protecting Small Stands of *Pinus contorta* in Montpelier Ranger District, Caribou-Targhee National Forest

Treatment	Number of Trees Mass Attacked[a]
SPLAT Verb (875 g/ha)	8
Verbenone pouch (875 g/ha)	18
Untreated control	50

Note: Trees were evaluated 3 months after treatment for signs of attack.
[a] Total number of trees attacked in five 1-acre square plots per treatment.

subsequent analyses. Treatments were applied before the start of *Dendroctonus ponderosae* flight, as confirmed by the lack of captures in pheromone-baited traps (Contech). In the center of each plot, the nearest tree was baited with a *Dendroctonus ponderosae* tree bait (Contech). Thus, baiting provides a rigorous evaluation of efficacy at the expense of detecting any subtle treatment effects.

Preliminary assessments of tree health were performed in September 2012 by visually examining trees for signs of *Dendroctonus ponderosae* attack (as in Study 1). Each *Pinus contorta* within each experimental plot was evaluated for signs of attack, and the number of mass-attacked trees in all five replicates (0.4 ha plots) was tallied for each treatment (Table 16.3). In all SPLAT Verb–treated plots, only eight trees were mass attacked by *Dendroctonus ponderosae*. A total of 18 trees were mass attacked in the plots treated with the 7-g verbenone pouch, and 50 trees experienced mass attacks in the untreated control plots.

Summary: SPLAT Verb

Over several decades, substantial research has focused on the use of antiaggregants, primarily verbenone but also other inhibitory compounds,[15] to disrupt the responses of *Dendroctonus ponderosae* to attractant-baited traps and attractant-baited and attractant-unbaited trees in hopes of developing tactics to reduce levels of tree mortality attributed to *Dendroctonus ponderosae*. Early experiments showed that there was significantly less tree mortality on verbenone-treated plots than untreated plots.[4,90] Later studies yielded inconsistent or ambiguous results over time,[92,100] geographical area,[100,101] outbreak intensity,[72,102] dose,[101,103] or tree species,[4,90] with studies indicating that verbenone is largely ineffective for reducing levels of tree mortality in *Pinus ponderosa*. Progar et al.[90] identified nine factors limiting the effectiveness and utility of semiochemical treatments for the management of *Dendroctonus ponderosae*, at least three of which are relevant to the performance of SPLAT Verb compared to conventional release devices: (1) enhanced chemical stability, (2) an increased range of inhibition, and (3) the ability to achieve more uniform coverage per unit area. Furthermore, dollops of SPLAT Verb biodegrade rapidly and, therefore, do not need to be retrieved from the field as do conventional release devices. This potentially represents significant labor cost savings in the execution of operational *Dendroctonus ponderosae* suppression campaigns (A. S. Munson, unpublished data). Although development of SPLAT Verb uses in forests is ongoing, we believe this product represents an important contribution to the relatively few effective tools that forest health specialists have available to protect trees from mortality attributed to *Dendroctonus ponderosae*.

ASIAN CITRUS PSYLLID, *DIAPHORINA CITRI*

The U.S. citrus industry faces a serious threat from the invasive *Diaphorina citri*, which was first established in the United States in 1998[104–106] in Miami. Although feeding and/or oviposition by these insects may result in direct damage to plant tissue,[107] the primary impact of this invasion is the psyllid's role in the transmission of Huanglongbing (HLB), one of the world's most serious diseases of citrus[108] (Figure 16.6 and Figure 16.7). Also known as citrus greening, HLB is associated with the presence of the pathogen *Candidatus Liberibacter* spp.[108] With the recent invasion of California by

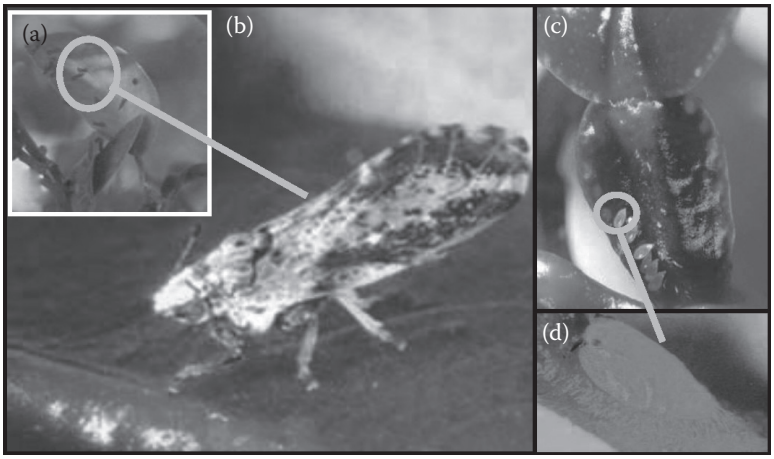

Figure 16.6 **(See color insert.)** *Diaphorina citri*, Asian citrus psyllid, the vector of the *Candidatus Liberibacter* spp. that causes the devastating and irreversible Huanglongbing (HLB), or citrus greening disease. Today, Asian citrus psyllid (ACP) is present in every citrus-producing state in the United States. ACP is a very effective vector of HLB because *Candidatus Liberiobacter* grows extremely fast inside the infected nymphs (c, d), amplifying their presence and thus increasing their hosts' ability to vector HLB as adults. Thus, the effective control of *Diaphorina citri*, and keeping population at extremely low levels, is very important to citrus growers. SPLAT ACP Repel represents an important tool for the management of *Diaphorina citri* (a, b).

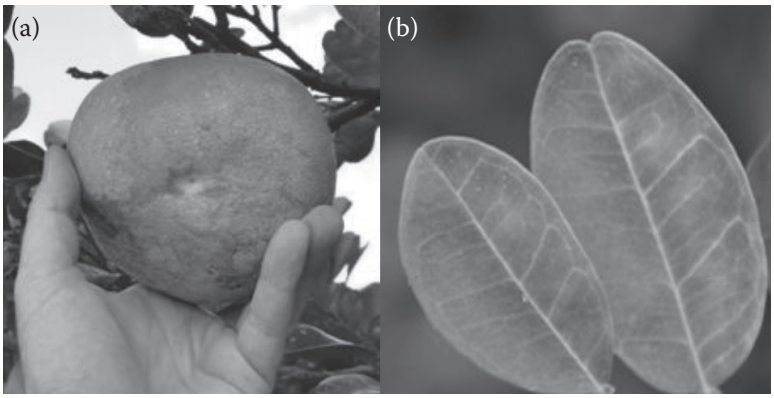

Figure 16.7 **(See color insert.)** Huanglongbing (HLB) is one of the most destructive citrus diseases worldwide. (a) A pomello fruit showing classic symptoms of infection including stunted growth and an irregular shape. The fruit itself is not palatable. (b) A leaf showing the corky veins and the unbalanced chlorosis, characteristic of HLB infection.

Diaphorina citri, this pest is now present in all major citrus-producing states in the United States. The HLB pathogen invariably follows the vector as it expands its range, with trees showing signs of the disease approximately 1–3 years after infection. Citrus trees infected with HLB produce unmarketable fruit and will ultimately die from the disease.

In 2005, the first detection of HLB-infected citrus trees in Florida[109] triggered a concerted effort to detect and remove HLB-infected trees, which has since become a monumental task due to the high rate of HLB infection. This detection and removal program peaked in 2009, when >1 million HLB-infected citrus trees were removed from Florida groves. As the prevalence of HLB increased in this area, growers began providing supplemental nutrition to their citrus plants. HLB reduces the availability of nutrients to the infected tree, causing a rapid decline in its overall health.

Careful supplementation of nutrients does not cure the disease but enables continued fruit production despite HLB infection. This, in turn, allows growers to maintain their productivity during the HLB epidemic. However, growers discovered that supplemented plants produce more and better fruit if they are not reinfected with HLB; so effective suppression of *Diaphorina citri* populations remains crucial, even in orchards where most of the plants are already infected with HLB and are being treated with supplemental nutrition.

Management of *Diaphorina citri* and HLB currently relies on high inputs of broad-spectrum insecticides, which almost certainly have adverse effects on natural enemies that might otherwise contribute to control of *Diaphorina citri* and other citrus pests.[107,110,111] Many stakeholders believe that this strategy is unsustainable, and there is growing concern over *Diaphorina citri*'s recently developed resistance to these insecticides.[112–114] There is an urgent need to develop novel and effective tools to manage *Diaphorina citri*. Furthermore, in response to consumer demand, citrus growers seek low-risk alternatives to broad-spectrum insecticides that can be applied using equipment they already possess. Although HLB is not completely successfully managed in any region of the world where the disease and the vector coexist, the most successful management efforts involve a combination of clean nursery stock, prompt removal of inoculum, and aggressive insecticides sprays against the psyllid.[109]

Solution: Develop an Effective Natural Repellent to Control *Diaphorina citri* and Mitigate Huanglongbing

Based on recent innovations in research that have shown great potential for use in *Diaphorina citri* management, we developed a botanically derived repellent, identified from guava leaves.[105] This compound, dimethyl disulfide (DMDS), was formulated into SPLAT and used to successfully repel *Diaphorina citri* from citrus groves.

Botanically Derived Semiochemicals: Repellents

Botanical insect repellents and insecticides have a prominent role in agricultural pest management. Classic examples include the neem tree–derived insecticide, the antifeedant, azadirachtin and the tobacco-derived insecticide nicotine. *Diaphorina citri*'s abundance in citrus groves is likely influenced by factors like host-plant suitability, mate-finding dynamics, and the presence of surrounding nonhost plants. Numerous chemical and environmental cues can also impact the behavior of adult insects, particularly those signals produced by host and nonhost plants. General models for host-searching behavior by herbivorous insects suggest that host location is a sequential process during which, at each successive step, the physiochemical signals associated with the plant may determine acceptance or rejection by the insect (for feeding or oviposition). Insects' detection of these cues, specifically by Hemipteran species, relies on a variety of sensory modalities, including vision, olfaction, and mechanoreception. Interfering with the host-finding process of the HLB vector is one potential method for reducing its spread. Development of an effective *Diaphorina citri* repellent formulation could improve citrus pest management in many ways. For one, a repellent formulation targeted specifically toward *Diaphorina citri* would not harm biological control agents, which are virtually eliminated from citrus groves by the frequent spraying of broad-spectrum insecticides. Adding an effective botanical repellent to a citrus management program could lessen the number of insecticide applications needed to achieve control, improve insecticide rotation, and reduce the potential for insecticide resistance.

The volatiles released by common guava, *Psidium guajava* L., have garnered intense interest because a recent report showed that guava grown in proximity to, or intercropped with, citrus had a repellent effect toward *Diaphorina citri*.[115] Citrus groves interplanted with guava were devoid of *Diaphorina citri* infestation, whereas nearby citrus groves without guava were heavily infested.[116]

Given that the presence of guava appears to reduce *Diaphorina citri* populations and subsequent incidence of HLB, the cause of this effect was investigated. Leaf volatiles released by white guava leaves, both crushed and intact,[105] collected using static headspace solid-phase microextraction (SPME), were identified using gas chromatography with pulsed flame photometric detection and gas chromatography–mass spectrometry. Leaf volatiles from four common guava cultivars were examined via a similar process to identify the potential components responsible for guava's repellency effect.[105] A total of 54 leaf volatiles were isolated by linear retention index (LRI) and mass spectrometry (MS) data in the crushed guava leaf headspace, including seven sulfur volatiles: hydrogen sulfide, sulfur dioxide, methanethiol, dimethyl sulfide (DMS), DMDS, methional, and dimethyl trisulfide (DMTS).[105] Identifications were based on matching LRI values on ZB-5, DB-Wax, and porous-layer open tubular columns and MS spectra in the case of DMDS and DMS. The DMDS is formed immediately after the guava leaf is crushed and becomes the predominant headspace volatile within 10 minutes and is an insect-toxic, defensive volatile produced only by wounded guava, and it is not found in citrus leaves. Consequently, DMDS was selected as the component most likely to be responsible for the repellent effect of guava against *Diaphorina citri*. Laboratory investigations subsequently proved that DMDS from guava was highly repellent toward adult *Diaphorina citri*. In Y-tube bioassays, DMDS- and guava-related compounds showed toxic effects to *Diaphorina citri*, resulting in knockdown of exposed adults.[117,118]

Laboratory Work with Botanical Volatiles

We examined the effect of a series of potential botanical repellents on *Diaphorina citri* in behavioral assays. Repellents were also evaluated in combination with citrus odors to verify their repellency in the presence of these attractive compounds. Finally, we examined the repellent effects of a select number of plant volatiles formulated in SPLAT.

Treatments tested included combinations of whole plants, leaves, and leaf extracts. Valencia orange, *Citrus sinensis* L., plants were grown in 3.78-L pots in a temperature-controlled greenhouse. Plant samples were composed of 10-week-old whole plants or 2-g samples of fresh leaves. DMDS (≥98% purity), DMTS (≥98.5% purity), and allyl methyl sulfide (AMS) (≥98% purity) were obtained from Sigma-Aldrich, St. Louis, Missouri. Allyl methyl disulfide (AMDS) (≥98% purity) and allyl disulfide (ADS) (~80.0% purity) were obtained from Frutarom Ltd., Billingham, United Kingdom, and Penta Chemical Company, United States.

A custom-designed two-port divided T-olfactometer (Analytical Research Systems, Gainesville, Florida) was used to evaluate behavioral responses. The olfactometer consisted of a 30 cm–long glass tube with 3.5 cm internal diameter that is bifurcated into two equal halves with a Teflon strip, forming a T-maze. Each half served as an arm of the olfactometer, enabling *Diaphorina citri* to choose between two potential odor fields. Its arms were connected to odor sources placed in guillotine volatile collection chambers or SPME chambers, through Teflon glass tube connectors. Its olfactometer was housed within a temperature-controlled room and positioned vertically under a fluorescent 900-lx lightbulb within a 1.0 × 0.6 × 0.6 m fiberboard box to achieve uniform light diffusion. This position took advantage of the negative geotactic and positive phototactic responses of *Diaphorina citri*. The olfactometer inlet adapter was covered with black cloth to facilitate insect movement toward odor sources. An odor source was randomly assigned to one of its arms at the beginning of each bioassay, and it was reversed after every 30 insects to eliminate positional bias.

A minimum of 120 adult female *Diaphorina citri* were examined per treatment combination (four replications with 30 psyllids per replication). *Diaphorina citri* females were released individually into the inlet adapter at the base of the olfactometer and given 300 seconds to show a behavioral response by entering either olfactometer arm. The number of adults entering the treatment arm or control arm or remaining in the inlet adapter (release port) or below the T-maze division was recorded. A treatment or control arm choice was recorded when an insect moved into either

olfactometer arm by crossing the division in the T-maze olfactometer. A release arm choice was recorded when an insect remained in the release port or below the T-maze division. All experiments were conducted at 26°C ± 1°C and 60% ± 2% relative humidity. The olfactometer and connecting tubes were thoroughly cleaned with 2% soap solution and baked at 93.3°C between treatment runs.

Behavioral bioassays with synthetic compounds. Allyl methyl trisulfide, diallyl trisulfide, DMTS, ADS, AMDS, DMDS, diallyl sulfide, and AMS were evaluated for their effect on *Diaphorina citri* behavior at 0.25%, 0.5%, and 1.0% concentrations, both individually and in combination with citrus leaves. The chemical samples were dissolved in 1 mL ethylene glycol (EG), to slow the release rate of the volatile sulfur compounds during the bioassays,[117,118] and pipetted onto a 5-cm Richmond cotton wick (Petty John Packaging Inc., Concord, North Carolina). The treated wick was then wrapped in laboratory tissue and placed in SPME chambers. The control treatment contained cotton wicks impregnated with 1 mL EG only. For evaluations of chemicals in the presence of citrus odors, approximately 2 g of fresh citrus leaves were placed in both chemical treatment and control arms of the olfactometer.

For assays in which putative repellent treatments were presented in the T-maze olfactometer with or without citrus and versus clean air, the number of *Diaphorina citri* remaining at the release point and not entering the olfactometer was compared between treatments using one-way analysis of variance, followed by Tukey's honest significant difference test ($p < .05$). For psyllids leaving the release arm, the number of psyllids choosing the control arm versus the treatment arm was compared with chi-square (χ^2) analysis at $p < .05$. The data from all four replicates were combined for the χ^2 analysis.

Significantly more *Diaphorina citri* remained at the release point in treatments where DMTS (at 0.25% concentration) was copresented with clean air or citrus odors than when clean air and citrus were presented simultaneously in both arms of the olfactometer (Figure 16.8a). No other synthetic sulfur chemical yielded significant differences from the control treatment (clean air) at the 0.25% concentration with respect to the number of *Diaphorina citri* leaving the release point. Significantly more psyllids entered the control arm than the treatment arm when 0.25% DMTS was compared with clean air (Figure 16.8a), indicating that DMTS exerts a high level of repellency on *Diaphorina citri* females.

Significantly more *Diaphorina citri* remained at the release point in treatments where DMTS, DMDS, AMDS, or ADS (all at 0.50%) was copresented with clean air or citrus odors compared to clean air alone, or citrus odor was simultaneously presented in both arms of the olfactometer (Figure 16.8b). The percentages of psyllids not moving from the release point for DMTS versus citrus and DMTS versus clean air ranged between 79% and 89%, respectively, whereas the percentages of psyllids not moving from the point of release for disulfides (DMDS, AMDS, and ADS) ranged between 41% and 60% (Figure 16.8b). Significantly more *Diaphorina citri* chose the arm with clean air compared to DMDS or AMDS and with citrus odors compared to DMDS or AMDS. The percentages of psyllids moving from the release point to DMTS versus clean air and DMTS versus citrus were 11% and 21%, respectively.

Significantly more *Diaphorina citri* remained at the release point in treatments where DMTS, DMDS, AMDS, or ADS (all at 1.0%) was copresented with clean air or citrus odors than when clean air alone or citrus was simultaneously presented in both arms of the olfactometer (Figure 16.8c). The percentages of *Diaphorina citri* not moving from the release point for DMTS versus citrus and DMTS versus clean air ranged from 84% to 92%. The percentages of psyllids not moving from the release point in treatments with disulfides (DMDS, AMDS, and ADS) ranged from 62% to 82%. Percentages of *Diaphorina citri* not moving from the release point were statistically equivalent for trisulfides and disulfides (Figure 16.8c). There were no differences between the number of *Diaphorina citri* entering the olfactometer arm containing AMS versus clean air. Significantly more psyllids remained in the release arm when the 0.25% blend of DMTS + DMDS versus clean air was

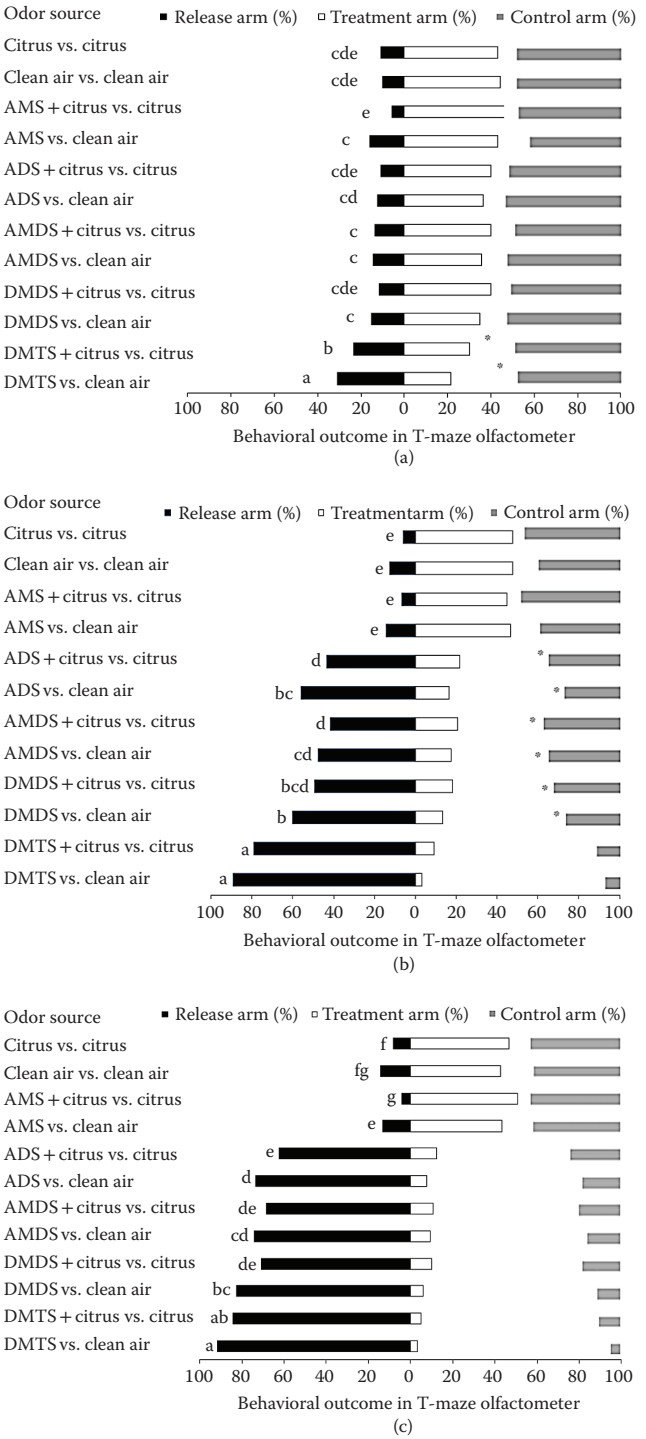

Figure 16.8 Responses of female adult *Diaphorina citri* presented with sulfur volatiles at (a) 0.25%, (b) 0.5%, or (c) 1.0% concentrations with or without citrus odors. Allyl methyl sulfide (AMS), allyl disulfide (ADS), allyl methyl disulfide (AMDS), dimethyl disulfide (DMDS), and dimethyl trisulfide (DMTS). Black bars followed by same letters are not significantly different (Tukey's honest significant difference, $p < .05$).

presented than when DMTS alone (0.25%) versus clean air was presented. It is possible, therefore, that blends, instead of single components, could provide optimal repellency to *Diaphorina citri*.

SPLAT ACP Repel

Fieldwork

Field tests of SPLAT formulations releasing DMDS were conducted in Florida in 20-tree blocks of mature sweet oranges, *Citrus sinensis* var. "Valencia," with four replicate blocks per treatment (SPLAT and control). Trees were 12 years old, planted with 3 × 6 m spacing, and averaged 4 m in height. Yellow sticky card traps were used to assess population densities of *Diaphorina citri*. SPLAT ACP Repel was applied at a rate of 6.2 kg/ha, that is, approximately 30 g of SPLAT per tree (six 5-g dollops per tree). This test proved that *Diaphorina citri* population densities can be significantly reduced by the application of DMDS to small plots of citrus (Figure 16.9). A follow-up field trial was performed with SPLAT ACP Repel, containing varying concentrations of the DMDS, and several different additives slowed the release rate of the active ingredient. This resulted in a formulation of SPLAT ACP Repel that provides 5 weeks of repellency toward *Diaphorina citri* in field conditions (Figure 16.10).

Diaphorina citri are known to recolonize citrus groves 1–2 weeks after an application of insecticide, because insecticide residues are relatively short-lived, and *Diaphorina citri* adults are highly mobile.[119,120] This creates the need for a high frequency of insecticide sprays. Some citrus growers in Florida and Brazil have been applying insecticides every 3–4 weeks during the field season to manage *Diaphorina citri*. To evaluate whether the application of SPLAT ACP Repel could help growers reduce the frequency of insecticide sprays, we designed the following experiment to determine whether *Diaphorina citri* recolonization in an area previously treated with insecticide was slowed by the presence of SPLAT ACP Repel compared to those areas receiving insecticide sprays alone.

Using a simple, randomized block design with two treatments, (1) insecticide followed by SPLAT ACP Repel and (2) insecticide only, all treatment plots received a label rate (40.0 oz./ha) application of the pyrethroid insecticide Danitol (Valent USA, Walnut Creek, California) at the start of the experiment to knock down *Diaphorina citri* population. After 2 weeks, half of the

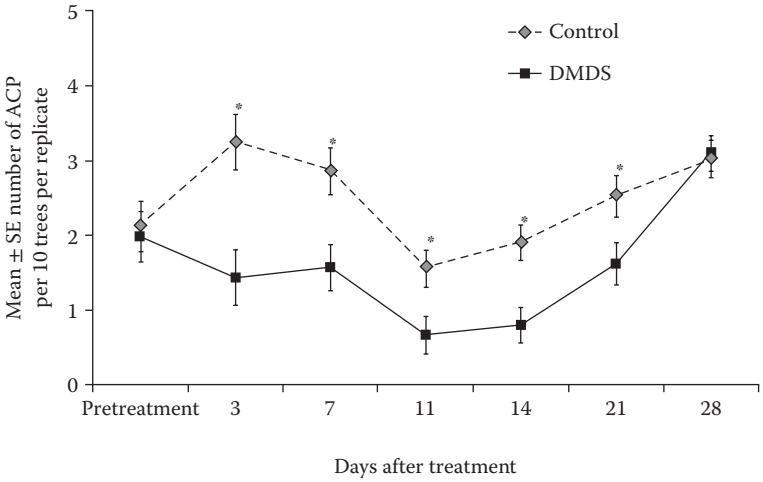

Figure 16.9 Reduction in *Diaphorina citri* populations following treatment with SPLAT containing dimethyl disulfide (DMDS). Results suggest that application of DMDS to an infested orchard can cause existing *Diaphorina citri* populations to disperse away from treated trees.

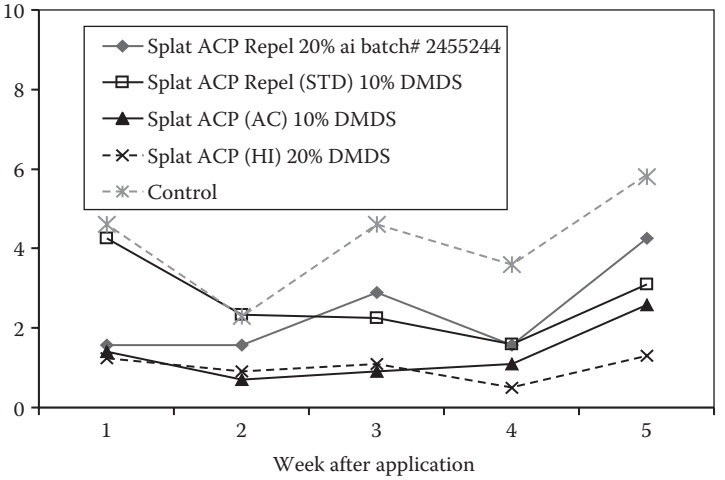

Figure 16.10 Optimization of SPLAT ACP Repel formulations in field testing. SPLAT was formulated with varying concentrations of dimethyl disulfide (DMDS) and with different additives to determine the ideal combination for maximum field longevity.

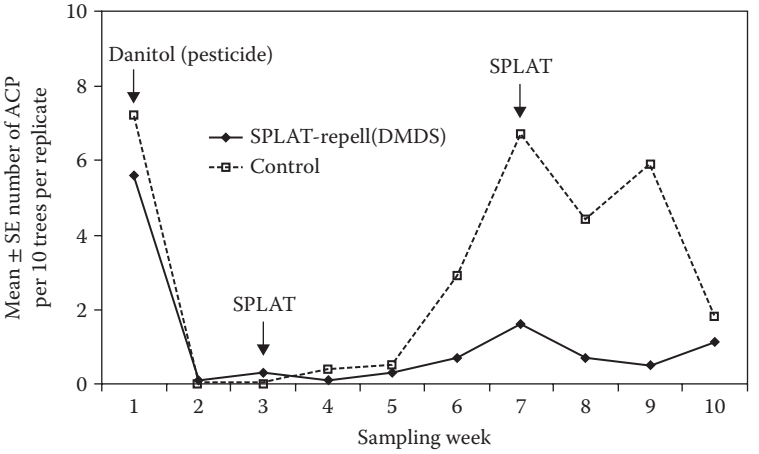

Figure 16.11 Delayed immigration of *Diaphorina citri* into insecticide-treated plots following application of SPLAT with dimethyl disulfide (DMDS). In untreated plots, populations of *Diaphorina citri* began to rebound 6 weeks after treatment, with populations becoming equivalent to pretreatment levels by week 7. In plots that received the SPLAT treatment 2 weeks after the insecticide spray, *Diaphorina citri* populations did not return to pretreatment levels for the entire 10 weeks of study 8.

plots were treated with SPLAT ACP Repel and the remaining half were left untreated. In untreated plots, populations of *Diaphorina citri* began to rebound 6 weeks after treatment, with populations returning to pretreatment levels by week 7. However, in those plots that received the SPLAT ACP Repel treatment 2 weeks after the insecticide spray, *Diaphorina citri* populations did not return to pretreatment levels for the entire 10 weeks of study (Figure 16.11). These data indicate that recolonization by *Diaphorina citri* was impeded by SPLAT ACP Repel and suggest that integration of this product into a *Diaphorina citri* management program has the potential to reduce the number of insecticide sprays needed while suppressing the *Diaphorina citri* population below critical thresholds.

RESULTS

This work indicates that the guava-derived semiochemical, DMDS, is an effective repellent for *Diaphorina citri* at concentrations as low as 10% in SPLAT ACP Repel. When incorporated into SPLAT, the resulting matrix demonstrated the capacity to extend the release period of this extremely volatile compound, prolonging the repellent's efficacy in the field. Field trials have shown that SPLAT ACP Repel with DMDS alone significantly reduces *Diaphorina citri* captures in infested orchards, indicating the potential for this formulation to reduce HLB infection rates in areas treated with SPLAT ACP Repel.

CONCLUSION

SPLAT formulations have been developed to release a variety of compounds, including sex pheromones, kairomones, attractants, phagostimulants, and insecticides. Several mating disruption and attract-and-kill formulations are commercially available. SPLAT Verb and SPLAT ACP Repel represent our first successful attempts at developing repellent formulations and will be registered and marketed in the United States.

Although we have demonstrated that SPLAT Verb is effective for protecting *Pinus contorta* from *Dendroctonus ponderosae*, we surmise this formulation will be useful for protecting other hosts of *Dendroctonus ponderosae* from successful colonization. To that end, additional studies are ongoing. In the future, we plan to evaluate SPLAT Verb against other bark beetles whose primary antiaggregation pheromone is verbenone, such as *Dendroctonus brevicomis* and the southern pine beetle *Dendroctonus frontalis* Zimmermann. Research also continues with SPLAT ACP Repel, specifically integrating it into pest management programs for the management of *Diaphorina citri* in commercial citrus production.

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