ANTIFEEDANT EFFECT OF COMMERCIAL CHEMICALS AND PLANT EXTRACTS
AGAINST *Schistocerca americana* (ORTHOPTERA: ACRIDIDAE) AND *Diaprepes*
abbreviatus (COLEOPTERA: CURCULIONIDAE)

By

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To my family, the source of my strength

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Abstract of Thesis Presented to the Graduate School
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I investigated the deterrent effect of seven botanical and three inorganic agricultural products against nymphs of the American bird grasshopper, *Schistocerca americana*, and adults of the sugarcane rootstock weevil, *Diaprepes abbreviatus*. Methanol and methylene chloride extracts of the Florida rosemary, *Ceratiola ericoides*, yellow star anise, *Illicium parviflorum*, and scratchthroat, *Ardisia crenata*, were also tested as potential feeding deterrents. Antifeedant activity was assayed using a leaf disk bioassay, in choice and no-choice tests. The residual activity of the agricultural products that showed a significant antifeedant activity in leaf disk bioassays was assayed by applying them in a no-choice test to foliage of *Citrus paradisi* plants exposed to three time intervals of sunlight. Sabadilla, azadirachtin and ryanodine effectively deterred *S. americana* whereas rotenone, sabadilla and ryanodine reduced the feeding activity of *D. abbreviatus* in choice and no-choice leaf disk bioassays. Rapid loss of effectiveness was observed under field conditions. Sabadilla was the only compound that maintained its antifeedant properties in the field, but only against *S. americana*. Methanol and methylene chloride extracts of *C. ericoides* deterred *D. abbreviatus* but only methylene chloride extract dissuaded *S. americana*. Methanol extract of *A. crenata* functioned as a feeding deterrent against both *S.*

americana and *D. abbreviatus*, whose was also deterred by methylene chloride extract of *A. crenata*. Extracts of *I. parvifolium* only dissuaded the insects in choice bioassays. Based on their deterrency, some of the agricultural botanical products and plant extracts have potential for use as substitute crop protectants against these two species.

CHAPTER 1 INTRODUCTION

Conceptual Framework

Feeding deterrents, or antifeedants, are chemical compounds that prevent or suspend the feeding behavior of an insect when they are detected (Schoonhoven 1982). They can be found amongst the major classes of secondary metabolites: alkaloids, terpenoids and phenolics (Koul 2008), but other organic and inorganic compounds can inhibit also food uptake by insects (Glen et al. 1999, Wei et al. 2000). Insects are able to detect antifeedants through contact chemoreceptors, characterized by the presence of a small number of bipolar sensory neurons (three to 10) within short hairs, spines or bristles (*sensilla trichoidea* or *sensilla chaetica*), pegs (*sensilla basiconica*), or cones (*sensilla styloconica*), with a single terminal pore. Sensory (gustatory) neurons can be phagostimulatory or deterrent cells. Activity of these neurons in response to appropriate stimuli would enhance or reduce feeding, respectively (Chapman 2003, Koul 2008). Feeding deterrents may be perceived by insects due to several chemoreception mechanisms (Schoonhoven 1982, Chapman et al. 1991):

1. Stimulation of specialized deterrent receptors
2. Distortion of the normal function of neurons that perceive phagostimulatory compounds.
3. Excitation of broad spectrum receptors.
4. Changes in complex and subtle sensory codes as a consequence of stimulation activity in some nerve cells and inhibition in others.
5. Production of a highly abnormal impulse pattern, often at high frequencies, and/or bursting activities.

Some antifeedants influence insect feeding activity through a combination of these modes of action (Schoonhoven 1982, Chapman 2003, Koul 2008).

Reduction or complete inhibition of feeding, using organic derivatives, crude plant extracts and/or pure allelochemicals as antifeedants, has been demonstrated in several orders such as Lepidoptera, Coleoptera, Hemiptera and Orthoptera.

Regarding Lepidoptera, Akhtar and Isman (2004) observed antifeedant effects of crude extracts of *Melia volkensii* (Meliaceae) on third instar larvae of the cabbage looper, *Trichoplusia ni* Hübner (Noctuidae), and the armyworm, *Pseudaletia unipuncta* Haworth (Noctuidae). Methanol, acetone and chloroform extracts of *Justicia adhatoda* L. (Acanthaceae), *Ageratum conyzoides* L. (Asteraceae) and *Plumbago zeylanica* L. (Plumbaginaceae), respectively, were strongly deterrent to feeding of *Spilarctia obliqua* Walker (Noctuidae) at a dose of 10 mg/ml (Prajapati et al. 2003). Ulrichs and collaborators (2008) detected antifeedant and toxic effects of *Porteresia coarctata* Takeoka (Poaceae) hexane extracts on *Spodoptera litura* F. (Noctuidae) third instar larvae due to the presence of terpenoids in the plant. Meliaceae and Labiatae also produce terpenes that modify the feeding behavior of insects: a mixture of limonoids, obtained from seeds of *Trichilia havanensis* Jacq. (Meliaceae), and the diterpene scutecyprol A isolated from *Scutellaria valdiviana* (Clos) Epling (Labiatae), reduced the feeding activity of *Spodoptera exigua* (Hübner) (Noctuidae) in choice and no choice bioassays (Caballero et al. 2008). Azadirachtin, a triterpenoid obtained from the neem tree, *Azadirachta indica* A. Juss (Meliaceae), reduced food consumption of *Heliothis virescens* (F.) (Noctuidae) and *Lymantria dispar* L. (Limantriidae) larvae (Yoshida and Toscano 1994, Kostic et al. 2008).

Concerning Coleoptera, extracts of *Humulus lupulus* L. (Cannabaceae) and *Xanthium strumarium* L. (Asteraceae) at a concentration of 20 g kg⁻¹, inhibited the feeding behavior of Colorado potato beetle larvae, *Leptinotarsa decemlineata* Say (Gökçe et al. 2006), which was also deterred by ichangensin, a terpenoid (limonoid aglycone) present in the family Rutaceae

(Murray et al. 1999). Terpenes isolated from *Lansium domesticum* Corr. Serr. (Meliaceae), as well as crude extracts of the plant, showed antifeedant activity against the rice weevil *Sitophilus oryzae* L. (Curculionidae) (Omar et al. 2007). Methanol extracts of *Momordica charantia* L. (Cucurbitaceae) strongly deterred cucurbitaceous feeding beetle species of the genus *Aulacophora* and *Epilachna* (Abe and Matsuda 2000). Villani and Gould (1985), after screening extracts from 78 plant species, discovered that *Asclepias tuberosa* L. (Asclepiadaceae) and *Hedera helix* L. (Araliaceae) provided high levels of feeding deterrence against the corn wireworm, *Melanotus communis* (Gyllenhal) (Elateridae).

Among the Hemiptera, the milkweed bug, *Oncopeltus fasciatus* Dallas (Lygaeidae), was deterred from feeding by extracts of dried roots of *Inula helenium* L. (Asteraceae) when applied to sunflower kernels (Alexenizer and Dorn 2007). Reuter and colleagues (1993), using an electronic feeding monitor, observed a reduction in the time spent in ingestion by the green peach aphid, *Myzus persicae* (Sulzer) (Aphididae), when it was feeding on lettuce plants treated with azadirachtin and an acrylic copolymer.

Within the Orthoptera, the graminivorous pest *Locusta migratoria* L. (Acrididae) is deterred by crude methanol extracts of *Cryptomeria japonica* (L. f.) D. Don (Taxodiaceae) (Kashiwagi et al. 2007), and also by azadirachtin, nicotine, coumarin and a mixture of phenolic compounds obtained from *Sorghum bicolor* (L.) Moench (Poaceae) (Adams and Bernays 1978). Gramine, 3-(dimethylaminomethyl)-indole, the principal alkaloid in barley, *Hordeum vulgare* L. (Poaceae), inhibits as well the feeding behavior of *L. migratoria* (Ishikawa and Kanke 2000). Rathinasabapathi et al. (2007) observed that arsenic (As) present in the Chinese brake fern, *Pteris vittata* L. (Pteridaceae), reduced frond tissue consumption by nymphs of the American bird grasshopper, *Schistocerca americana* Drury (Acrididae).

Feeding deterrents can protect plants from insect herbivory by making the hosts less palatable or even toxic to the arthropods. Therefore, antifeedants are a useful element for integrated pest management programs that involve behavioral manipulation of insect pests, like push-pull strategies where a protected plant resource is made unattractive or inappropriate to the pests (push) while drawing them into an attractive source (pull) from where the pests are subsequently removed (Jain and Tripathi 1993, Cook et al. 2007). Furthermore, antifeedants are usually safer alternative crop protectants than conventional synthetic pesticides due to their low toxicity, specificity, and protection to non-target organisms (insects, birds or mammals) that are indispensable for interactions like natural biological control and pollination (Isman 2006). Moreover, antifeedants are advantageous due to their effectiveness at small concentrations and quick degradation. This can result in lower non-target hazard and reduced pollution problems relative to conventional pesticides. Typical problems associated with conventional insecticides include ground water contamination, the development of pesticide resistant pest populations, and acute and chronic intoxication of applicators, farm workers and consumers (Isman 2006, Koul 2008, Rosell et al. 2008).

One of the weak points of investigations into the occurrence of antifeedant substances is that in most cases only one test insect species is used. In most papers the structure of the compound(s) found to be active in laboratory tests on one or a few insect species is reported, but no further attempts are made to find out against what other species the substance is active, or how stable the compound is under natural conditions (Jermy 1990).

The purpose of the present study was to test the antifeedant effect of the botanical derivatives azadirachtin, neem oil, sabadilla, rotenone, ryanodine, capsaicin and garlic juice, as well as inorganic substances, such as diatomaceous earth, elemental sulfur and kaolin clay. Tests

were conducted under laboratory and field conditions, using behavioral bioassays, against nymphs of the American bird grasshopper, *Schistocerca americana* (Drury) (Orthoptera: Acrididae), and adults of the West Indian sugarcane rootstock borer weevil, *Diaprepes abbreviatus* L. (Coleoptera: Curculionidae), two generalist herbivores. In addition, crude extracts of the Florida rosemary, *Ceratiola ericoides* Michx (Empetraceae), yellow star anise, *Illicium parviflorum* Michaux ex Ventenat (Illiciaceae), and scratchthroat, *Ardisia crenata* Sims (Myrsinaceae), were tested as potential antifeedants for both insect species.

Pest Insects

The American bird grasshopper, *Schistocerca americana* (Drury), is one of the most destructive grasshopper species in the southeastern United States. It is found throughout the eastern United States west to the Rocky Mountains. This grasshopper's range also includes the Bahamas and Mexico (Thomas 1991). It occasionally reaches outbreak status, at least locally, and can cause injury to corn, oats, rye, peanuts, sugarcane, tobacco, cotton, vegetables, flowers, ornamental shrubs and citrus. The American grasshopper caused, during 1991, significant loss to citrus and ornamental plants in west-central Florida and limited damage to field crops and ornamentals in north-central Florida (Capinera 1993a). Young citrus trees are damaged by the grasshopper's gnawing on the leaves and most of the feeding damage is caused by the third, fourth and fifth instars, which have a much larger appetite than the adults. Although a highly polyphagous acridid, *S. americana* does exhibit a clear preference (e.g., smooth crabgrass, bahiagrass, hyssop spurge, soybean, corn, pecan) and non-preference (e. g., cucumber, nandina, pepper) for certain host species (Capinera 1993a, Smith and Capinera 2005). The American grasshopper has two generations per year and overwinters in the adult stage; therefore, it is present throughout the year. Females lay their eggs in the soil, about 2 or 3 cm below the surface, and are able to deposit up to three egg clusters (each cluster pod usually consist of 60 to 80

eggs). The groups of eggs are held together by proteinaceous foam which serves to cement the surrounding soil particles together to form a more or less discrete capsule, or “pod”. Nymphs hatch from the eggs after three or four weeks and undergo between five and six molts before reaching adulthood (Thomas 1991, Capinera 1993b).

The West Indian sugarcane rootstock borer weevil, *Diaprepes abbreviatus* L., is native to the Lesser Antilles of the Caribbean region and has become an important long-term pest of citrus and ornamental crops in Florida (Hall 1995). Since its introduction to Florida in 1964, *D. abbreviatus* has spread into approximately 23,000 ha of commercial citrus in 17 counties and 94 commercial citrus and ornamental plant nurseries (Woodruff 1968, Hall 1995). It is a highly polyphagous species; Simpson et al. (1996) listed numerous host plants associated with this pest, including 270 species in 157 genera from about 59 families. Adult weevils feed on plant foliage, leaving characteristic semicircular notches along the edges of young leaves, and oviposit on preferred host plants. Females deposit clusters of eggs between leaves that are glued together by secretion of a sticky substance that cements the leaf surfaces. Each cluster contains around 50 eggs, and female weevils are able to deposit as many as 5,000 to 29,000 eggs during their lifespan (three to four months). As the eggs hatch, after seven to 10 days, the larvae fall to the soil surface where they burrow into the soil to feed on the roots of the plant where they remain for 8-12 months, until adult emergence (Woodruff 1968, Hall 1995, Mannion et al. 2003).

Commercial Chemicals Tested

Neem oil and azadirachtin are biopesticides obtained from the Indian neem tree, *Azadirachta indica* A. Juss (Meliaceae). Neem oil is acquired by cold-pressing the seeds of the neem tree and is useful in the control of soft-bodied insects (Bruce et al. 2004), mites and phytopathogens due to the presence of disulfides that contribute to its bioactivity. Azadirachtin, a limonoid or tetranortriterpenoid, is found in the extracts of the seed residues after removal of the

oil (Isman 2006). Several effects, both physiological and behavioral, have been reported on insects (Schmutterer 1990): disruption of growth, development and reproduction by blocking the synthesis and release of molting hormones, and repellency and oviposition deterrence.

Azadirachtin and neem seed extracts also seem to have a potent antifeedant effect on a large number of pest insects, including orthopterans (Aerts and Mordue 1997, Capinera and Froeba 2007), hemipterans (Kumar and Poehling 2007), coleopterans (Baumler and Potter 2007), and lepidopterans (Aerts and Mordue 1997, Yoshida and Toscano 1994, Liang et al. 2003, Showler et al. 2004, Charlston et al. 2005).

Sabadilla is a botanical insecticide obtained from the seeds of *Schoenocaulon officinale* Grey (Liliaceae). The major active ingredients are cevadine and veratradine, which are esters of a steroidal alkaloid named veracevine (Rosell et al. 2008). These alkaloids bind the activation gate of the sodium channels in the nervous system of insects, resulting in persistent neuroexcitation. Although sabadilla has a high mammalian toxicity, commercial preparations normally contain less than 1% active ingredient, providing a margin of safety (Isman 2006). Sabadilla is used mostly for control of thrips populations in citrus and avocado (Hare and Morse 1997, Humeres and Morse 2006) but it has potential as an antifungal agent (Oros and Ujvary 1999) and as a feeding deterrent as well (Yoshida and Toscano 1994).

Rotenone is one of several isoflavonoid compounds produced in the roots of the tropical legumes *Derris*, *Lonchocarpus* and *Tephrosia* (Leguminosae). Rotenone is a mitochondrial poison that blocks the electron transport chain and prevents energy production. It has been used as an insecticide, acaricide and piscicide and is commonly sold as a dust containing 1% to 5% active ingredients for home and garden use, though liquid formulations used in organic agriculture can contain between 8% and 15% (Isman 2006, Rosell et al. 2008). Rotenone can act

as a feeding deterrent against stored product insect pests (Nawrot et al. 1989) and polyphagous noctuid species (Wheeler et al. 2001).

Ryanodine is an alkaloid obtained by grinding the wood of the Caribbean shrub *Ryania speciosa* Vahl (Flacourtiaceae) (Isman 2006). This botanical pesticide is active on the muscular system, specifically neuro-muscular calcium channels. Ryanodine acts as an agonist by enhancing calcium output of the sarcosome tubule network that surrounds muscle fibers, resulting in continual calcium availability and a continual state of muscle contraction (Nauen 2006). Ryanodine and related ryanoids deter feeding by lepidopteran and coleopteran pests (Yoshida and Toscano 1994, Gonzalez-Coloma et al. 1999).

Capsaicin, extracted from the hot cayenne pepper, *Capsicum annuum* L. (Solanaceae), is a derivative of vanillyl amine (8-methyl-*N*-vanillyl-6-noneamide), a compound that produces the “hotness” in some species of the plant genus *Capsicum*. Capsaicin is currently registered by the US Environmental Protection Agency (EPA) for use as an insect repellent and toxicant as well as a vertebrate repellent for dogs, birds, voles (*Microtus* spp), deer (*Odocoileus* spp), rabbits (*Sylvilagus* spp.) and squirrels (*Sciurus* spp.) (EPA 1996). Capsaicin has been tested as a leaf protector against scarab pests, including the rose chafer, *Macrodactylus subspinosus* (F.) (Isaacs et al. 2004) and the Japanese beetle, *Popillia japonica* Newman (Baumler and Potter 2007) but without consistent results.

Garlic extracts derived from *Allium sativum* L. (Liliaceae) have shown insecticidal activity to dipteran pests (Prowse et al. 2006) as well as antifeedant effects on coleopteran stored-products pests (Chiam et al. 1999) and moths (Gurusubramanian and Krishna 1996).

Diatomaceous earth is composed of the fossilized skeletons of various species of marine and fresh water phytoplankton, predominantly diatoms and other siliceous algae which existed

during the Eocene and Miocene periods. The diatomaceous earth active ingredient is amorphous silicon dioxide (silica), which can damage the insect's epicuticular lipids by hydrocarbon absorption and abrasion, making the cuticle permeable and therefore causing death due to water loss and desiccation (Korunic 1997). Diatomaceous earth has proved to be useful in the control of stored-products insects such as *Cryptolestes ferrugineus* Stephens (Laemophloeidae) (Fields and Korunic 2000), *Rhyzopertha dominica* F. (Bostrichidae) (Ferizli and Beris 2005) and the confused flour beetle, *Tribolium confusum* Jacquelin du Val (Tenebrionidae) (Dowdy and Fields 2002, Vayias et al. 2006).

Sulfur is a non-systemic contact and protectant fungicide with secondary acaricidal activity. It is used primarily to control powdery mildews, certain rusts, leaf blights and fruit rots. Spider mites, psyllids and thrips are also vulnerable to sulfur. This chemical is known to be of low toxicity and poses little if any risk to human and animal health (Lamberth 2004).

Kaolin is a soft, white, clay mineral that can be mixed with water and sprayed on plants in order to form a protective particle film. Kaolin is a nonabrasive aluminosilicate ($\text{Al}_4\text{Si}_4\text{O}_{10}(\text{OH})_8$), that can reduce feeding and oviposition of arthropods by entangling mouthparts and restraining mobility over treated plant surfaces (Glenn et al. 1999). Furthermore, kaolin may interfere with insect's contact chemoreceptors, causing plants to be unrecognizable as a host (Puterka et al. 2000). Spraying kaolin on crops has been effective against pests belonging to different orders including Hemiptera (Glenn et al. 1999, Puterka et al. 2000, Cottrell et al. 2002, Daniel et al. 2005), Coleoptera (Lapointe 2000) and Lepidoptera (Knight et al. 2000).

Plant Species Used To Obtain The Extracts

The Florida rosemary, *Ceratiola ericoides* Michx, is an indigenous plant restricted to the Florida scrub community, growing on excessively to well-drained sandy soils. This plant is a dioecious perennial evergreen shrub which can grow as tall as 2 m in height. *Ceratiola ericoides*

has a whorled branching pattern, with leaves strongly revolute (needle-like) of 8-12 mm (Wunderlin and Hansen 2002). The absence of herbaceous growth around *C. ericoides* plants demonstrates the allelopathic effects that this species exerts on others. Examples include suppressing germination of *Eryngium cuneifolium* Small (Apiaceae) and *Hypericum cumulicola* (Small) P. Adams (Hypericaceae) by leaf and litter leachates (Hunter and Menges 2002), and affecting radicle growth and germination of sandhill grasses such as little bluestem, *Schizachyrium scoparium* (Michx.) Nash (Poaceae) and green sprangletop, *Leptochloa dubia* (Kunth) Ness (Poaceae), (Fischer et al. 1994). Several chemicals have been isolated from the Florida rosemary including the dihydrochalcone flavonoid ceratiolin (Tanrisever et al. 1987, Tak et al. 1993) which seems to be the precursor of the photochemically activated hydrocinnamic acid, a germination and growth inhibitor of grasses and pines (Fischer et al. 1994).

Illicium parviflorum Michaux ex Ventenat, commonly known as swamp star anise, or yellow star anise, is an indigenous species of moist forests and swamps of central Florida. It is a broadleaf evergreen large shrub or small tree with highly aromatic anise-scented foliage that can grow up to 15 feet in height (Osorio 2001). *Illicium parviflorum* is used in landscapes because of its considerable drought tolerance, capacity to grow under a broad range of light conditions, and resistance to pests. Sharma and Rich (2005) assessed reproduction of three root-knot nematode species (*Meloidogyne arenaria*, *M. incognita* and *M. javanica*) on five native plants and three non-native plants to the southeastern USA and observed very few or no galls on roots of *I. parvifolium*. Secondary metabolites such as sesquiterpene lactones (Schmidt 1999) have been isolated from leaves of *I. parviflorum*, as well as safrole ($68.14 \pm 0.88\%$), linalool ($13.18 \pm 1.01\%$) and methyl eugenol ($11.89 \pm 0.87\%$), the main components of the essential oil of yellow anise tree (Tucker and Maciarello 1999).

Ardisia crenata Sims, or scratchthroat, is an evergreen small shrub (0.5-1 m in height) native to Japan to north India that grows in multi-stemmed clumps. It has alternate leaves (dark green above, waxy, glabrous with crenate margins) of 21 mm (Wunderlin and Hansen 2002). *Ardisia crenata* was introduced to the USA for ornamental purposes and has become established in much of northern and central Florida. In some areas, it is a serious pest, displacing native species in the understory of hardwood forests by creating dense local shade (Kitajima et al. 2006). Several studies have revealed the presence, in the genus *Ardisia*, of phytochemicals such as triterpenoid saponins, isocoumarins, quinones and alkylphenols (Kobayashi and de Mejia 2004, Liu et al 2007) that exhibit a wide range of bioactivities such as uterus contraction, inhibition of cyclic adenosine monophosphate phosphodiesterase, cytotoxicity, anti-HIV and anti-cancer among others (Kobayashi and de Mejia 2004). The presence of these secondary metabolites may cause *A. crenata* to be an unsuitable host for arthropod feeders. For example, Neal and colleagues (1998) observed a reduction in the number of eggs laid by the twospotted spider mite, *Tetranychus urticae* Koch, as well as a higher nymphal mortality of the whiteflies *Bemisia argentifolii* Bellows & Perring and *Trialeurodes vaporariorum* (Westwood) when developing on *A. crenata* leaves, compared with other three host plants of the same genus.

Objectives

- To test the antifeedant properties of azadirachtin, neem oil, sabadilla, rotenone, ryanodine, capsaicin, garlic juice, diatomaceous earth, elemental sulfur and kaolin clay against fifth instar nymphs of *S. americana* and adults of *D. abbreviatus* using behavioral bioassays.
- To test the residual activity under field conditions of the commercial formulations that are the most deterrent to the feeding of the two species of insects in the laboratory.
- To obtain crude extracts from *C. ericoides*, *I. parviflorum*, and *A. crenata* plants, and to test their antifeedant properties against nymphs of *S. americana* and adults of *D. abbreviatus* using behavioral bioassays.

CHAPTER 2 MATERIALS AND METHODS

Insect Material

The American grasshoppers used in this study were from a laboratory colony that has been maintained for approximately 10 years in the Entomology and Nematology Department at the University of Florida. The insects had free access to water and a dry diet consisting of whole wheat flour (one part), soy flour (one part) and wheat bran (two parts), supplemented with romaine lettuce, *Lactuca sativa* var. *longifolia* Lam, (Asteraceae). The nymphs and adults were maintained at 27°C, although they had access to 90 W light bulbs, approximately 10 cm away from the cages, so they could attain a warmer temperature if desired. They also were provided with a photoperiod of 16:8 (L:D) and a relative humidity of about 58%.

Adult sugarcane rootstock borer weevils were from a rearing facility of the Florida Department of Agriculture & Consumer Services, Division of Plant Industry (DPI), at Gainesville, Florida, where they had been maintained for 5 years. The insects were maintained in plastic cages of 30 x 30 x 30 cm, 60 weevils per cage, with free access to water and a diet consisting of romaine lettuce (*Lactuca sativa* var. *longifolia* Lam) and store-bought carrot roots [*Daucus carota* L. (Apiaceae)]. The adult weevils were maintained at 28°C and a relative humidity of about 58%.

Chemicals Tested

Ten bioinsecticides and putative antifeedants/repellents were evaluated for deterrence to *S. americana*, and *D. abbreviatus* (Table 1). They included seven botanical derivatives (azadirachtin, neem oil, sabadilla, rotenone, ryanodine, capsaicin and garlic juice) and three inorganic materials (diatomaceous earth, elemental sulfur and kaolin clay). Products were applied at label rates, using the highest concentration commercially recommended.

Plant Extracts Tested

The plant extracts were prepared based on the procedures described by Gökçe and collaborators (2005). Samples of Florida rosemary, *C. ericoides*, were collected in the Ordaway-Swisher Biological Station (Putnam County, Florida), during fall of 2007 and 2008. Samples of yellow star anise tree, *I. parviflorum* and scratchthroat, and *A. crenata*, were obtained in the city of Gainesville (Alachua County, Florida) during fall of 2008. Leaf samples were dried at room temperature in the dark for 3 weeks and afterward ground in a Wiley mill (Model 3383-L10, Thomas Scientific, Swedesboro, NJ) using a mesh size of 40. The ground leaf material was stored in plastic containers at -80°C. Ten-gram samples of dried plants were placed into 125 ml Erlenmeyer flasks and treated with 100 ml of methanol (Fisher Scientific, Fair Lawn, NJ) or methylene chloride (Fisher Scientific, Fair Lawn, NJ). Flasks were covered with aluminum foil, placed on an orbital shaker (Model 361, Fisher Scientific, Pittsburgh, PA), and shaken (120 oscillations/min) for 24 h in the dark at 24°C. The suspension was filtered first through two layers of cheese cloth (XL-400, CottonTM, Worcester, MA) followed by vacuum filtration. The filtrate was transferred into a 250 ml evaporating flask and dried at 40°C in a rotary evaporator (RE 111 Büchi, Switzerland), in order to evaporate the excess methanol or methylene chloride. The resulting residues were weighed and mixed with acetone to yield a 20% (w/w) plant suspension.

Behavioral Bioassay

Antifeedant activity of test substances was assayed using a leaf disk bioassay, in both choice and no-choice formats. The bioassays were conducted in round transparent plastic containers of 18 cm diameter and 8 cm height. A moist paper towel (Prime Source) was placed at the bottom of each container in order to maintain high humidity and to keep the foliage fresh. The leaf disks were pinned on a cork base of 1.3 cm height.

Leafs were removed from healthy romaine lettuce plants purchased from a grocery store, and leaf disks were cut using a no. 15 cork borer (2 cm diameter, 3.14 cm² area). Foliage disks were cut immediately before application of treatment solutions, minimizing changes in leaf quality. After leaf disks were cut, they were immediately dipped into one of the treatments solutions for 10 sec. The disks were left to dry under a fume hood for 15 min at room temperature. Control disks were dipped into water and were left to dry at room temperature. In the case of the plant extracts, the control disks were dipped into acetone. A single fifth instar nymph of *S. americana*, 2-3 days after molting from fourth instar, was added to each container and allowed to feed for 24 h at 27°C. Nymphal instars were determined according to the methods of Capinera (1993b.). For *D. abbreviatus*, one 7-10 day-old adult was placed into each container and allowed to feed, also for 24 h at 27°C. The test insects were starved for 12 h prior to the experiments. For every chemical substance evaluated, in both choice and no-choice situations, twenty replicate containers were tested on three different days (n=20, N=60). On each day a different set of insects was assayed and a fresh preparation of each compound was tested.

In choice tests, one treated and one control disk were placed in each container. The distance between the two disks was 13 cm. In order to ensure that bioassays were not hunger-biased, experiments were stopped when grasshoppers or weevils had consumed 50% of either disk. The bioassays were checked periodically. If the insects had not consumed 50% of either disk after 24 h, the experiment was terminated. In no-choice tests, either two treated leaf disks or two untreated (control) disks were placed in each container. The average of the leaf area consumed in both disks was used for data analysis. No-choice tests were only stopped after 24 h. Control and treatment evaluations were done simultaneously. Only the biological pesticides that demonstrated a significant antifeedant activity in the choice tests were evaluated using no-choice

tests. The remaining area of the disks (treated and controls) was measured using a leaf area meter (LI-3000A, LI-COR, Lincoln, Nebraska, USA).

In order to determine the most effective insect antifeedant, multiple-choice bioassays that included three treatments in the same container were carried out for 24 h. The treatments chosen for comparison showed a significant reduction in the consumed leaf area in the control vs. treatment no-choice assays.

The consumed area of the leaf disks (treated and controls) in both the choice and the no-choice tests were used for the data analysis. This was obtained by subtracting the remaining area, calculated with the leaf area meter, from the total area of each leaf disk (3.14 cm²). Shapiro–Wilk normality tests and Levene tests of homogeneity of variances were employed to determine the type of distribution for the data obtained in every experiment. For choice tests, paired *t*-tests or sign tests, depending on data distribution, were used to test for significant differences in consumption levels of treatment and control disks (Horton 1995). In no-choice bioassays, *t*-tests for independent samples (parametric data) or Mann-Whitney *U* test (nonparametric) were used to evaluate the dissimilarities in area consumed in the control and treatment disks. Parametric one-way ANOVAs or Kruskal-Wallis nonparametric one-way ANOVAs were used to compare the leaf area consumed (control and treatment) between the three replicates (n=20, N=60) utilized to assess every treatment in choice and no-choice bioassays. A Friedman ANOVA was used to analyze the results of the multiple-choice bioassays, separating means with Mann-Whitney *U* test. *P* values <0.05 were considered to be statistically significant. STATISTICA 6.0 (Stat Soft, Inc., Tulsa, OK) software was used for the data analysis.

Field Trial

To test the residual activity under field conditions of the chemicals that showed a significant antifeedant activity against *S. americana* and *D. abbreviatus*, applications of the most

effective treatments were made to 5-year-old, randomly selected, *Citrus paradisi* MacFad (Rutaceae) potted plants. For each treatment, applications were made at 7 a.m, 11 a.m and 3 p.m. At every time interval, treatments were applied by a number 4 flat brush to the adaxial surface of five leaves (per treatment), on the same plant. The painted leaves were collected at 7 p.m. on the day of application in order to obtain 4 h (3 p.m), 8 h (11 a.m) and 12 h (7 a.m) of sunlight exposure. Also, five unpainted leaves were collected at 7 p.m. as controls. The selected leaves were undamaged and apparently of the same age, based on size, coloration and turgidity. From the painted leaves for each treatment, 10 leaf disks (2.25 cm, diameter, 3.97 cm² area) were cut and provided in pairs to individual fifth instar nymphs of *S. americana* or pairs of adults of *D. abbreviatus* in a no-choice format (five replicates per time application, per treatment). Control leaves were handled in the same way. After 24 h the average of the leaf area consumed in both disks was used for data analysis. The field experiment was repeated twice for a total of three trials, for both insect species, using a different citrus plant each time. Applications were made on November 17, 23 and 27 of 2008 for the *S. americana* trials, and on February 17, 21 and 23 of 2009 for the *D. abbreviatus* trials. There was no precipitation on any of the application dates. The temperature profile on the six dates is presented in the Table 3-2.

Factorial ANOVAs were used in order to analyze the individual and interactive effects of the hours of sunlight exposure and the three trials made per treatment, on leaf consumption. Means were separated with Tukey's test ($P=0.05$). STATISTICA 6.0 (Stat Soft, Inc., Tulsa, OK) software was used for the data analysis.

Table 2-1. Chemicals evaluated for antifeedant activity against *S. americana* nymphs and *D. abbreviatus* adults.

Active ingredient (ai)	Trade name	AI (% in product)	Application rate (ml or g/liter)	Product Source
Neem oil	Pure Neem Oil	100	8 ml	Dyna-Gro, San Pablo, CA
Azadirachtin	Azatrol EC	1.2	5.6 ml	PBI/Gordon, Kansas City, MO
Sabadilla alkaloids	Sabadilla Pest Control	8	30 g	Necessary Organics, New Castle, VA
Rotenone	Rotenone 5	5	48 g	Bonide Products, Yorkville, NY
Ryanodine	Ryan 50	0.10	18 g	Dunhill Chemical, Rosemead, CA
Capsaicin and other capsaicinoids	Hot Pepper Wax	0.00018	31.3 ml	Hot Pepper Wax, Greenville, PA
Garlic juice and oil	Garlic Guard	40	50 ml	Super-Natural Gardner, Exeter, NH
Diatomaceous earth	Mother Earth D	100	NA	Whitmire Micro-Gen, St. Louis, Mo
Elemental sulfur	Liquid Sulfur	52	19.5 ml	Bonide Products, Oriskany, NY
Kaolin clay	Surround WP	95.0	60 g	Extremely Green Gardening, Abington, MA

Table 2-2. Temperature (°C) profile of the days on which antifeedant's residual activity was tested.

Date	Max °C	Min °C	Average °C
11/17/08	18.33	0.00	9.44
11/23/08	20.00	0.00	10.00
11/27/08	21.67	1.11	11.67
02/17/09	19.44	0.56	10.00
02/21/09	19.44	-3.33	8.33
02/23/09	16.67	0.56	9.44

CHAPTER 3 RESULTS

Schistocerca americana

Behavioral Bioassays: Commercial Formulations

In choice tests (Figure 4-1), American grasshoppers consumed significantly more untreated (control) leaf disk material when presented simultaneously with leaf disks treated with either azadirachtin ($t = 12.6$; $df = 59$; $P < 0.001$), sabadilla ($z = 7.62$; $P < 0.001$), ryanodine ($t = 6.89$; $df = 59$; $P < 0.001$), neem oil ($t = 9.50$; $df = 59$; $P < 0.001$), capsaicin ($t = 2.50$; $df = 59$; $P = 0.02$), or kaolin clay ($t = 2.63$; $df = 59$; $P = 0.01$). There was not a significant difference in the leaf area consumed between the untreated and the disks treated with elemental sulfur ($z = 7.62$; $P = 0.52$), diatomaceous earth ($t = 1.33$; $df = 59$; $P = 0.19$), garlic juice ($z = 7.62$; $P = 0.79$), or rotenone ($t = 0.86$; $df = 59$; $P = 0.39$).

In no-choice tests (Figure 4-2), azadirachtin ($U = 388.00$; $Z = -7.41$; $P < 0.001$), sabadilla ($U = 0.00$; $Z = -9.45$; $P < 0.001$) and ryanodine ($U = 546.50$; $Z = -6.58$; $P < 0.001$) were confirmed to be statistically significant antifeedants against *S. americana*. Sabadilla showed a strikingly potent inhibition effect (Figure 4-2). Neem oil did not reduce the feeding behavior of the American grasshoppers significantly ($U = 1707.50$; $Z = 0.49$; $P = 0.63$). Capsaicin and kaolin clay were discarded as potential *S. americana* feeding deterrents after the first trial because nymphs completely consumed the leaf disks treated with these chemicals.

The multiple-choice bioassays demonstrated that sabadilla was the most effective feeding deterrent for *S. americana* fifth instar nymphs. It exerted a strong feeding inhibition effect that was statistically superior to the one produced by ryanodine and azadirachtin ($\chi^2_F = 51.11$; $df = 2$; $P < 0.001$). Although the average leaf consumption of azadirachtin treated leaf disks was lower

than in the ryanodine treated disks (Figure 4-3), there was not a statistically significant difference between these treatments ($U=1566.00$; $Z = 1.23$; $P = 0.22$).

Field Trial

Persistence of the potential antifeedants under field conditions was variable. There was no effect of sunlight exposure intervals on consumption of foliage treated with azadirachtin ($F = 0.06$; $df = 2$; $P = 0.95$) or ryanodine ($F=3.06$; $df = 2$; $P = 0.053$). Azadirachtin (Figure 4-4) did not significantly reduce herbivory by *S. americana* nymphs during any of the exposure periods in each of the three trials. Ryanodine (Figure 4-5) only exerted significant leaf protection after 8 h of exposure to sunlight, during the second evaluation. Sabadilla (Figure 4-6) was the only treatment that reduced significantly the feeding by *S. americana* at 4 h, 8 h and 12 h of sunlight exposure, and did so in all the three trials. Consumption of leaf material in foliage treated with sabadilla did not differ statistically among the hours of exposure ($F = 2.24$; $df = 2$; $P = 0.11$). There were no differences among replicates for consumption of foliage treated with azadirachtin ($F = 1.33$; $df = 2$; $P = 0.27$), ryanodine ($F=1.15$; $df = 2$; $P = 0.32$) or sabadilla ($F=3.00$; $df = 2$; $P = 0.06$), as well as the interaction of this factor with the hours of exposure (azadirachtin: $F = 0.33$; $df = 4$; $P = 0.86$; ryanodine: $F=1.83$; $df = 4$; $P = 0.13$; sabadilla: $F = 0.68$; $df = 4$; $P = 0.61$).

Behavioral Bioassays: Plant Extracts

The plant extracts obtained from *C. ericoides*, *I. parviflorum* and *A. crenata* significantly reduced the consumption of treated leaf disks, compared to untreated (control) disks in choice tests (Figure 4-7). Both methanol (*C. ericoides*: $z = 2.34$; $P = 0.02$; *I. parviflorum*: $t = 11.31$; $df = 59$; $P < 0.001$; *A. crenata*: $t = 5.70$; $df = 59$; $P < 0.001$) and methylene chloride extracts (*C. ericoides*: $z=5.29$; $P < 0.001$; *I. parviflorum*: $t = 14.40$; $df = 59$; $P < 0.001$; *A. crenata*: $z = 4.26$; $P < 0.001$) significantly protected the treatment disks in this type of bioassay (Figure 4-7).

In no-choice bioassays, only the *C. ericoides* methylene chloride extract ($U = 1294.50$; $Z = -2.65$; $P = 0.008$) and the *A. crenata* methanol extract ($t = -2.1$; $df = 118$; $P = 0.04$) functioned as antifeedants that significantly reduced herbivory by nymphs of *S. americana* (Figure 4-8). Leaf disks treated with *I. parviflorum* extracts were consumed in higher proportion than the control disks (methanol: $t = 3.28$; $df = 118$; $P < 0.001$; methylene chloride: $t = 2.21$; $df = 118$; $P = 0.03$). Treatment of leaf disks with *C. ericoides* methanol extract ($t = -0.48$; $df = 118$; $P = 0.63$) and *A. crenata* methylene chloride extract ($t = -0.58$; $df = 118$; $P = 0.56$) did not statistically modify the feeding behavior of the grasshoppers (Figure 4-8).

Diaprepes abbreviatus

Behavioral Bioassays: Commercial Formulations

Feeding by the sugarcane rootstock borer weevils in choice tests was reduced in leaf disks treated with azadirachtin ($t = 4.26$; $df = 59$; $P < 0.001$), neem oil ($t = 2.25$; $df = 59$; $P = 0.03$), sabadilla ($z = 2.47$; $P = 0.01$), rotenone ($z = 6.17$; $P < 0.001$) and ryanodine ($z = 2.76$; $P = 0.01$), as compared with untreated disks (Figure 4-9). Elemental sulfur ($z = 1.04$; $P = 0.30$), diatomaceous earth ($t = -1.05$; $df = 59$; $P = 0.29$), garlic juice ($t = 0.07$, $df = 59$; $P = 0.95$), kaolin clay ($t = -1.08$; $df = 59$; $P = 0.86$) and capsaicin ($t = 1.82$; $df = 59$; $P = 0.07$) did not significantly modify the feeding behavior of the weevils (Figure 4-9).

Ryanodine ($t = -6.02$; $df = 118$; $P < 0.001$), rotenone ($U = 1403$; $Z = -2.08$; $P = 0.04$) and sabadilla ($U = 627$; $Z = -2.08$; $P < 0.001$) were the treatments that caused a significant antifeedant effect over *D. abbreviatus* adults in no-choice bioassays (Figure 4-10). There was not a significant difference between the area consumed of azadirachtin-treated leaf disks and untreated ones ($t = 1.87$; $df = 118$; $P = 0.06$). Neem oil-treated disks were eaten in a higher proportion than control disks ($U = 1319.50$; $Z = 2.52$; $P = 0.01$).

The multiple-choice bioassays (Figure 4-11) did not expose a significant difference amongst ryanodine, rotenone and sabadilla in terms of leaf area consumed by the weevils ($\chi^2_F = 1.78$; $df = 2$; $P = 0.41$).

Field Trial

The hours of sunlight exposure did not affect significantly the herbivory of citrus leaves treated with rotenone ($F = 0.28$; $df = 2$; $P = 0.75$), ryanodine ($F = 1.21$; $df = 2$; $P = 0.30$) or sabadilla ($F = 0.70$; $df = 2$; $P = 0.5$). At each time interval (4 h, 8 h and 12 h) these chemicals were ineffective as feeding deterrents. Rotenone (Figure 4-12), ryanodine (Figure 4-13) and sabadilla (Figure 4-14) failed to reduce leaf consumption, compared to untreated leaves, during the field evaluations. Replication did not have an effect on the leaf area consumed in foliage treated with rotenone ($F = 2.62$; $df = 2$; $P = 0.08$), ryanodine ($F = 2.09$; $df = 2$; $P = 0.13$) or sabadilla ($F = 2.52$; $df = 2$; $P = 0.09$), as well as the interaction of this factor with the hours of sunlight exposure (rotenone : $F = 1.55$; $df = 4$; $P = 0.20$; ryanodine: $F = 0.68$; $df = 4$; $P = 0.61$; sabadilla: $F = 0.62$; $df = 4$; $P = 0.65$).

Behavioral Bioassays: Plant Extracts

The plant extracts reduced feeding by sugarcane rootstock borer weevils. Regardless of the solvent used, weevils consumed significantly more of the untreated lettuce leaf disks than disks treated with *C. ericoides* (methanol: $z = 7.62$; $P < 0.001$; methylene chloride: $z = 7.29$; $P < 0.001$), *I. parviflorum* (methanol: $t = 4.88$; $df = 59$; $P < 0.001$; methylene chloride: $z = 3.88$; $P < 0.001$) and *A. crenata* (methanol: $z = 7.10$; $P < 0.001$; methylene chloride: $z = 5.56$; $P < 0.001$) extracts, in choice tests (Figure 4-15).

In no-choice bioassays, both extracts of *C. ericoides* (methanol: $U = 144.50$; $Z = -8.69$; $P < 0.001$; methylene chloride: $U = 58.00$; $Z = -9.14$; $P < 0.001$) and *A. crenata* (methanol: $t = -34.54$; $df = 118$; $P < 0.001$; methylene chloride: $U = 89.50$; $Z = -8.98$; $P < 0.001$) were confirmed to be

feeding deterrents against the sugarcane rootstock borer weevils (Figure 4-16). Leaf disks treated with *I. parviflorum* extracts were consumed in the same proportion as the control disks (methanol: $t = -1.14$; $df = 118$; $P = 0.25$; methylene chloride: $t = -1.45$; $df = 118$; $P = 0.15$).

Figure 3-1. Total area consumed of untreated and treated leaf disks, by *S. americana* nymphs, in choice bioassays.

Figure 3-2. Total average area consumed of untreated and treated leaf disks, by *S. americana* nymphs, in no-choice bioassays.

Figure 3-3. Total leaf area eaten (cm^2) by *S. americana* nymphs when exposed to the most effective feeding deterrents in multiple-choice situations.

Figure 3-4. Consumption of azadirachtin treated and control citrus disks after three time intervals of sunlight exposure on three trials. Disks were provided to *S. americana* nymphs in no-choice situations. Vertical bars denote 0.95 confidence intervals.

Figure 3-5. Consumption of ryanodine treated and control citrus disks after three time intervals of sunlight exposure on three trials. Disks were provided to *S. americana* nymphs in no-choice situations. Vertical bars denote 0.95 confidence intervals.

Figure 3-6. Consumption of sabadilla treated and control citrus disks after three time intervals of sunlight exposure on three trials. Disks were provided to *S. americana* nymphs in no-choice situations. Vertical bars denote 0.95 confidence intervals.

Figure 3-7. Total area consumed of untreated and plant extracts-treated leaf disks, by *S. americana* nymphs, in choice bioassays.

Figure 3-8. Total average area consumed of untreated and plant extracts-treated leaf disks, by *S. americana* nymphs, in no-choice bioassays.

Figure 3-9. Total area consumed of untreated and treated leaf disks, by *D. abbreviatus*, in choice bioassays.

Figure 3-10. Total average area consumed of untreated and treated leaf disks, by *D. abbreviatus* adults, in no-choice bioassays.

Figure 3-11. Total leaf area eaten by *D. abbreviatus* adults when exposed to the most effective feeding deterrents in multiple-choice situations.

Figure 3-12. Consumption of rotenone treated and control citrus disks after three time intervals of sunlight exposure on three trials. Disks were provided to *D. abbreviatus* adults in no-choice situations. Vertical bars denote 0.95 confidence intervals.

Figure 3-13. Consumption of ryanodine treated and control citrus disks after three time intervals of sunlight exposure on three trials. Disks were provided to *D. abbreviatus* adults in no-choice situations. Vertical bars denote 0.95 confidence intervals.

Figure 3-14. Consumption of sabadilla treated and control citrus disks after three time intervals of sunlight exposure on three trials. Disks were provided to *D. abbreviatus* adults in no-choice situations. Vertical bars denote 0.95 confidence intervals.

Figure 3-15. Total area consumed of untreated and plant extracts-treated leaf disks, by *D. abbreviatus* adults, in choice bioassays.

Figure 3-16. Total average area consumed of untreated and plant extracts-treated leaf disks, by *D. abbreviatus* adults, in no-choice bioassays.

CHAPTER 4 DISCUSSION

Behavioral Bioassays: Commercial Formulations

In this research, sabadilla was the most effective feeding deterrent against *S. americana*. Alkaloids, the active ingredient in sabadilla, have previously been shown to produce an antifeedant effect on grasshoppers. Nicotine hydrogen tartrate, as well as gramine, were able to reduce the feeding behavior of *S. americana* (White and Chapman 1990, Chapman et al. 1991, Bernays 1991), *L. migratoria* (Ishikawa and Kanke 2000), *Ageneotettix deorum* Scudder and *Phoetaliotes nebrascensis* Scudder (Mole and Joern 1994). The two former species were also deterred by the alkaloid eserine (Mole and Joern 1994). Alkaloids seem to be perceived in the American bird grasshopper by the stimulation of a specialized deterrent receptor. Prior electrophysiological studies have shown that contact chemoreceptors on the tibia and tarsus of *S. americana* are stimulated by alkaloids, and have demonstrated an association between the neuron activity and the antifeedant response (White and Chapman 1990, Chapman et al. 1991). Therefore, it is possible that the sabadilla alkaloids cevadine and veratradine were detected by the alkaloid-sensitive neurons. Based on the rapid and persistent rejection of sabadilla-treated leaf disks by *S. americana* nymphs observed during the bioassays, it is plausible that the sensory coding that elicited the sabadilla-deterrent effect resulted from “labeled line” responses (Schoonhoven 1982, van Loon 1996, Koul 2008). This means that each neuron in a contact chemoreceptor conveys a specific message, in this case a deterrent signal, which can be interpreted by the central nervous system without additional information from other neurons, leading to immediate rejection of a food source without feeding.

Sabadilla also effectively reduced the feeding behavior of *D. abbreviatus*. The feeding inhibition that this chemical elicited on the weevils was not as potent as the deterrence observed

for the grasshoppers; thus, the sensory input that the sabadilla alkaloids generated in *D. abbreviatus* and *S. americana* nervous system, is perhaps different. Instead of a labeled line response, the reduction in feeding behavior by *D. abbreviatus* could be produced by an across-fiber pattern (Schoonhoven 1982, van Loon 1996, Koul 2008), in which the combined input of two or more receptors, with different stimulus thresholds, determines the acceptance or rejection of a host. For example, alkaloids from the family Solanaceae do not have any specific disruptive effects on several taste neurons nor inhibit the activity of the taste cell sensitive to phagostimulants in the red turnip beetle, *Entomoscelis americana* Brown (Mitchell and Gregory 1979), suggesting an across-fiber pattern as sensory coding.

Although not as potent as sabadilla, azadirachtin caused a significant reduction in the feeding behavior of *S. americana*. Previous studies had documented the antifeedant properties of azadirachtin against different orders of insects, including Orthoptera (Mordue (Luntz) and Blackwell 1993, Aerts and Mordue 1997, Capinera and Froeba 2007). Inhibition of feeding behavior by this triterpenoid could be the result of stimulation of a deterrent receptor, or blockage of the input from neurons that detect phagostimulatory compounds such as carbohydrates, or both. Winstanley and Blaney (1978) studied the behavioral and sensory response of *Schistocerca gregaria* Forskal to a set of solutions including azadirachtin. They proposed that the deterrent effect of azadirachtin on this grasshopper was caused by the stimulation of a specialized deterrent cell. *Pieris brassicae* L. larvae also possess a deterrent receptor, located in the medial sensilla styloconica on the maxilla, which is excited by azadirachtin (Schoonhoven 1982). Charleston and colleagues (2005) observed feeding deterrent activity by plants extracts from *Melia azedarach* L. (Meliaceae) and *Azadirachta indica* against the diamondback moth, *Plutella xylostella* L. The authors suggested that triterpenoids present in

the plant extracts, including azadirachtin, disrupted the normal function of chemoreceptors responsible for perceiving glucosinolates, a strong phagostimulant for this lepidopteran.

The antifeedant effect of azadirachtin varies among insect species. In this study, *D. abbreviatus* was deterred by azadirachtin during the choice tests, consuming more control disks than treated ones. But in the no-choice bioassays the terpenoid did not reduce herbivory of the treated leaf disks. These results concurred with the observations of Showler and collaborators (2004), who evaluated three commercial neem-based insecticides (Agroneem, Ecozin and Neemix) as potential feeding and oviposition deterrents against gravid female boll weevils, *Anthonomus grandis grandis* Boheman on cotton plants. They found that in choice assays, only Ecozin deterred the weevils from feeding, whereas in no-choice tests, none of the products reduced the consumption of cotton leaves after 24 h. It is probable that *D. abbreviatus* had habituated to azadirachtin due to repeated exposure to the chemical during the 24 h time interval of the experiments. Desensitization to azadirachtin has been reported previously. Fifth instar *S. litura* larvae became desensitized to pure azadirachtin, in both choice and no-choice assays, after being exposed to the terpenoid for 2 h (Bomford and Isman 1996). Similarly, Held and colleagues (2001) observed adults of the Japanese beetle, *P. japonica*, to habituate to a commercially formulated neem extract, applied to linden, *Tilia cordata* L., in a series of 4-h choice or no-choice tests over four successive days.

Neem oil reduced feeding of treated leaf disks by *S. americana* and *D. abbreviatus* in paired choice assays but was unable to deter both insects in no-choice tests. *D. abbreviatus* even consumed significantly more neem oil-treated leaf disks than untreated ones. The increased acceptability of neem oil by the insects may be due to desensitization. The biological activity of neem oil is closely related to its azadirachtin content (Isman et al. 1990, Isman 2006). Neem

seeds normally contain 0.2% to 0.6% azadirachtin by weight, while azadirachtin formulations contain 10% to 50% (Isman 2006). Desensitization occurs more frequently when an antifeedant exerts weak inhibitory stimuli (Held et al. 2001), in this case lower concentrations of azadirachtin. Although neem oil includes considerable quantities of other triterpenoids such as salannin and nimbin (Schmutterer 1990, Isman 2006) that can act as antifeedants as well (Koul et al. 2004), the feeding strategy of *S. americana* and *D. abbreviatus* may have counteracted their effect. The capacity for habituation in insects may be greater in polyphagous than in oligophagous or monophagous species (Jermy 1990, Held et al. 2002) because the taste sensitivity of insect herbivores to deterrents is lower in generalists than in specialists (Bernays et al. 2000).

Rotenone was ineffective as a feeding deterrent of *S. americana* in this research. There is evidence that some species of grasshoppers are able to detect flavonoids (Bernays and Chapman 2000). Chapman and co-workers (1991) detected that salicin, a phenolic glycoside that stimulates a deterrent neuron in *S. americana*, had a phagostimulatory effect on this grasshopper at low concentrations. But it seemed that the activity of the deterrent cells at higher concentrations of salicin was sufficient to override the phagostimulatory effects of the sucrose-sensitive cells, producing an antifeedant effect (Chapman et al. 1991). Therefore, the inefficiency of rotenone in reducing herbivory by *S. americana* may be a consequence of the imbalance between the weak deterrent stimuli generated by the isoflavonoid and the strong phagostimulatory effect of the carbohydrates present in the lettuce leaf disks.

Contrary to the effect observed with the grasshoppers, rotenone applied to lettuce leaf disks effectively deterred *D. abbreviatus* adults in both choice and no-choice bioassays. This result supports previous studies that tested rotenone against curculionids and other coleopterans.

Rotenone showed a strong antifeedent effect against adults and larvae of the wheat weevil, *Sitophilus granarius* L., adults of the confused flour beetle, *Tribolium confusum* Jacquelin du Val, and larvae of the khapra beetle, *Trogoderma granarium* Everts (Nawrot et al. 1989). The grass grub, *Costelytra zealandica* (White) was also deterred by rotenone in an artificial diet (Lane et al. 1985).

Ryanodine was the only chemical tested other than sabadilla that significantly reduced the feeding behavior of *S. americana* and *D. abbreviatus* in both choice and no-choice tests. The antifeedant effect of ryanodine on insects has been proven previously. Ryanodine reduced the food consumption of the tobacco budworm, *Heliothis virescens* (F.), and the tobacco cutworm, *Spodoptera litura* F., under laboratory conditions (Yoshida and Toscano 1994, Gonzalez-Coloma et al. 1996). Larvae of the African cotton leafworm, *Spodoptera littoralis* Boisduval, were more sensitive to the deterrent action of ryanodine than adults of the Colorado potato beetle, *L. decemlineata* (Gonzalez-Coloma et al. 1999). The observed antifeedant action of the alkaloid ryanodine potentially implicates the involvement of a common ligand-gated ion channel that mediates the taste response to these compounds in both the grasshoppers and weevils nerve cells. A possible candidate could be a ryanodine receptor. Ryanodine receptors are ryanodine-sensitive intracellular Ca^{2+} release channels (Nauen 2006). Ca^{2+} is an intracellular messenger which intercedes in many cellular and physiological activities such as neurotransmitter release, hormone secretion, gene expression and muscle contraction (Nauen 2006).

In this study, capsaicin did not exert a relevant antifeedent effect against *S. americana* or *D. abbreviatus*. The ineffectiveness of capsaicin as an insect feeding deterrent has been previously documented. Capsaicin did not decrease herbivory of grape leaves by the rose chafer, *Macrodactylus subspinosus* F. (Isaacs et al. 2004). Potato plots treated with a capsaicin extract

did not decrease the incidence of the Colorado potato beetle, *Leptinotarsa decemlineata* Say (Moreau et al. 2006). Baumler and Potter (2007) also reported the inefficacy of capsaicin in reducing defoliation of linden (*Tilia cordata* L.) by the Japanese beetle.

Kaolin clay, an aluminosilicate material, did not effectively deter any of the insects tested in this study. *Schistocerca americana* consumed less kaolin-treated leaf disks than untreated ones in choice bioassays, but in a no-choice scenario the presence of the aluminosilicate did not prevent the entire consumption of the treated disks. Against *D. abbreviatus*, kaolin clay did not even exert a significant protection for treated leaf disks, in choice tests. Other studies have observed successful suppression of arthropod pests on fruit plants coated with kaolin (Glenn et al. 1999, Puterka et al. 2000). According to these studies, a plant coated with a hydrophobic particle film barrier, such as kaolin clay, can repel arthropods not only by making the plant visually or tactilely unrecognizable as a potential host but also by restraining mobility and snaring the arthropod's mouthparts. But kaolin clay as a plant protector is maybe only effective against small arthropods with piercing-sucking mouthparts, such as the ones tested in these studies (pear psylla, *Cacopsylla pyricola* Foerster, spirea aphid, *Aphis spirecola* Patch, potato leaf hopper, *Empoasca fabae* Harris, twospotted spider mite, *Tetranychus urticae* Koch and pear rust mite, *Epitrimerus pyri* Nalepa). For large insects with chewing mouthparts, such as *D. abbreviatus* or *S. americana*, kaolin clay does not represent a physical barrier. Our results also contrast with those of Lapointe (2000), who observed a reduction in feeding by *D. abbreviatus* adults on citrus plants treated with kaolin clay under laboratory conditions. Lapointe suggested that the antifeedant effect was caused by interference with tactile recognition of citrus plants as hosts, using the research of Puterka and colleagues (2000) as a reference. This mode of action is probably correct against specialist arthropods, like the pear psylla and the pear rust mite, which

depend on very specific visual, olfactory, gustatory and mechano-sensory stimuli in order to select a host plant, but this may not apply to generalist insects that rely more, at short distances, on tasting most plants and eating those that lack strong feeding deterrent compounds in order to select a host plant (Schoonhoven et al. 2005). The contrasting results suggest a necessity for further studies of the antifeedant effect of kaolin clay on *D. abbreviatus*.

Field Trial

Residual activity of the tested antifeedants under field conditions proved to be quite brief. All the chemicals tested did not significantly reduce herbivory by *S. americana* or *D. abbreviatus* after 4 h of exposure to sunlight. The only exception was sabadilla, which showed an antifeedant effect against nymphs of *S. americana* after 4 h, 8 h and 12 h of exposure. Photodegradation of botanical pesticides under sunlight has been reported previously (Liang et al. 2003, Showler et al. 2004, Capinera and Froeba 2007) and represents one of several problems affecting plant-based insecticides under field conditions.

Behavioral Bioassays: Plant Extracts

In this study, both methanol and methylene chloride extracts from the Florida rosemary, *C. ericoides*, deterred *D. abbreviatus* adults; whereas only the methylene chloride extracts from this plant species exerted an antifeedant effect on *S. americana* nymphs. Chemical analysis of *C. ericoides* has previously demonstrated the presence of several classes of flavonoids including, dihydrochalcones, flavones, catechins and epicatechins (Tanrisever et al. 1987). Flavonoids may affect the feeding behavior of insects by stimulation of a specialized deterrent receptor (Simmonds 2001, Koul 2008). Flavonoids are compounds (Ding 1998) that are only soluble in a polar solvent such as methanol. Therefore, these phenolic compounds may be the basis of the antifeedant effect of *C. ericoides* methanol extracts on *D. abbreviatus*. The feeding inhibition that the *C. ericoides* methylene chloride extracts observed against *S. americana* and *D.*

abbreviatus suggests the presence of a non-polar chemical that interacts with the chemosensory system of the two insect species. A possible candidate would be a class of terpenoid, due to the relative non-polar character of these plant chemicals (Fischer et al. 1994) and their potential as feeding deterrents (Koul 2008).

Methanol extracts from *A. crenata* functioned as a feeding deterrent against both *S. americana* nymphs and *D. abbreviatus* adults. The latter insect species was also deterred by methylene chloride extracts from *A. crenata*. Triterpenoid saponins have been isolated from *A. crenata* plants (Liu et al. 2007). These non-polar terpenoids are able to modify the feeding behavior of insects. Larvae of the diamondback moth, *Plutella xylostella* L., were deterred by a triterpenoid saponin extracted using chloroform from wintercress, *Barbarea vulgaris* W. T. Aiton (Shinoda et al. 2002). Terpenoids can inhibit insect feeding by distortion of the normal function of phagostimulant receptors, excitation of deterrent receptors and/or stimulation of broad spectrum receptors, among others (Koul 2008). Isocoumarins are another type of phytochemical that has been identified in members of the genus *Ardisia* (Kobayashi and de Mejia 2004). Isocoumarins are phenolic compounds that can produce a feeding deterrent effect by antagonizing γ -aminobutyric acid (GABA) (Ozoe et al. 2004). GABA and related aminobutyric acids have been shown to stimulate feeding and induce taste cell responses among herbivorous insects of four orders including Orthoptera and Coleoptera (Mullin et al. 1994). GABA-gated chloride channels in the peripheral nervous system of insects participate in chemoreception. In excitable cells, binding by a ligand (GABA, in this case) changes channel conformation, which leads to an opening of the ion pore and an inhibitory inward Cl^- movement, due to high external Cl^- . But, according to Mullin et al. (1994), opening of these channels under low Cl^- concentrations of plant tissues, in the absence of a mechanism to maintain high

extracellular Cl^- in the sensillar fluid (which is a mucopolysaccharide material surrounding the tip of the dendrites of the contact chemoreceptors), could result in an outward Cl^- movement leading to depolarization of the sensory neuron. Thus, if a phytochemical (i.e. isocoumarins) antagonizes GABA at the binding site of a ligand-gated chloride channel in a gustatory cell, feeding deterrence will be induced.

In summary, of the ten chemicals tested only sabadilla, azadirachtin and ryanodine deterred *S. americana* under laboratory conditions. Sabadilla was the only compound that maintained its remarkable antifeedant properties against the grasshoppers after 12 h of exposure to sunlight. Sabadilla's deterrent effect and relative durability under field conditions makes it a potential tool for integrated management of the American bird grasshopper. Against *D. abbreviatus*, ryanodine, rotenone and sabadilla acted as feeding deterrents, but only in the laboratory bioassays. The stability of these chemicals in the field must be improved if effective protection against the sugarcane rootstock borer weevil is desired. The effectiveness of the extracts obtained from *C. ericoides* and *A. crenata* in reducing herbivory of the two insect species tested is an indication that many plants contain phytochemicals that could potentially be developed as antifeedants, and examination of these compounds is a logical next step for this research.

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BIOGRAPHICAL SKETCH

Andres Felipe Sandoval Mojica was born on 1981 in Bogota, Colombia. The youngest of four children, he grew up in Tunja, Boyaca, Colombia graduating from Colegio de Boyacá in 1997. He obtained his undergraduate degree in biology from the Pontificia Universidad Javeriana, where he developed an interest in entomology after taking the course “Biology of Arthropods”. Due to an excellent academic performance in this subject, he became the teaching assistant for the same course in the year 2001. His undergraduate thesis identified the pattern of altitudinal variation in richness, abundance, diversity and composition of the Orthoptera community in an altitudinal gradient between 2,000 m and 3,000 m. in an Andean cloud forest. This research contributed to understand the controversial relationship that exists between altitude and species richness. It also provided information about factors affecting insect distribution in environmental gradients. Due to the significance of this work, he received financial support, as a scholarship, from the *Colombian Society of Entomology* (SOCOLEN). The research was nominated as the best student paper at the XXXII meeting of the same institution in 2005 and the results published in the 32nd volume of *Revista Colombiana de Entomologia* in 2006. In 2005, he was hired by *Fundación OMACHA* (OMACHA Foundation, an organization committed to the sustainable development, research and conservation of the Colombian natural resources, with emphasis in aquatic ecosystems), where he studied the entomological fauna that exist in the *Bojonawi Natural Reserve*, in the Colombian Orinoquia. He proposed and developed a research subject that compared the structure and composition of dung beetles, ants and butterflies communities in three land units that are present at the reserve: savannah, galleria forest and palm trees. This study, which was presented at the XXXIII meeting of the *Colombian Society of Entomology*, was nominated as the best study presented by a professional in 2006. A new report of *Phanaeus haroldi* Kirsch (Coleoptera: Scarabaeidae) for the Department of Vichada

(Colombia) was also obtained. During 2006, he worked as a volunteer at the *Colombian Corporation of Agricultural Research (CORPOICA)*.