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Evaluation of Control Tactics for Management of Sweetpotato Weevil (Coleoptera: Curculionidae)

Jie Chen
Louisiana State University and Agricultural and Mechanical College

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EVALUATION OF CONTROL TACTICS FOR MANAGEMENT OF SWEETPOTATO WEEVIL (COLEOPTERA: CURCULIONIDAE)

A Dissertation

Submitted to the Graduate Faculty of the Louisiana State University and Agricultural and Mechanical College in partial fulfillment of the requirements for the degree of Doctor of Philosophy

in

The Department of Entomology

by

Jie Chen
B.S., Southwest University, 2010
M.S., University of Maine, 2013
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ABSTRACT

Sweetpotato weevil (SPW), *Cylas formicarius elegantulus* (Summers), is the most damaging root-feeding insect of sweetpotato, *Ipomoea batatas* L. (Lam), worldwide. The efficacy and compatibility of host plant resistance and insecticide control were evaluated in this dissertation. cv. Beauregard is the most susceptible cultivar for oviposition and offspring performance of SPW compared to cvs. Evangeline and Murasaki. The oviposition preference was shaped by the larval experience of SPW, providing evidence in support of the Hopkins’ Host-Plant Selection Principle. Although oviposition on cv. Murasaki was reduced, the egg capacity of SPW developed on cv. Murasaki was not decreased, indicating an adaptive behavior of egg-resorption to compensate encountering an inferior host. The larval performance was not influenced by the previous experience nor related to oviposition preference. Our study highlighted the importance of considering previous experience in host plant resistance studies. It is the first time such studies were conducted on SPW.

Induced host plant resistance was found in SPW-infested plant against above-ground virus vectors, green peach aphid (GPA), *Myzus persicae* (Sulzer) and cotton aphid (CA), *Aphis gossypii* Glover. SPW infestation decreased the fitness and inhibited the feeding activities of above-ground virus vectors. When SPW population are controlled in the field, the virus epidemiology may be altered.

Insecticide resistance was not detected in field-collected populations in Louisiana. Sweetpotato cultivars did not reduce insecticide efficacy against SPW, indicating compatibility of host plant resistance and insecticide control.
CHAPTER 1. INTRODUCTION

1.1 Sweetpotato Distribution and Economic Importance

Sweetpotato, *Ipomoea batatas* L. (Lam), was originally discovered 5000 years ago in northwestern South America (O’Brien 1972, Austin 1988, Yen 1982). The cultivation of sweetpotato spread to other parts of the world including Africa, Europe and Asia during 14th to 16th century (Yen 1982). Nowadays, sweetpotato is an important world crop. Asia accounts for 85% of sweetpotato annual production, followed by Africa with 11% (FAO 2013). In China, 70% of the sweetpotato production goes to animal feed, especially for swine production (Loebenstein 2009). The need of sweetpotato for animal feed and industrial starch continues to increase (Huang et al. 2003). Sweetpotato-swine system is in many Asian countries and plays a critical role in rural agriculture (Scott 1992). In sub-Saharan Africa, the production area of sweetpotato has increased by four million hectares since 2010 (FAO 2013). Sweetpotato is a ‘poor man’s crop’ with most production by subsistent farmers (Loebenstein 2009). In the United States, the annual production of sweetpotato is over 134 million metric tons and California, North Carolina, Louisiana, and Mississippi account for over 90% of the overall production (FAO 2013). In Louisiana, the sweetpotato yield is over 4 million bushels with 23 kg per bushel (USDA 2016).

Unlike tuber-propagated Irish potatoes, *Solanum tuberosum* L., sweetpotato can be propagated from vine, root slips or storage roots (Loebenstein 2009). Farmers in the U. S. often use vine cuttings for propagation. Sweetpotato can be used as staple food, vegetable, snack food, animal food and raw material for industrial produce (Bouwkamp 1985). Given the same cultivation time and input, sweetpotato returns the highest yield among all cultivated crops (Woolfe 1992). The role of sweetpotato is critical in developing countries because of its resilient
performance under barren agricultural conditions (Jansson and Raman 1991). Sweetpotato can be considered as a permaculture plant since all plant parts are consumable.

1.2 Pests on Sweetpotato

1.2.1. Insect Pests

Sweetpotato is under attack by a broad spectrum of insects that feed on flowers, foliage, stems, vines, and roots. Most recently, the described insect pests on sweetpotato include 270 insect and 17 mite species worldwide (Talekar 1991). In the U. S., over 19 insect species can attack sweetpotato fields and reduce production (Cuthbert 1967). The foliage feeding insects include cabbage looper, *Trichoplusia ni* (Hübner); soybean looper, *Chrysodeixis includens* (Walker); sweetpotato hornworm, *Agrius cingulata* (F.); beet armyworm, *Spodoptera exigua* (Hübner); sweetpotato whitefly, *Bemisia tabaci* (Gennadius); green peach aphid, *Myzus persicae*, and cotton aphid, *Aphis gossypii* (Smith and Beuzelin 2015). Aphids and whiteflies are also virus vectors on sweetpotato (Talekar 1991). Foliage feeders are comparably easier to manage than root feeders in terms of control accessibility.

The root feeders include the larvae of sweetpotato weevil (SPW), *Cylas formicarius elegantulus* (Summers), rootworms, *Diabrotica balteata* LeConte and *Diabrotica undecimpunctata howardi*, white grubs, primarily *Phyllophaga ephilida* (Say), the larvae of white-fringed beetles, *Naupactus spp.*, wireworms, *Conoderus spp.* and *Melanothus communis* (Gyllenhal), larvae of flea beetles, *Systena spp.*, and adult sugarcane beetles, *Eutheola humilis* (Burmeister) (Chalfant et al. 1990, Smith 2006, Smith and Beuzelin 2015). Plants injured by foliage feeders can compensate for intensive defoliation and exhibit tolerance to foliage injury (Chalfant et al. 1990). Root feeders, on the other hand, are challenging to manage due to their cryptic living environment and their direct feeding on the harvestable tissue (Chalfant et al. 1990,
Sorensen 2009). Among the insect pests on sweetpotato, SPW is the most devastating pest affecting sweetpotato production worldwide (Talekar 1991). Regionally, SPW is a quarantinable pest and currently under regulation by the Louisiana Department of Agriculture and Forest (LDAF 2014).

1.2.2 Diseases

Plant diseases were once a large problem that limited sweetpotato production in the U. S. in the early 20th century (Sorensen 2009). Currently, many of the plant diseases can be successfully managed without causing significant production loss (Sorensen 2009). Sweetpotato cultivars with resistance to plant disease, such as Fusarium wilt (*Fusarium oxysporum f. sp. batatas* and *nicotianae*), Streptomyces soil rot (*Streptomyces ipomoeae*), and Rhizopus soft rot (*Rhizopus spp.*), have been developed and effectively control these pests together with fungicides (Clark and Moyer 1988). Viruses occurring in sweetpotato include *Sweet potato feathery mottle virus*, *Sweet potato leaf curl virus*, *Sweet potato virus G*, *Sweet potato chlorotic stunt virus*, and *Ipomoea vein mosaic virus* (Clark and Hoy 2006). The infection of a single virus and mixed viruses can lead to yield loss from 14% to 44% (Clark and Hoy 2006). Nematodes are another disease challenge for sweetpotato (Sorensen 2009). The average damage to sweetpotato yield by nematodes is estimated at 10% worldwide (Whitehead 1998). Root-knot nematode (*Meloidogyne incognita* Kofoed & White (Chitwood)) is the primary nematode and capable of reducing yield under high populations (Sorensen 2009). The control of root-knot nematode can be achieved through resistant cultivars (Cervantes-Flores et al. 2008). In general, the pathogens that can pass through clonal reproduction can be controlled using virus-tested seed. Other control methods including sanitation, chemical control and deployment of resistant cultivars can control systemic pathogens and reduce virus re-infection (Clark and Hoy 2006).
1.2.3 Weeds

Weeds in sweetpotato fields compete for resources with sweetpotato and impair crop yield and quality. In the U.S., the problematic weeds include morning glory (*Ipomoeas spp.*), prickly sida (*Sida spinosa* L.), common cocklebur (*Xanthium strumarium* L.), hophornbeam copperleaf (*Acalypha ostryifolia* Riddell), hemp sesbania (*Sesbania exaltata*), smellmelon (*Cucumis melo* L. Var. dudaim Naud.), groundcherry (*Physalis spp.*), nutsedge (*Cyperus spp.*), common ragweed (*Ambrosia artemisiifolia* L.), and pigweeds (*Amaranthus spp.*) (Monks et al. 1998, Curtis 2003, Kelly et al. 2006). In early sweetpotato production, weed management relies primarily on mechanical cultivation with hand-weeding in early sweetpotato production (Welker 1967). Nowadays, mechanic weeding remains as a critical tactic in weed management with an average of three cultivations per season on a national scale (Haley and Curtis 2006). Herbicide application is also a control method for weed management that requires less labor, time and expense from hand-weeding (Sorensen 2009). Nowadays, clomazone (Commond®) is adopted by over 82% of growers in the Southern states of the U.S. for weed management (Haley and Curtis 2006). In 2003, the U.S. Environmental Protection Agency (EPA) granted several states a Section 18 Emergency Exemption for the use of metolachlor and flumioxazin in sweetpotato fields and provided rotational choices of herbicides (Sorensen 2009). Other herbicides include fluazifop (Fusilade®), sethoxydim (Poast®) and clethodim (Select®) that are efficient in reducing postemergence grasses (Sorensen 2009).

1.3 Sweetpotato Weevil (SPW)

1.3.1 Pest Status

SPW is distributed globally as heterogeneous populations categorized by different names and biological characters (Chalfant et al. 1990). Publications often define these geographically
different populations as subspecies. *C. f. elegantulus* occurs in the New World throughout most of the southern U. S. from southern Texas to the coastal regions of North Carolina, while *C. f. formicarius* occurs in the Old World (Chalfant et al. 1990). *C. puncticollis* and *C. brunneus* species complexes occur in continental Africa and Madagascar (Chalfant et al. 1990).

The economic damage caused by SPW varies in different countries (Hue and Low 2015). In China, yield reduced due to SPW infestation was 1-5% in research farmland and could increase up to 18% in commercial farmland (Hue and Low 2015). In the Philippines, SPW could reduce sweetpotato yield by 50%, while yield loss by SPW was recorded as 15% in Japan (Gapasin 1989, Miyaji and Tanaka 1998). In Cuba, SPW is prevalent in all provinces and cause yield loss up to 45% (Alcazar et al. 1997). SPW damage was more severe in Africa than in other continents. The yield loss was 73% in Uganda and could reach up to 100% in other areas of Africa (Smit 1997, Fuglie 2007, Nderitu et al. 2009). In the U.S., yield loss to insect injury could reach up to 80% (Jansson et al. 1987). SPW is considered the most damaging root herbivore (Smith and Beuzelin 2015). Nowadays, SPW is under quarantine regulations in parts of Asia and in Southern U.S. (Zhang et al. 2009, Smith and Beuzelin 2015).

### 1.3.2 Pest Biology

SPW feeds on a wide range of host plants, including carrot (*Dacus carota* L.), radish (*Raphanus sativus* L.), rhubarb (*Rheum rhaponticum* L.), and plants in the families of Convolvulaceae (Muruvanda et al. 1986). For species in Convolvulaceae, sweetpotato is a primary host for SPW, whereas other species of *Ipomoea*, such as *I. pes-caprae*, *I. hederacea* var. *integriuscula*, *I. hederifolia*, *I. triloba*, *I. horsfalliae* and *I. obscura* can serve as alternative host for SPW (Chittenden 1919, Cockerham 1943, Muruvanda et al. 1986, Jansson et al. 1989, Reddy...
and Chi 2015). Louisiana Department of Agriculture and Forestry listed all the *Ipomoea* species as hosts for SPW (LDAF 2014).

SPW are multivoltine with four successive stages. The overall developmental time of the life cycle is 33 days, and the optimal temperatures for growth are 27 to 30°C (Mullen 1981). The average life span of an adult is 3 months (Mulan 1981). Females go through preoviposition, oviposition and postoviposition periods with time lengths of 4, 64 and 8 days, respectively (Jansson and Hunsberger 1991). Eggs are creamy white and oval, and rarely produced in clusters. Eggs are laid in the root, foliage, and vines, and covered with a fecal plug for protection (Smit and Matenogo 1995). Eggs hatch within two weeks and the larvae go into pupation after two to four weeks (Mullen 1981). Larvae tunnel and develop in the roots, leaving frass, causing thickening and malformation of the roots (Korada et al. 2010). Larval injury coincides with odor release and a bitter taste rendering the SPW damaged roots unacceptable for human consumption (Uritani 1975). Pupae are about 5mm long, white in color. Pupation takes place inside of the roots. While the immatures stay in the roots, adults can feed on all parts of sweetpotato (Korada et al 2010). Both male and females are attracted to the leaf volatiles of sweetpotato, while only females are attracted to root volatiles (Nottingham et al. 1989). The females have capitate antennae while the males have filiform antennae. This distinguishing character provides an observable method for morphological identification of the sexes. The adults preferentially attack the root near the soil surface instead of feeding on the vines (Sutherland 1986). Both adult and larval injury cause qualitative and quantitative economic loss in storage roots and in field from the direct feeding on the root (Talekar 1982).

1.4. Sweetpotato Weevil Management

1.4.1 Cultural Control
Cultural control includes practices that prevent population increases of insect pests including intercropping, modified planting time, deployment of trap crops, and sanitation (Koradao et al. 2010). In India, intercropping is adopted by farmers in north eastern India to suppress populations of SPW (Korada et al. 2010). Planting time can affect yield and root quality significantly (Teli and Salunkhe 1994). From previous studies, sweetpotatoes planted during June to August yielded higher with less insect damage compared to the ones planted during January to April in India (Teli and Salunkhe 1994). Delayed harvest from 108 to 133 days after planting also increased the total yield but reduced the percentage of marketable value due to internal damage from SPW (Korada et al. 2010). Deep planting into the ground and uplifting soil after 6 weeks can significantly protect the plant against SPW (Macfarlane 1987). Other practices including removal of host and crop debris after harvesting, planting away from infested fields, and use of noninfested planting materials can help reduce SPW population in the field (Sorensen 2009).

1.4.2 Chemical Control

Chemical control using synthetic insecticides is an important tactic in SPW management in the U.S. Studies have evaluated the efficacy of insecticides for control of SPW. Mason et al. (1991) evaluated the baseline toxicity of SPW to the technical grade of five insecticides: parathion, carbamate methomyl, chlorpyrifos, endosulfan, and carbaryl using topical applications. Chlorpyrifos had the highest toxicity in both studies, but is no longer registered for SPW management. A more recent study evaluated five formulated insecticides using adult vial test (Smith and Hammond 2006). Methyl parathion had the highest toxicity and was recommended for SPW management. The current insecticides registered for SPW management in Louisiana include beta-cyfluthrin, bifenthrin, carbaryl, imidacloprid, and phosmet (Smith and Beuzelin 2015). All the registered insecticides target the insect Central Nervous System and are fast acting. The
Louisiana Department of Agriculture and Forestry (LDAF) is monitoring SPW populations using pheromone traps in all commercial fields in Louisiana (Smith and Beuzelin 2015). It is mandatory to spray fields in which SPW is previously detected using a rotation of insecticides (LDAF 2014). Due to the differences in population abundance and management, SPW control in other continents relies more on alternative practices than on chemical control (Hue and Low 2015).

1.4.3 Biological Control

Biological control agents, including nematodes, fungi, parasitoids and predators, have been tested on SPW. Mannion and Jansson (1992) compared ten species of entomopathogenic nematodes in the family of Heterorhabditidae and Steinernematida against SPW. All tested nematodes could kill SPW in all life stages. Nematode strains in the family Heterorhabditidae were more efficient against larval SPW than strains in the family of Steinernematida. Besides nematodes, the entomopathogenic fungi *Beauveria bassiana* and *Metarhizium brunneum* have insecticidal effect against SPW (Yasuda 1999, Reddy et al. 2014a). A recent study found that the combination of the two entomopathogenic fungi resulted in less root damage and more adult cadavers in the field (Reddy et al. 2014a). However, to achieve sufficient inoculum, adults must encounter a large number of conidia, which is often difficult in practice (Yasuda 1999). Field trapping using pheromone traps containing fungal conidia could significantly reduce SPW population (Yasuda 1999). However, the time gap occurring before the infected adults transfer the fungus to healthy adults renders this practice less effective in the field. An eulophid wasp parasitoid, *Euderus sp.*, was reported to attack SPW in Florida (Jansson and Lecrone 1992). The reported parasitism rate ranged from 0.4% to 1.4%. The control efficacy was questionable since the parasitoid was found in very low abundance in the field.
Sterile insect technique (SIT) is considered as augmentative biological control tactic by releasing radiation-sterilized males to the area-wide population (Klassen 2005). The effectiveness of SIT depends on the mating performance and fitness of the released sterile male (Knipling 1979). Gamma irradiated SPW males stay active with less death-feigning behavior within the first two days after irradiation (Kuriwada et al. 2010a). However, gamma irradiation decreases the mating frequency of irradiated males one week after release (Kumano et al. 2008). Gamma irradiation can severely damage somatic cells, decreasing mating ability (Bakri et al. 2005). SIT may result in inbreeding depression and inhibit the efficiency of SIT, as demonstrated in other *Cylas spp.* (Kuriwada et al. 2010b). Considering the limitation of gamma radiation, fractionated-dose irradiation is more effective with less adverse effects on the fitness and behavior of the insects. Fractionated-dose irradiation is a series of irradiations with the same dosage of irradiation in total but lower dosages at each irradiation (Bakri et al. 2005). The mating propensity of sterile males was prolonged in this fashion (Kumano et al. 2011). Future studies on the efficiency of fractionated-dose irradiation against SPW under the field conditions could help understand the effectiveness of SIT before implementing SIT in a management plan.

1.4.4 Host Plant Resistance

Painter (1951) defined host plant resistance as the heritable characters of a plant that enables it to avoid, tolerate, or recover from herbivore attack and reduce the injury compared to the same plants without these characters. Using resistant cultivars to manage SPW can significantly reduce the labor and expense from the farmer. Selection of cultivars resistant to insects and diseases has been carried out by the Louisiana State University AgCenter Sweetpotato Breeding Program through an open-pollinated polycross nursery (LaBonte et al. 2009a, LaBonte et al. 2009b). Commercial cultivars, such as Beauregard, Evangeline, and Murasaki, were released with
sweetpotato disease resistance to soil rot, fusarium wilt, fusarium root rot, and rhizopus soft rot (Rolston et al. 1987, LaBonte et al. 2008a, LaBonte et al. 2008b). Beauregard is a leading commercial cultivar with orange flesh in Louisiana that was developed in the Louisiana State University breeding program (Reames and Smith 2015). Beauregard was released in 1987 for the superior taste and texture (Rolston et al. 1987). Evangeline was released more recently and exhibits similar production characteristics to Beauregard (LaBonte et al. 2008a). Beauregard and Evangeline are susceptible to the injury of root herbivores (Thompson et al. 1999, Jackson and Bohac 2006, Jackson et al. 2012). Murasaki was released in 2008 with a white flesh, purple skin and high dry matter, which resulted in large demand in the Asian market (LaBonte et al. 2008b). Like Beauregard, Murasaki is resistant to soil rot and fusarium root rot (LaBonte et al. 2008b). Unlike other dark-tone skin cultivars, such as O'Henry and Kotobuki, Murasaki is resistant to southern root-knot nematode (LaBonte et al. 2008b). Murasaki exhibited lower root injury level of WDS (wireworms, Diabrotica, and Systena) complex and resistance to rootworm, SPW, and white grubs (Story et al. 2010, Jackson and Harrison 2013). The average yield of Murasaki is 13.9 (Mt ha$^{-1}$), while that of Beauregard is 16.9 (Mt ha$^{-1}$). Although Murasaki has lower yield and inconsistent performance compared to Beauregard, Murasaki is profitable with good marketable value (LaBonte et al. 2008b).

Understanding the effect and mechanism of resistant cultivars can help identify the role of host plant resistance in SPW management. Smith (2005) defined three categories of host plant resistance against herbivores: non-preference (antixenosis), antibiosis, and tolerance. Antixenosis is defined as the plant traits that lead the herbivores away from the host. Cultivars with repellent or lack of attractant surface chemicals can demonstrate antixenosis on herbivores. Boehmeryl acetate, an oviposition stimulant, was found in higher concentrations of the root surface of
susceptible cultivars compared to resistant cultivars (Son et al. 1991, Marti et al. 1993). Epidermal thickness can also affect cultivar preference by inhibition of mouthpart penetration, which could also influence the feeding site decision of SPW (Korada et al. 2010). Antibiosis is defined as the plant defense that reduces the fitness of the herbivores. The epidermis of sweetpotato roots contains various secondary components that inhibit development and fitness of the insect (Kay 1992). Concentrations of caffeic acid, resin glycosides, and hydroxycinnamic acid esters from the epidermis of storage roots vary among cultivars (Mao et al. 2001, Stevenson et al. 2009). Caffeic acid is a phenolic stress metabolite and is associated with reduction in larval survivorship (Stange et al. 2001, Harrison et al. 2003, Harrison et al. 2008). Resin glycosides have shown insecticidal properties by reducing larval mortality and life expectancy of diamondback moth, *Plutella xylostella* L. (Peterson and Jackson 1998, Jackson and Peterson 2000). Octadecyl and hexadecyl esters of hydroxycinnamic acids inhibit the larval development of SPW and can even be lethal to larvae (Stevenson et al. 2009). Host plants can also compensate for insect injury without significant yield loss. However, compensation can be difficult to identify and can be confused with insect escape. Overall, host plant resistance shows great potential in managing SPW. In developing countries, where sweetpotato production is on small-scale farms owned by subsistence farmers, implementing host plant resistance can help bring more benefit to the farmers. Host plant resistance is also more compatible with the environment and alleviates the concerns on insecticide use. Future research should focus on identifying the resistance mechanism and utilizing resistant cultivars efficiently.

1.4.5 Other Tactics

Using transgenic plants with *Bacillus thuringiensis* (Bt) has been studied against SPW. Currently, seven Cry protoxins have been tested in diet bioassays for toxicity against *Cylas* spp.
Three of the Cry protoxins, Cry7Aa1, Cry3Ca1, and ET33-34, have shown higher toxicity due to larval mortality (Ekobu et al. 2010). Inconsistent results were found by Rukarwa et al. (2013). Only pupation rates were reduced rather than larval and pupation survivorship. Bt transgenic plants have the potential to control SPW. Unfortunately, current Bt toxins expression in the roots is low and considered insufficient to control SPW (Rukarwa et al. 2013).

A sampling plan is always needed to determine if a control tactic should be implemented or not. Adult males of SPW locate females for copulation through pheromone cues (Reddy et al. 2012). A synthesized female pheromone lure has been developed for trapping in the field (Reddy et al. 2014b). The green lights together with the pheromone lure showed increased attractiveness to the males (McQuate 2014). In the sweetpotato fields, SPW normally infest along the field edge. Pheromone traps can be placed along the field edge to reduce the field population of males.

1.5 Experience Modulated Host Preference

Root tunneling insects, such as the larva of SPW, typically have limited choice of host due to immobility. Thus, oviposition preference by females primarily determines the host preference of the insect. Host plant resistance is a study of insect-plant interaction. However, it often overlooks the effect of previous experience of the herbivore on future host choice. Hopkins (1917) stated that “a species which breeds in two or more hosts will prefer to continue to breed in the host to which it has become adapted”, which is referred to as the Hopkins’ Host Plant Selection Principle (Hopkins’ HSP). Hopkins drew this conclusion based on the observation that mountain pine beetle, Dendroctonus monticolae, preferred to breed on the pine tree species that they had become adapted to, even when another optimal host species was available. Since the initial introduction, subsequent studies have been carried out to test Hopkins’ HSP. The testing protocol in general
involves rearing the insects on a host plant and test the host preference of their offspring (Wiklund 1975, Chow et al. 2005, Rietdorf and Steidle 2002, Midega 2011). Janz et al. (2009) collected a field population of polyphagous butterfly, *Polygonia c-album* L., and reared them on three natural hosts, *Urtica dioica* (stinging nettle), *Salix cinerea* (grey sallow), and *Ribes uva-crispa* (gooseberry), for one generation. The F1 females of the butterflies were split tested for oviposition preference in pairwise choice trials. Mader et al. (2012) collected field populations of eastern spruce budworm, *Choristoneura fumiferana* (Clem.) from two types of hosts and reared the budworm from eggs to F1 generation in the laboratory without exposure to any food source. The F1 population was transferred to two hosts in the field for future observation of oviposition preference. Additionally, offspring behaviors were also evaluated in this study, including feeding duration, number of probing events, number of meals etc. Olfactory preference of the offspring was also reported for testing Hopkins’ HSP. Rietdorf and Steidle (2002) evaluated the induction of olfactory preferences by larval and early adult experience for odor from wheat or maize grain for the granary weevil, *Sitophilus granarius* L. in the laboratory. The weevils were reared on wheat or maize for five generations before testing. The F5 larvae and adults were exposed to the odor of the hosts and tested for host preference. The rearing period of insects on host plant varies from one generation to multiple generations (Rietdorf and Steidle 2002, Janz et al. 2009). However, a span of many generations of the insects is more convincing to rule out random fluctuations in host preference (Jaenike 1978). Evidences both in support of (Phillips 1977, Rietdorf and Steidle 2002, Coyle et al. 2011) and against (van Emden 1996, Janz et al. 2009) Hopkins’ HSP have been found in many insects.

There are several underlying mechanisms that govern this phenomenon. Genetic variation commonly exists in many insect populations and is found to influence oviposition preference
Through parental effects, the genetic variance can be passed on to the offspring through glandular products and influence the size and quality of the eggs (Chen 1984). However, evidence for genetic variation is often difficult to identify due to compounding micro-habitat factors (Barron 2001). Imaginal conditioning is rarely studied but there is some evidence in *Drosophila*. During the early stage of metamorphosis, Kenyon cell bodies in the mushroom bodies remain alive and form new connections from the regenerated fibers of the new adults (Technau and Heisenberg 1982, Truman 1990). The mushroom bodies are associated with insect memory (Barron 2001). Behavioral bioassays have confirmed the possibility of larval learning and memory retention, especially when conditioning is associated with reinforcement (Tully et al. 1994). Imaginal conditioning can reinforce larval stage memory retention. The critical period of imaginal conditioning is the early post-emergence period (Corbet 1985).

1.6 Relationship between Oviposition Preference and Offspring Performance

While Hopkins’ HSP focuses on the effect of larval experience to adult oviposition, the experience of adulthood can affect the performance of the next generation. According to the “Mother Knows Best” hypothesis, or preference performance hypothesis (PPH), the female makes oviposition choices to maximize offspring fitness (Jaenike 1978, Valladares and Lawton 1991). Evidence supporting the hypothesis has been found in the tortoise beetle (*Cassida canaliculata* L.) and the *P. xylostella* L. (Heisswolf et al. 2005, Zhang et al. 2012). The testing protocol contains three essential steps: 1) to evaluate oviposition preference of the females among various hosts, 2) to estimate the offspring performance on the hosts, 3) to compare if the optimal host for oviposition is consistent with the one for offspring performance. On the other hand, the “Bad Motherhood” hypothesis states that the female chooses oviposition sites to optimize her fitness (Scheirs et al.
A weak linkage was found between oviposition preference and offspring performance in grass miner, *Chromatomyia nigra* (Scheirs et al. 2000). Understanding the effect of host plant on all life stages is important in obtaining a whole picture of host influence on population dynamics. However, the adaptation to host plant can take place in any life stage. From Hopkin’s HSP, the previous living experience could influence the oviposition preference. It is likely that the preference-performance linkage can be affected by the previous experience as well. Thus, considering previous experience of the parents is necessary in the study of PPH, as well as in Hopkin’s HSP. However, the studies of PPH often do not consider the effect of previous experience (Verschut et al. 2017).

1.7 Objectives

The specific objectives in this study are:

1. Determine if Hopkin’s HSP applies to SPW

   Null hypotheses:
   
   a. The previous larval experience has no effect on oviposition of SPW.
   
   b. Female feeding on different cultivars does not affect oviposition preference among the cultivars.
   
   c. No interaction between previous larval experience and female feeding site on oviposition preference of SPW

2. Test if Preference-Performance Hypothesis applies to SPW and whether this relationship was modified by previous larval experience

   Null hypotheses:
   
   a. The cultivars preferred for oviposition site does not favor the larval performance of SPW
b. The relationship between oviposition preference and larval performance is not modulated by previous living environment.

3. Evaluate induced host plant resistance of SPW infested roots against the population dynamics and feeding behavior of above-ground virus vectors

Null hypotheses:

a. The SPW larvae infestation has no effect on the population dynamics of colonizing aphids.
b. The SPW larvae infestation has no effect on the feeding behavior of the above-ground aphid species.

4. Evaluate the baseline susceptibility of SPW populations to selected chemicals

Null hypothesis: There is no difference in the baseline susceptibility of chemicals among SPW populations with different previous experience of insecticide exposure.

5. Test the cultivar effect on insecticide susceptibility of SPW

Null hypothesis: The cultivar has no effect on the insecticide susceptibility of SPW.

1.8 References


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CHAPTER 2. HOST PREFERENCE OF CYLAS FORMICARIUS ELEGANTULUS (SUMMERS): AN EXAMPLE OF HOPKINS’ HOST-PLANT SELECTION PRINCIPLE

2.1 Introduction

Sweetpotato, *Ipomoea batatas* L. (Lam) was originally discovered and cultivated in Central or South America (O’Brien 1972) and current cultivation occurs in tropical and subtropical areas in Africa, Asia, and America (FAO 2013). Sweetpotato weevil (SPW), *Cylas formicarius elegantulus* (Summers), is one of the major insect pests of sweetpotato in storage and field (Talekar 1991). Adults prefer to feed on the roots and oviposit in punctures on the root surface and close with a fecal plug. Larvae tunnel through the roots and spend the entire life stage in the roots, causing storage root thickening and malformation (Korada et al. 2010). In addition to direct damage of roots, sweetpotato responds to SPW injury by producing terpenoids that result in an unpalatable taste (Uritani et al. 1975). In Louisiana, SPW is primarily distributed in the Southern region of the state, referred to as “pink-tag zone”. Fields in this area are under a mandatory insecticide spray schedule to slow down SPW expansion as outlined by Louisiana Department of Agriculture and Forestry (LDAF) (Smith and Hammond 2006, Smith and Beuzelin 2015). However, the mandatory spraying program contains an intensive insecticide spraying interval (10-to 14-day spraying schedule), which could result in the development of insecticide resistance from selection pressure, and ultimately failure of chemical control (Roush and McKenzie 1987).

Host plant resistance is a more environmentally friendly and low-cost control tactic for producers. Recurrent selection efforts have been carried out since the early 20th century to breed cultivars of sweetpotato resistance to pests. Pest resistant cultivars have been released by the U.S. Department of Agriculture-Agriculture Research Service, U.S. Vegetable Laboratory (USDA-ARS, USVL), Charleston, SC, and by the Sweetpotato Breeding Program at Louisiana State University Agricultural Center, Baton Rouge, LA. The cultivars Charleston, Scarlet, Sumor,
Resisto, Regal, Ruddy, and Murasaki have all displayed levels of resistance to sweetpotato insects, including WDS (wireworms, *Diabrotica, Systena*) complex, SPW, sweetpotato flea beetle (*Chaetocnema confinis* Crotch), and white grub larvae (including *Plectris aliena* Chapin and *Phyllophaga* spp.) (Jones et al. 1983, 1985, Mullen et al. 1985, Dukes et al. 1987, Bohac et al. 2002, LaBonte et al. 2008, Jackson et al. 2010). The type of resistance found in resistant cultivars has been identified as antixenosis by field evaluation (Jackson 2009, 2010, Jackson and Harrison 2013). Antixenosis is a form of resistance that results in reduced preference for a host plant by herbivores, while resistance that affects herbivore development or physiology is termed antibiosis (Smith 2005). However, the low yields of resistant cultivars have resulted in reduced profits, which have resulted in poor grower acceptance (Jackson and Harrison 2013). Murasaki is the only commercial resistant cultivar that is widely cultivated, because it meets the needs of Asian markets and has high economic potential (LaBonte et al. 2008). In contrast, high yielding, susceptible cultivars to sweetpotato insects include cvs. Centennial, Jewel, Beauregard, Evangeline, and SC1149-19 (Mullen et al. 1985, Jackson and Harrison 2013). In fact, cvs. Beauregard and SC1149-19 are often used as susceptible controls in laboratory and field evaluations of insect injury (Jackson and Bohac 2006, Jackson et al. 2012, Jackson and Harrison 2013). Beauregard is a predominant cultivar in Louisiana (Reames and Smith 2015).

For insects like SPW, root-tunneling larvae are incapable of switching hosts and develop exclusively on the host chosen by their mother. Although the mother chooses the developmental location and host for the next generation, larval experience can lead to oviposition preference for the host that the insects have become developed on (Hopkins 1917). This is defined as the Hopkins’ Host Selection Principle (Hopkins’ HSP) in the entomological literature, or ‘habitat imprinting’ in the vertebrate literature (Hopkins 1917, Immelmann 1975). Studies on Coleoptera have found
evidence in support of Hopkins’ HSP, including the granary weevil (*Sitophilus granaries*) and the black vine weevil (*Otiorhynchus sulcatus*) (Rietdorf and Steidle 2002, Coyle et al. 2011). Rietdorf and Steidle (2002) evaluated the induction of olfactory preferences by larval and early adult experience for odor from wheat or maize grain for the granary weevil, *Sitophilus granaries* L. in a static olfactometer. The weevils were originally collected from the field and reared on wheat in the laboratory over generations. Two groups of weevils were developed from two iso-female lines reared on wheat or maize for five generations before testing. The F5 larvae and adults were exposed to the odor of the two hosts and tested for host preference using mean allocation time. The host preference was shaped by both larval experience and early adulthood odor exposure, providing evidence supporting Hopkins’ HSP. In the study of black vine weevil, the researcher collected adult weevils from a field site containing black currant, raspberry and strawberry. A total of 150 weevils were collected and randomly divided into three groups. Each group was assigned to one host plant, black currant, raspberry or strawberry, and fed with excised leaves of the host plant for four weeks. The host preference was tested for each group in a choice bioassay with one plant of each host available. The weevils discriminated among host plants and preferred to oviposit on plants which they were fed on, as predicted in Hopkins’ HSP.

However, the validity of this principle remains ambiguous. Evidence against Hopkins’ HSP was also found in the flea beetle (*Altica [Haltica] lythr*) and in other herbivores and parasitoids (Phillips 1977, van Emden 1996, Janz et al. 2009). In the study of flea beetle, the eggs of the beetles were collected from a colony kept on flowering plants, *Epilobium hirsutum* L. and placed on moist filter paper in plastic Petri dishes (Phillips 1977). Larvae were then collected and divided into two groups: one group reared on *E. hirsutum* as control plant, the other group reared on alternative plants *Oenothera biennis* L., *Circaea lutetiana* L. or *Lythrum salicaria* L. (Phillips
The final-instar larvae, newly emerged adults prior to feeding, and adults tested one week and one month after emergence were given a choice of the control plant and one alternative plant. Overall, no significance was found in recorded feeding behaviors of all insect stages, indicating no evidence in support of Hopkins’ HSP. For SPW, the effect of previous experience on host preference is still unknown.

Although antixenosis effect was identified on resistant cultivars in field evaluation, the mechanisms of resistance in cv. Murasaki is still not clear. Field studies are helpful in evaluating field injury and yield loss, but requires an extended period of time for study. The laboratory study has the advantage of eliminating chaos from environmental changes. The current study was designed to categorize the resistance mechanism of sweetpotato cultivars under laboratory condition; 1) categorize the resistance mechanisms of three commercial sweetpotato cultivars, Beauregard, Evangeline, and Murasaki and 2) evaluate if Hopkins’ HSP applies to the oviposition behavior of SPW.

2.2 Materials and Methods

2.2.1 Insect Source

Three colonies of SPW were established with different histories of exposure to sweetpotato cultivars. Sweetpotato weevil colony BEAX was originally collected from a field population in South Louisiana and maintained under dark conditions at 27.0 ± 1°C with 65.0 ± 5% RH in the laboratory. BEAX has been maintained on cv. Beauregard storage roots for over four years. Another two colonies, EVAN and MURA, were developed by randomly selecting 200 adults (F0 generation) from the BEAX colony and rearing new generations of SPW on storage roots of cvs. Evangeline and Murasaki, respectively. Adults were reared in 14 L plastic containers (Sterilite®, Townsend, MA). A 21.0 by 25.0 cm section of the top lid of each container was cut and replaced
with screen wire mesh (Saint Gobain ADFORS, Grand Island, NY) to provide airflow. The inside bottom edge (approx. 2.5 cm) of each container was coated with Vaseline Petroleum Jelly (Vaseline®, Trumbull, CT) mixed with mineral oil (Top Care®, Skokie, IL) at a 3:1 ratio to prevent escape of adult SPW. Storage roots of all cultivars were provided by the Louisiana State Agricultural Center Sweetpotato Research Station located at Chase, LA. Storage roots were grown under recommended production conditions (Smith et al. 2012). For colony maintenance, two to three fresh storage roots were added to the colony twice a week, providing a food source and oviposition site for the adults. Infested roots were incubated at 27.0 ± 1°C in one-quart, cylindrical paper containers (Ridgid Paper Tube Corporation, Wayne, NJ), 17.5 cm in depth and 8.6 cm in diam., until adults of the next generation emerged. Gravid females of the F2 generation were collected for the following test.

2.2.2 No-Choice Oviposition

A no-choice test was conducted following the protocol of Story et al. (2000) with three trials. In each trial, ten storage roots of each cultivar were prepared for each colony. A single root was placed in a one-quart, cylindrical paper container (described above in Insect Sources) with six gravid females (two to three weeks old) randomly selected from a colony. In total, 180 containers were prepared with 10 containers per trial per colony per cultivar. All paper containers were placed in the same laboratory conditions described previously. After five days, all adults were removed from the paper containers. Eggs oviposited in each root were recorded by gently removing the epidermis of the roots near the feeding punctures with forceps. The total number of eggs per root were counted and recorded.

2.2.3 Choice Oviposition
The choice bioassay was tested with three trials. The test arenas of this bioassay were 14 L plastic containers (41.9 × 33.0 × 16.8 cm, Sterilite®), Townsend, MA). All containers were glued with a screen cover and coated with Vaseline-mineral oil mix as described above. In each trial, five plastic containers were prepared for each colony. For a single container, three roots (one of each cultivar) were placed 30 cm apart in the plastic container equilateral from each other. Six two to three weeks old gravid females were randomly selected from a colony and released into the center of each test arena which was kept in complete darkness. After five days, all females were removed, and the number of eggs oviposited per root were counted and recorded. A total number of 45 containers were prepared with five containers per trial per colony.

2.2.4 Data Analysis

In both bioassays, the experimental design was a randomized block design with trial as a block effect. The fixed effects, colony and cultivars, were in a factorial arrangement. The effect of the colony, cultivar, and the interaction was estimated using the number of eggs recorded per root. The analytical procedure was performed by Analysis of Variance, followed by Tukey’s HSD (Honest Significant Difference) test for pairwise comparison (PROC GLMM, SAS Institute, 2013).

2.3 Results

2.3.1 No-choice Oviposition

The number of eggs oviposited on a single root was significantly different among colonies ($F = 4.86; \text{d.f.} = 2, 232; P = 0.0086$). BEAX and MURA adults oviposited more eggs than EVAN adults (Fig. 2.1). The eggs deposited per root were also different among cultivars ($F = 49.66; \text{d.f.} = 2, 232; P < 0.0001$). Murasaki had fewer eggs deposited compared to cvs. Beauregard and Evangeline (Fig. 2.2). Colony and cultivars had an interaction ($F = 4.95; \text{d.f.} = 4, 232; P = 0.0008$). The significant interaction indicated the oviposition preference was affected by both colony
Fig. 2.1. Average (± se) numbers of eggs deposited on individual storage roots of sweetpotato by gravid females of SPW from the three colonies, BEAX, EVAN, and MURA, in a no-choice bioassay. Different letters present significantly different means from each other (Tukey HSD test, \( P < 0.05 \)).

(Previous experience) and cultivar (available host). For BEAX, females oviposited the most eggs on cv. Beauregard, followed by cv. Evangeline, and least on cv. Murasaki (Fig. 2.3). For EVAN, females oviposited the most eggs on cv. Evangeline, followed by cv. Beauregard, and least on cv. Murasaki. For MURA, females oviposited similar amounts of eggs on cvs. Beauregard and Evangeline, but fewer eggs on cv. Murasaki. Oviposition legacy to previous experience was found in colonies reared on cvs. Beauregard and Evangeline.

### 2.3.2 Choice Oviposition

The number of eggs oviposited on a single root was not different among colonies (\( P=0.98 \)), but different among cultivars (\( F = 14.94 \); d.f. = 2, 102; \( P < 0.0001 \)). Murasaki had fewer eggs deposited compared to cv. Beauregard and Evangeline (Fig. 2.4). The colony and cultivar had an interaction (\( F = 3.13 \); d.f. = 4, 102; \( P < 0.0181 \)), indicating the oviposition preference was shaped by previous experience and current host. For BEAX, females oviposited the most eggs on cvs. Beauregard and Evangeline, but least on cv. Murasaki (Fig. 2.5). For EVAN, females oviposited...
Fig. 2.2. Average (± se) number of eggs deposited on individual storage roots of the sweetpotato cultivars, Beauregard, Evangeline, or Murasaki, by gravid females of SPW from all colonies in a no-choice bioassay. Different letters present significantly different means from each other (Tukey HSD test, \( P < 0.05 \)).

Fig. 2.3. Average (± se) number of eggs deposited on individual storage roots of sweetpotato cultivars, Beauregard, Evangeline, or Murasaki, by gravid females of SPW from three colonies, BEAX, EVAN, and MURA in a no-choice bioassay. Different letters present significantly different means from each other (Tukey HSD test, \( P < 0.05 \)).
more eggs on cv. Evangeline than cvs. Beauregard and Murasaki (Fig. 2.5). For MURA, adults oviposited more eggs on cv. Beauregard than cv. Murasaki (Fig. 2.5). Oviposition legacy to previous experience was found in colonies reared on cv. Evangeline.

![Graph showing average number of eggs deposited on individual storage roots of sweetpotato cultivars, Beauregard, Evangeline, or Murasaki, by gravid females of SPW of all colonies in a choice bioassay.](image)

Fig. 2.4. Average (± se) number of eggs deposited on individual storage roots of sweetpotato cultivars, Beauregard, Evangeline, or Murasaki, by gravid females of SPW of all colonies in a choice bioassay. Different letters present significantly different means from each other (Tukey HSD test, $P < 0.05$).

### 2.4 Discussion

Our study found antixenosis as a resistance effect of cv. Murasaki against SPW due to the reduced oviposition on cv. Murasaki regardless of the source of the breeding host. The oviposition behavior is an example of an antixenosis host plant resistance effect (Smith 2005). In general, the proximal cause of oviposition preference could be genetic variation, environmental cues, the role of learning, and physiological process of egg maturation and resorption (Hahn and Leuschner 1981, Kay 1992, van Alphen and Visser 1990, Barron 2001, Minkenberg et al. 1992). In our study, these factors could all be the cause of the oviposition preference in SPW.
Fig. 2.5. Average (± se) number of eggs deposited on individual storage roots of sweetpotato cultivars, Beauregard, Evangeline, or Murasaki, by gravid females of SPW from three colonies, BEAX, EVAN, and MURA in a choice bioassay. Different letters present significantly different means from each other (Tukey HSD test, \( P < 0.05 \)).

Epidermal chemicals of sweetpotato roots contain secondary components that influence behavior and inhibit fitness of herbivores (Kay 1992). Hexadecyl, heptadecyl, octadecyl, quinic acid esters of caffeic and coumaric acid, resin glycosides, and hydroxycinnamic acid esters were found more abundant in resistant cultivars (Mao et al. 2001, Stevenson et al. 2009, Anyanga et al. 2013). Applying these chemicals to the root surface of susceptible cultivars reduced oviposition behavior of \textit{C. puncticollis} and \textit{C. brunneus} (Anyanga et al. 2013). Boehmeryl acetate, an oviposition stimulator, was found in higher concentrations of the root surface in susceptible cultivars compared to resistant cultivars (Son et al. 1991, Marti et al. 1993). Caffeic acid is a phenolic stress metabolite and is associated with resistance to larval survivorship (Stange et al. 2001, Harrison et al. 2003, Harrison et al. 2008). Resin glycosides are toxic to larvae and reduce the lifetime fecundity of herbivores, such as \textit{P. xylostella} (Peterson and Jackson 1998, Jackson and...
Octadecyl and hexadecyl esters of hydroxycinnamic acids inhibit the larval development of SPW and can even be lethal to larvae (Stevenson et al. 2009). Future work should test the surface chemical contents of cv. Murasaki and identify secondary plant compounds that may be contributing to antixenosis.

Hopkins’ HSP has been found operating when SPW were breeding on cvs. Beauregard and Evangeline. Barron discussed the possible underlying mechanisms of Hopkins’ HSP including genetic variation and conditioning (2001). Conditioning is more likely to be the cause in the current study, since we used insects from the same colony and only reared them on different host for two generations. Conditioning could be achieved through memory retention and chemical legacy by storing and maintaining information during metamorphosis (Barron 2001). Studies in *Drosophila* showed that Kenyon cell bodies in the mushroom bodies remain alive and form new connections from the regenerated fibers of the new adults (Technau and Heisenberg 1982, Truman 1990). This indicates that memory retention during metamorphosis is achievable through the central nervous system. Chemical legacy is another way of memorizing the larval experience through chemical compounds derived from the food and stored in puparium (Corbet 1985). Similar studies have also found the larval experience influence oviposition in cabbage looper (*Trichoplusia ni*), diamondback moth (*P. xylostella*), African cotton leafworm (*Spodoptera littoralis*), the granary weevil (*Sitophilus granaries*), and the black vine weevil (*Otiorhynchus sulcatus*) (Anderson et al. 1995, Rietdorf and Steidle 2002, Akhtar and Isman 2003, Coyle et al. 2011).

When reared on cv. Murasaki, a resistant cultivar, oviposition preference of SPW did not follow Hopkins’ HSP. Similar studies found no larval experience on the host preference among hosts with different quality for insects (van Emden 1996, Janz et al. 2009). van Emden et al. (1996)
reported the disappearance of Hopkins’ HSP on the parasitic wasps (*Aphidius rhopalosiphi* De Stef.) when aphids (*Metopolophium dirhodum* Walk.) were reared on wheat cultivars Rapier and Maris Huntsman together. Rapier has partial resistance to aphids, while cv. Maris Huntsman is susceptible (van Emden 1999). Janz et al. (2009) conducted similar experiments with oviposition preference of the polyphagous butterfly (*Polygonia c-album* L.) among three breeding hosts in the family of Urticaceae differing in susceptibility to the larvae of *P. c-album*. Similar to our result, the oviposition preference did not follow the larval experience when a non-suitable host was included; resistant hosts were not preferred by polyphagous butterfly for oviposition when being reared on.

Originally, Hopkins’ HSP was developed from the observation of host preference of mountain pine beetle (*Dendroctonus monticolae*) between two suitable hosts. However, later publications included hosts with resistance to insects, which expanded the host range from the original Hopkins’ HSP (Janz et al. 2009). Resistant hosts can lead to non-preference and antibiosis on insects, which could lead to reduced fitness (Smith 2005). If adults stay on the inferior breeding host, it is hardly a survival strategy that will be favored by natural selection. In fact, insects do not only react to a preference of host but also avoid it when experience provides negative reinforcement. Bernays (1993) defined aversion learning as the ability to avoid sources with negative consequences, and it is widespread among insect species. In *Drosophila*, both larvae and adults learn to avoid the odor paired with electrical shock from the previous larval experience (Tully et al. 1994). Woolly bear caterpillars (*Diacrisia virginica* and *Estigmene congrua*) learn to avoid host plants that induced a malaise from previous larval experience (Dethier 1980). In our study, feeding on cv. Murasaki could have provided the negative reinforcement during larval stage and contributed to the memory retention that later guided the females to avoid ovipositing on cv. Murasaki. Thus, if
the resistance hosts are included, the insects will intend to avoid the inferior host through aversion learning. This behavior is not proof against Hopkins’ HSP, but a different outcome of insect learning from previous experience.

From the physiological perspective, reduced oviposition could be a result of reduced reproductive capacity or/and increased oosorption. Reproductive capacity, also termed as egg load, is the number of mature oöcytes available for oviposition and is considered a primary source of variability for oviposition behavior (Minkenberg et al. 1992). In our study, the number of eggs oviposited by colonies were not significantly different in the choice bioassay. The similar oviposition level of all colonies indicates that oviposition capability was not the cause of oviposition preference in our study. Instead, adult experience influenced oviposition capacity. Insects are known to resorb eggs and reclaim nutrients needed for somatic maintenance when under nutrient stress (Bell and Bohm 1975, Rosenheim et al. 2000). For instance, starved females of *Nasoniu vitripennis* resorb eggs when a foodsource is lacking (Edward 1954). A similar case was found on *Aphytis melinu* females when facing a protein-starved condition (Collier 1995). In our study, it is possible that cv. Murasaki could not provide enough nutrition for SPW females. As a result, the adults feeding on the inferior host exhibited reduced oviposition by oosorption in response to stress.

In conclusion, our study supported Hopkins’ HSP and found the correlation between oviposition preference and host suitability. When being bred on a suitable host, the insect will prefer the host that they have been developed on. However, when the host suitability varies for insects, it will cause mixed results of adult host preference. Memory retention of insects can be through perceived both positive and negative reinforcement from the host plant. Based on our study, we recommend farmers incorporate host plant resistance for SPW management. Breeders
could also use cv. Murasaki to introgress resistance into orange flesh cultivars and breed more commercial cultivars with insect resistance. Host plant resistance has the potential to reduce farmer input, especially in developing countries where sweetpotato is the principle food and insecticides are not available.

### 2.5 References


Chapter 3. EFFECTS OF PARENTAL EXPERIENCE AND OVIPOSITION PREFERENCE ON OFFSPRING PERFORMANCE IN SWEETPOTATO WEEVIL (COLEOPTERA: CURCULIONIDAE)

3.1 Introduction

Sweetpotato, *Ipomoea batatas* L. (Lam), is a major staple food worldwide and is known as a crop that can alleviate food insecurity (Mukhopadhyay et al. 2011). Sweetpotato grows well in unfavorable environmental conditions and has high yields with limited inputs. Over 96% of the sweetpotato production worldwide occurs in Asia and African (FAO 2013). Surveys from International Potato Center (CIP) conducted in the developing countries indicate that the leading problem in sweetpotato production is yield (Fuglie 2007). Sweetpotato production is at risk from insect foliage feeders, root feeders, and disease transmitters (Smith and Beuzelin 2015).

Sweetpotato weevil (SPW), *Cylas formicarius elegantulus* (Summers), is one of the most destructive root feeders of sweetpotato internationally, and is widely distributed (Talekar 1991). In Louisiana, SPW is endemic to the Southern regions (Smith and Beuzelin 2015). However, range expansion is still occurring due to abundant alternative hosts, such as such as *I. tribola* and *I. hederifolia* in the family of Convolvulaceae (Jansson et al. 1989, Reddy and Chi 2015).

SPW can cause significant yield loss. Both adult and larva stages feed on storage roots and cause direct damage on harvestable tissues. When larvae tunnel inside of the storage root, they induce secondary compounds such as terpenes which result in an unpalatable taste (Uritani et al. 1975). Current control tactics for SPW in Louisiana primarily rely on in storage and post-planting insecticide application, together with other practices, such as destroying overwintering sites, destroying plant beds, and population monitoring (Smith and Beuzelin 2015). Over-reliance on insecticides can lead to resistance. Due to the cryptic living condition of SPW, the efficacy of the current control tactics is often questionable. Thus, a control tactic that is both efficacious and sustainable is desired.
Host plant resistance, an alternative control tactic, shows potential in the management of insects and is also more environmental friendly compared to chemical control (Smith 2005). Host plant resistance is the deployment of plant cultivars that possess heritable traits that reduce insect damage (Smith 2005). In sweetpotato, commercial cultivars are released for disease resistance and yield performance. For instance, commercial cvs. Beauregard, Evangeline, and Murasaki, have been released for disease resistance to soil rot, fusarium wilt, fusarium root rot, and rhizopus soft rot (Rolston et al. 1987, LaBonte 2008a, LaBonte 2008b). However, the development of insect resistant cultivars is lacking. Previous studies have shown that cv. Murasaki has lower root injury level of WDS (Wireworm, Diabrotica, and Systena) complex in field experiments (Story et al. 2010, Jackson and Harrison 2013). Laboratory studies have shown that some cultivars have few eggs deposited on and less adult emergence (Story et al. 2000, Chapter 2). Reduced oviposition indicates that the resistance category is antixenosis (Smith 2005). Reduced adult emergence could be a factor of either antixenosis from reduced oviposition or antibiosis from reduced fitness of immatures. The underlying resistance mechanism is still unclear.

Immatures of insects, such as SPW, have limited mobility and are incapable of making host choices. Instead, female adults must be able to discriminate and select a suitable host for larval development (Gripenberg et al. 2010). Jaenike (1978) first posits that a female will oviposit on the host plant that maximizes the fitness of the offspring known as Preference-Performance Hypothesis (PPH). Thus, the oviposition preference and offspring performance are positively related. Interchangeable concepts were developed in later literature such as naïve adaptionist theory, optimal oviposition theory, or the “mother knows best” hypothesis (Jaenike 1978, Courtney and Kibota, 1990, Valladares and Lawton 1991). In a study of the bronze bug, Thaumastocoris peregrinus, male and female adults were obtained from a stable indoor mass rearing maintained
on *Eucalyptus tereticornis* at their last molt (Martínez et al. 2017). The collected individuals were reared on plants of *E. globulus*, which is not included in future experiments, for one week. The insects were tested for oviposition preference in a pairwise choice study with several plant genera in the *Eucalyptus*. The nymph survivorship was recorded daily as offspring performance. In this study, the oviposition preference of the adults correlated with the offspring performance positively. Evidences in support of PPH has also been found in many other insect species, such as *Liriomyza trifolii* (Diptera: Agromyzidae), *Aphrophora pectoralis* (Hemiptera: Aphrophoridae), *Euura amerinae* (Hymenoptera: Tenthredinidae), *Galerucella nymphaeae* (Coleoptera: Chrysomelidae), and *Homoeosoma electellum* (Lepidoptera: Pyralidae) (Minkenberg and Fredrix 1989, Craig et al. 1989, Kouki 1993, Craig and Ohgushi 2002, Mphosi and Foster 2010). In these examples, offspring performance was positively related to the adult oviposition preference, supporting PPH. However, evidences to the contrary of PPH were also found in insects, such as *Otiorhynchus sulcatus* (Coleoptera: Curculionidae), *Epiphyas postvittana* (Lepidoptera: Tortricidae), *Leptinotarsa decemlineata* (Say) (Coleoptera: Chrysomelidae), and *Liriomyza huidobrensis* (Diptera: Agromyzidae) (Martin et al. 2005, Clark et al. 2011, Rizvi et al. 2016, Hufnagel et al. 2017). Females often make choices which do not favor or, in some cases, strongly conflict with the offspring fitness in these studies. Grinpenberg et al. (2010) proposed a few alternative hypotheses that explain the weak linkage between preference and performance. One of the alternative hypotheses states that the females will oviposit on the host that maximizes their fitness instead of their offspring’s, which is defined as “optimal bad motherhood” (Mayhew 2001). For instance, grass miner, *Chromatomyia nigra*, oviposit preference is favored on the host that favors adult performance instead of larval performance, indicating the females are “bad mothers” (Scheirs et al. 2000). The weak linkage (negative correlation or no correlation) between female oviposition
and offspring performance lead to unpredictable population fitness and unknown host preference over a population. To better utilize HPR, the understanding of cultivar effects on all insect stages and the relationship between preference and performance are critical in providing system-level management guidance.

Insect behavior and adult fitness are often modulated by larval experience (Barron 2001, Gripenberg et al. 2010). It is likely that larval experience will also shape the relationship between oviposition preference and offspring performance. In our Chapter 2, SPW females preferred to oviposit on cultivars that they experienced during the larval stage when the host was suitable. However, studies on the correlation between oviposition preference and offspring performance often neglect the effect of previous experience (Verschut et al. 2017).

In this study, we tested the offspring performance of SPW on three different cultivars of sweetpotato. In addition to estimating the previous experience of the parental generations, we used SPW from the three colonies that were reared on three sweetpotato cultivars, respectively. We predicted: 1) the sweetpotato cultivar preferred for oviposition by the females would also be the optimal host of the offspring, 2) and this predicted relationship would be shaped by the previous experience of the SPW.

3.2 Materials and Methods

3.2.1 Insect and Storage Root Source

Fresh storage roots of cvs. Beauregard, Evangeline, and Murasaki were provided by the Louisiana State Agricultural Center Sweetpotato Research Station located at Chase, LA. Storage roots were cultivated under typical production conditions (Smith et al. 2012). A laboratory colony, BEAX, was developed with adults of SPW collected in southern Louisiana four years ago. BEAX is reared at 27.0 ± 1°C with 65.0 ± 5% RH in 14 L screened plastic containers (Sterilite®, Townsend,
MA). The center square (21.0 by 25.0 cm) of the cover was removed and screen wire mesh (Saint Gobain ADFORS, Grand Island, NY) was glued in its place to provide airflow. To avoid SPW escape, the edge of each container (approx. 2.5 cm) was pasted with a mixture of Vaseline Petroleum Jelly (Vaseline®, Trumbull, CT) and mineral oil (Top Care®, Skokie, IL) at a 3:1 ratio. Storage roots of cv. Beauregard were placed in the containers on a weekly basis. Infested roots were either kept in cylindrical paper containers for the adult emergence or discarded after being frozen. Two new colonies, EVAN and MURA, were developed from BEAX, using the adults from BEAX reared on cvs. Evangeline and Murasaki respectively. Initially, four hundred adults (F0) including both males and females were selected randomly from BEAX and evenly split into two containers. Storage roots of cv. Evangeline or Murasaki were then assigned to one container and replaced with fresh roots of the same cultivar every other day. The eggs oviposited by the F2 generation were used in the following bioassays.

### 3.2.2 Offspring Performance

Offspring performance was tested using a bioassay developed from methods described in Nottingham et al. (1989). A randomized block design was used with three trials. In each trial, three colonies and three cultivars were the two main effects and were arranged in a factorial design. The treatment unit was a 24-well Falcon™ tissue culture plate (12.5 by 8.5 by 2.0 cm; Falcon model 3047, Becton Dickenson, Lincoln Park, NJ). One plate was filled with root cores of one of the three cultivars; Beauregard, Evangeline, or Murasaki. The storage roots were peeled and then cored using a No.9 cork borer (diam. 1.4 cm, depth 2.0 cm) (Humboldt, Raleigh, NC) which provided a tight fit into the wells. In one trial, three plates filled with root cores of a cultivar were prepared for each colony. In total, 27 plates (9 plates per cultivar) were prepared for one trial. On each root core, a single egg collected from the assigned colony that was oviposited within 24 hours
was added to the surface of the root core. In this way, eggs from the three colonies were randomized to the 27 plates per trial. After the placement of the eggs, all the plates were closed with the plate cover and kept under laboratory conditions (27±1°C and 65± 5% RH) in complete darkness. Since eggs do not hatch in the first two days after deposition, the eggs were checked every other day for hatching starting on day 3. The number of eggs that hatched on each plate and the number of days to eclosion were recorded. One week after hatching, all root cores were checked every other day for adult emergence. Root cores infested with fungus were either replaced with a new root core or removed from the study if the larva was dead. The developmental time from egg to adult emergence and the number of adults emerged per plate were recorded. Emerged adults were sexed and weighed.

3.2.3 Data Analysis

Colony effect and cultivar effect were analyzed for survivorship, developmental time, and adult weight using Analysis of Variance followed by Tukey’s Honest Significance Difference test for mean separation in PROC GLMM (SAS Institute 2013). One plate was excluded from analysis due to fungal infection one week after egg placement. The different sample size was adjusted using Kenward-Roger degrees of freedom approximation. The sex ratio of the adult emergence per colony was tested using chi-square goodness-of-fit test in PROC FREQ (SAS Institute 2013).

3.3 Results

The percentage of eggs hatched per plate was significantly different among colonies ($F = 4.42; \text{d.f.} = 2, 64; P = 0.0159$). Colony BEAX had more hatched eggs compared to EVAN and MURA (Fig. 3.1). Cultivar also influenced the percentage of egg-hatching ($F = 6.42; \text{d.f.} = 2, 64; P =$
Fig. 3.1. The average proportion (± se) of hatched eggs per plate from three colonies, BEAX, EVAN, and MURA.

0.0092). Fewer eggs hatched on cv. Evangeline than on cvs. Murasaki and Beauregard (Fig. 3.2). Colony and cultivar also had an interactive effect (Fig. 3.3; $F = 2.55$; d.f. = 4, 64; $P = 0.0472$). Colony had effects on percent adult emergence ($F = 7.96$; d.f. = 2, 63; $P = 0.0008$). Colony BEAX had more adult emergence than EVAN and MURA (Fig. 3.4).

Fig. 3.2. The average proportion (± se) of hatched eggs per plate when tested on cvs. Beauregard, Evangeline, and Murasaki.
Fig. 3.3. The average proportion (± se) of hatched eggs of the three colonies, BEAX, EVAN, and MURA, on three cultivars, Beauregard, Evangeline, and Murasaki.

Fig. 3.4. The average proportion (± se) of adult emergence out of the hatched eggs of the three colonies, BEAX, EVAN, and MURA.

Cultivar affected adult emergence as well ($F = 9.37; \text{d.f.} = 2, 63; P = 0.0003$). Murasaki had the least adults emerge (Fig. 3.5). Colony and cultivar did not have an interaction effect ($P = 0.1504$). The developmental time from egg to adult emergence was different among cultivars ($F = 19.34; \ldots$
d.f. = 2, 1248; \( P = < 0.0001 \). Offspring that developed on cv. Beauregard had the shortest developmental period, followed by cv. Evangeline, with the longest on cv. Murasaki (Fig. 3.6).

![Fig. 3.5. The average proportion (± se) of adult emergence out of the hatched eggs tested on cvs. Beauregard, Evangeline, and Murasaki.](image1)

![Fig. 3.6. The developmental days (± se) of SPW on three cultivars, Beauregard, Evangeline, and Murasaki.](image2)
Overall, females were heavier than males (Fig. 3.7; \( F = 4.35; \) d.f. = 1, 1213; \( P = 0.0371 \)).

Adult weight was significant among colonies (\( F = 10.19; \) d.f. = 2, 1238; \( P = < 0.0001 \)). Adults of colony EVAN weighed less than BEAX and MURA (Fig. 3.8). Adult weight was also different among cultivars (\( F = 18.8; \) d.f. = 2, 1238; \( P = < 0.0001 \)). Adults that emerged from cv. Beauregard weighed less than those from cvs. Evangeline and Murasaki (Fig. 3.9). When adult was analyzed by sex, both males and females tested on cvs. Evangeline and Murasaki were heavier than those
tested on cv. Beauregard (Fig. 3.10; d.f. = 2, 605.9; $F_{\text{female}} = 7.22; P_{\text{female}} = 0.0008; F_{\text{male}} = 14.88; P_{\text{male}} < 0.0001$). There was no interaction between colony and cultivar ($P=0.1744$). The sex ratios were 1:1 for the three colonies.

![Graph showing individual adult weight of SPW reared on cvs. Beauregard, Evangeline, and Murasaki.](image1)

**Fig. 3.9.** Individual adult weight (mg) ± se of SPW reared on cvs. Beauregard, Evangeline, and Murasaki.

![Graph showing single adult weight of SPW reared on the three cultivars tested for female and male separately.](image2)

**Fig. 3.10.** Single adult weight (mg) ± se on the three cultivars tested for female and male separately. Both cultivar was significantly for both sex.
3.4 Discussion

From our study, cv. Beauregard is the optimal host for offspring survivorship, including the percentage of eggs hatched and adults emerged out of viable eggs. Our study is consistent with previous report on the cultivar effect (Mao et al. 2001). In Mao’s study, larval survivorship of SPW was tested on five sweetpotato genotypes; Beauregard, Excel, W-244, W-250, and Sumor. Larval survivorship was the highest on cv. Beauregard for two years.

The hatchability of eggs either collected from or tested on cv. Evangeline decreased. One of the major components that influences insect eggs is relative humidity and microclimate (Woods 2010). Extremely low and high humidity can lead to egg mortality. Low humidity can result in egg desiccation, inhibited embryo development, and reduced egg hatchability, while high humidity can cause drowning and increase chances of disease infection (Guarneri et al. 2002, Gullan and Cranston 2005). In plates filled with cv. Evangeline root cores, we observed a higher number of disease infection at the end of the experiment. Additionally, one plate was so severely infested that we were unable to check the development of the eggs. The higher infection rates in cv. Evangeline could have contributed to higher egg mortality. Although cv. Evangeline is a susceptible cultivar with high adult oviposition, it appears to be vulnerable to disease infection and not suitable for offspring development. Thus, cv. Evangeline has an indirect antibiotic effect on egg survivorship from the increased chance of disease infection.

In previous studies, the periderm was found to be responsible for effects on insect behavior and survivorship (Peterson et al. 2003, Jackson and Bohac 2007). In general, the periderm of the sweetpotato was found to have an antibiotic effect on larval survivorship and governs the storage roots from pathogenic fungal infections (Peterson et al. 2003, Jackson and Bohac 2007). Surface chemical contents, such as caffeic acid, resin glycosides, and hydroxycinnamic acid esters, from
the epidermis of storage root, have been tested on the SPW (Mao et al. 2001, Stevenson et al. 2009). Caffeic acid is a phenolic stress metabolite and is associated with resistance to larval survivorship (Harrison et al. 2003, Harrison et al. 2008). Resin glycosides are toxic to larvae and reduce the lifetime fecundity of diamondback moth, *Plutella xylostella* L. (Jackson and Peterson 2000). Octadecyl and hexadecyl esters of hydroxycinnamic acids inhibit the larval development of SPW and can even be lethal to larvae (Stevenson et al. 2009). The periderm was believed to be the only root part that has a significant effect on the larval performance of SPW. However, in our study, we used peeled storage roots, which did not contain the epidermis. Thus, the antibiosis effect for larval survivorship is affected by the flesh of the cultivar instead of the periderm. Harrison et al. (2008) evaluated the contents of caffeoylquinic acid compounds, including chlorogenic acid and the 3,4-, 3,5-, and 4,5- isomers of dicafeoylquinic acid (DCQA) in the storage roots of sweetpotato. The average contents of the four compounds were found higher in cortex and stele, which comprise of the flesh of the storage root. Thus, the chemical contents in the flesh are also responsible for the survivorship and development of SPW larvae.

Our study did not support PPH; offspring performance was not always optimized by the host preferred by the females as an oviposition site. From Chapter 2, SPW reared on cv. Evangeline preferred to oviposit on cv. Evangeline. In this Chapter, the offspring performance on cv. Evangeline was not the best among the three hosts. Similarly, SPW reared on cv. Murasaki chose to oviposit on cvs. Beauregard and Evangeline, but cv. Evangeline was not the optimal host for larval performance. Only in SPW reared on cv. Beauregard, the oviposition preference was the same as the optimal host for offspring performance. Thus, our study did not find evidence of positive correlation between preference and performance. For West Indian sweetpotato weevil, *Euscepes postfasciatus*, larval performance was not related to the oviposition preference of the
females (Okada et al. 2014). The body size of the adults was larger on the hosts that were not preferred by the adults. Similarly, the adult weight was heavier on the inferior host in this study. The adult weight often indicates egg carrying capacity (Bennettova and Fraenkel 1981, Wiktelius and Chiverton 1985). The increased body weights on inferior host could lead to higher oviposition capacity, which is an advantage for future fitness. In our previous study, SPW colonies reared on cvs. Evangeline and Murasaki had the same egg carrying capacity as those reared on cv. Beauregard. Thus, the larval stage could compensate for poor host quality by acquainting more food and increasing body weight for the adult stage. Such survival strategies on inferior hosts could lead to host shift when limited food sources are available. It is also capable of providing mixed results between adult preference and offspring performance when the larvae are going through different physiological adaptations when reared on different hosts.

Although insect colony was significant on most recorded parameters in this study, the previous experience was not found to be correlated to the offspring performance as oviposition preference. Similar results were found for the leaf beetle, *Galerucella calmariensis* (Coleoptera: Chrysomelidae) (Verschut et al. 2017). The influence of previous experience on the linkage of preference-performance was only found when the leaf beetle was reared on tufted loosestrife (*Lysimachia thyrsiflora*) but not on purple marshlocks (*Potentilla palustris*). This shows that previous experiences serve as an indicator for female oviposition preference but cannot be consistently maintained and transmitted to the next generation. It is possible that the memory retention or chemical legacy can only be retained within a generation and regained accordingly in the next generation. Previous host experience could provide mixed results to the fitness performance of future generations.
In general, Beauregard is the most optimal cultivar for offspring performance. Evangeline and Murasaki have antibiosis based on the longer development and reduced survivorship of the offspring. However, there is a chance of adaptation to inferior host from the increased adult weights when reared on cv. Murasaki (Okada et al. 2014). From Chapter 2, the egg capacity of SPW reared on cv. Murasaki was the same as reared on other cultivars. In this Chapter, the adult weight was increased when reared on cv. Murasaki. Egg capacity and adult weight could contribute to the adaptation by saving eggs and energy to distribute offspring on optimal host. If SPW adapts to a resistant cultivar, the efficacy of host plant resistance would be jeopardized. Additionally, it also indicates the possibility of host shift when only resistant cultivars are available. Thus, future studies should focus on the physiological changes during larval stages and select cultivars that do not favor larval adaptations. For instance, breeders could select cultivars that constantly inhibit population growth to all life stages. Our study highlighted the difficulties in answering the relationship of insect fitness between and within generations. It is critical to understand the whole picture of host plant resistance against all life stage before the future deployment, but often difficult to find concordant patterns among all recorded life history parameters.

3.5 References


CHAPTER 4. BELOW-GROUND SWEETPOTATO HERBIVORY BY SWEETPOTATO WEEVIL (COLEOPTERA: CURCULIONIDAE) ALTERS POPULATION DYNAMICS AND FEEDING BEHAVIOR OF ABOVE-GROUND HERBIVORES

4.1 Introduction

Sweetpotato, *Ipomoea batatas* L. (Lam), is one of the most important staple foods worldwide and can alleviate food security concerns in the world (Mukhopadhyay et al. 2011). Currently, sweetpotato is widely planted in tropical and subtropical areas in the world (Loebenstein and Thottappilly 2009). Like Irish potato, *Solanum tuberosum* (L.), sweetpotato is propagated vegetatively using root generated cuttings and is prone to accumulate virus (Clark et al. 2010). In the U. S., the most problematic viruses in sweetpotato fields are the potyviruses: *Sweet potato feathery mottle virus*, *Sweetpotato virus G*, and *Sweet potato virus 2* (synonym= *Ipomoea vein mosaic virus*) (Clark et al. 2012). Sweetpotato potyviruses often infect the field plants as a mixture of all three viruses and can reduce yield and marketable value (Clark et al. 2010). The transmission of these viruses can be completed by several aphid species in a nonpersistent manner, but *Myzus persicae* (Sulzer) and *Aphis gossypii* Glover, are the most efficient vectors (Wosula et al. 2014). Potyviruses are noncirculative and stylet borne (Watson and Roberts 1939). Green peach aphid (GPA), *M. persicae*, is the dominant species that colonizes sweetpotato fields in Louisiana (Wosula et al. 2013a). Cotton aphid (CA), *A. gossypii*, is also an efficient vector that is commonly captured in sweetpotato fields in Louisiana (Wosula et al. 2012). The behavior and fitness of these vectors are important factors that determine plant virus epidemiology (Jeger et al. 2004).

Aphids acquire virus particles together with virus-encoded proteins that attach the virus to the stylets from plants (Berger and Pirone López-Abella et al. 1988). Probing behaviors are difficult to visualize but can be monitored and presented as waveforms using the electrical penetration graph (EPG) technique (Tjallingii 1993). The EPG technique was first developed in the early
1960s by McLean and Kinsey to study the insect-plant interactions of aphids (McLean and Kinsey 1964). Today, the EPG technique has been widely adopted in studies of the feeding behaviors of soft-body insects with piercing-sucking mouthparts (Backus and Bennett 2009). The EPG technique uses an electrical circuit that connects plant and insect using wires. The circuit incorporates an electrical resistor (Ri), a voltage source (V), a thin wire that glued to the back of the insect, and another wire inserted to the soil of the plant pot. When the aphid stylet penetrates the plant tissue, the circuit is initiated and starts to generate fluctuating voltage which is amplified and translated into waveforms. The EPG system is categorized into three types: AC, DC, and AC-DC. The AC-EPG system is the first generation of the EPG system using an alternating current as voltage source (McLean and Kinsey 1964). This system is not sensitive to the fluctuating ‘generated’ voltages but only respond to the fluctuating electrical resistance. The DC-EPG system was the second generation that replaced the alternating current with a direct current (DC) as voltage source (Tjallingii 1985). Using DC system, the intra- and extracellular stylet tip positioning could be distinguished and recognized, which is an improvement from the AC-EPG system. The third generation consists of a combination of fluctuating electrical resistance and fluctuating ‘generated’ voltages (Backus and Bennett 2009). Among the three generations, the DC-EPG system is commonly referred to as ‘EPG’. The waveforms in this system has been characterized and correlated with the positioning of the stylet tips and the insect feeding activities (van Helden and Tjallingii 2000, Tjallingii 2006). The waveforms are distinguished into three main behavioral phases: pathway, phloem and xylem (Tjallingii 2006). During the pathway phase, several stylet penetration activities occur, including intercellular stylet insertion and withdrawal, no-stylet movement, and brief intracellular punctures by stylet tips, also named as potential drops (pds) (Tjallingii 1985, 2006). The potential drops last for a short period (3 to 15s) but are critical for
acquisition and inoculation of nonpersistent viruses. Three pd subphases have been categorized; II-1, II-2, and II-3 (Powell et al. 1995, Collar and Fereres 1997). Virus acquisition occurs at II-3 phase, where the aphid ingests cytosolic fluid (also known as ‘helper proteins’) together with virus articles from the source plant (Powell et al. 1995, Martín et al. 1997). The inoculation of viruses occurs during the subphase II-1 (Martín et al. 1997, Powell 2005). The intercellular stylet insertion and withdrawal reveals if the insect accepts the host or not (Jiang and Walker 2001). The follow-up phase includes phloem and xylem feeding behaviors (Tjallingii 2006). The phloem feeding starts with a salivation period (E1) and followed by phloem sap ingestion (E2) (Tjallingii 2006). The xylem feeding phase (G) allows the insects to regain water and remain osmotically balanced (Pompon et al. 2010).

Management efforts to control plant viruses rely on the understanding of the three-way interaction of virus-plant-vector (Alvarez et al. 2007). For the potyvirus on sweetpotato, studies have found that plant species and virus-infected condition of the plant changes the vector behavior and fitness (Wosula et al. 2012, 2013b, 2014). The virus acquisition of SPFMV by GPA is reduced on sweetpotato cultivars Beauregard and Evangeline compared to the morning glory species, *I. cordatotriloba* and *I. hederacea* (Wosula et al. 2012). The infected conditions of the sweetpotato also lead to different probing behaviors of the GPA by the plant species (Wosula et al. 2013b. For *I. cordatotriloba*, *I. hederacea*, and cv. Evangeline, the stylet penetration behaviors increased on virus-infected plants vs. noninfected (Wosula et al. 2014). However, feeding behaviors also decreased when cv. Beauregard was infected with potyvirus (Wosula et al. 2014).

Plant-mediated interactions between below- and aboveground insects can influence the fitness and behaviors of the virus vector and indirectly influence virus epidemiology. In the field, sweetpotato are under attack by a wide spectrum of herbivores including both above- and below-
ground insects (Smith and Beuzelin 2015). When the host plant is exposed to herbivore injury, the plant induces chemistry that impacts the behavior and fitness of another herbivore (Karban and Myers 1989, Bezemer et al. 2003). One mechanism to explain interactions between below- and aboveground herbivores is that the injured plant reallocates primary metabolites such as nitrogen and carbohydrates of the host plant away from the injured sites (Gange and Brown 1989, Masters et al. 1993). When root herbivores attack the plant, the stress response of the plant is triggered and starts to accumulate amino acids and carbohydrates in the foliage, which later facilitate the performance of foliage feeders (White 1984, Chapin 1991). However, the induced response could also be harmful to foliage feeders since many plant secondary metabolites are toxic or deterrent to insect herbivores (Karban and Baldwin 1997). Thus, the performance and behaviors of the virus vector on herbivory injured plants could be affected positively, negatively, or left unaffected. In this study, we use sweetpotato infested with sweetpotato weevil (SPW), *Cylas formicarius* (Fab.), as a root herbivory injured plant and tested the performance of two aphid species that transmit potyvirus on sweetpotato. The population dynamics is only estimated for GPA, since CA do not reproduce on sweetpotato (Wosula et al. 2013b). The aphid feeding behaviors of both species were evaluated using electrical penetration graph during 30 min (behaviors related to potyvirus acquisition and inoculation) and 6 hr (behaviors related to host acceptance and colonization).

### 4.2 Materials and Methods

#### 4.2.1 SPW Colony

Fresh storage roots of cv. Beauregard were provided by the Louisiana State Agricultural Center Sweetpotato Research Station located at Chase, LA. Storage roots were cultivated under typical production conditions (Smith et al. 2012). A laboratory colony of SPW was developed with adults collected in southern Louisiana four years ago. The colony was reared at 27.0 ± 1°C
with 65.0 ± 5% RH in 14 L screened plastic containers (Sterilite®, Townsend, MA). The center square (21.0 by 25.0 cm) of the cover was removed and screen wire mesh (Saint Gobain ADFORS, Grand Island, NY) was glued in its place to provide airflow. The edge of each container (approx. 2.5 cm) was pasted with a mixture of Vaseline Petroleum Jelly (Vaseline®, Trumbull, CT) and mineral oil (Top Care®, Skokie, IL) at a 3:1 ratio to avoid escaping. The storage roots were provided for the colony and replaced on a weekly basis. Infested roots were either kept in cylindrical paper containers for the adult emergence or discarded after being frozen.

4.2.2 Aphid Colony

The lab colony of GPA was formerly collected from eggplant, Solanum melongena L., and developed from a single aptera in 2009. The colony of CA was developed from a single aptera collected from a cotton plant, Gossypium hirsutum L., at LSU AgCenter Macon Ridge Research Station, Winnsboro, LA, in 2006. The GPA colony was reared on cultivar Tendergreen (Seed Savers, Decorah, IA) of mustard, Brassica cretica L., a sweetpotato virus free host, while the CA colony was reared cv. DP174RF of Gossypium spp., DeltaPine (Monsanto Company, St. Louis, MO), also a non-host of sweetpotato viruses. The colonies were maintained in screened cages (30 cm × 30cm × 30 cm) assembled with Plexiglas and nylon mesh fabric under laboratory condition at 20 to 22°C and photoperiod of 14:10 (L: D) h. All host plants were grown in growth chambers (Percival Scientific Inc., Perry, Iowa) at 25°C and 50% RH, in 10-cm-diameter plastic pots filled with soil mix, Miracle-Gro® Potting Mix (Miracle-Gro Lawn Products Inc, Marysville, OH) and 3.5 g per pot Osmocote 14-14-14 (Scotts-Sierra Horticultural Products Company, Marysville, OH). Fresh host plants were provided to the colonies every 2 to 3 wk with 5 to 10 aphids of the same cohort placed on them.

4.2.3 Laboratory Population Dynamics
Sweetpotato roots (cv. Beauregard) were placed to sprout under the laboratory conditions (20 to 22°C) for one to two weeks. Sprouted roots were selected for this study. Sprouts were covered by aluminum foil wrap (Raynolds®) and secured with masking tapes to protect the sprouts. Eight wrapped roots were prepared for one replication, with three replications in total. A single root was kept in one paper cylinder container and stored under laboratory conditions (27±1°C and 65± 5% RH) for one week. Four containers had 6 gravid females of SPW released per container, while the rest were kept without SPW as controls. The females and the aluminum foil were removed from the roots after one week. All roots were planted in the greenhouse (20 - 32°C and 21 - 98% RH) in 20-cm-diameter clay pots filled with soil mix, Miracle-Gro® Potting Mix (Miracle-Gro Lawn Products Inc, Marysville, OH) and 3.5 g per pot Osmocote 14-14-14 (Scotts-Sierra Horticultural Products Company, Marysville, OH). All plants were caged in a plastic bucket sealed with a screen cover to prevent possible escape of emerging SPW. Two weeks after planting, leaves of each plant were taken at random, and cored using a No.9 cork borer (diam. 1.4 cm, depth 2.0 cm) (Humboldt, Raleigh, NC). For each plant, ten leaf cores were prepared in one replication. A single leaf core was placed in a diet cup with agar bedding at weight ratio 1:20 (agar powder: water), providing a solid base and attachment for the leaf core. In total, 240 diet cups with leaf cores was provided for both treatment and control. During the test, leaf cores and agar bedding were replaced with leaf cores from the same plant when needed. One adult of GPA was added to one diet cup and allowed to larviposit for 24 hr. After nymphs were deposited, all but one first instar was removed in each diet cup. All aphids were checked daily for molting, survivorship and fecundity till death. The offspring produced after adulthood was recorded and removed from the leaf cores daily. The experiment was processed under laboratory condition at 20 - 22°C. All diet cups were stored in a growth chamber (Percival Scientific Inc., Perry, Iowa) at 25 ±1°C and 16:8 (L:D) h.
Life tables were developed for each treatment following the methods described in Birch (1948). Intrinsic rate of population growth ($r_m$) was calculated using the following equation:

$$\sum e^{-r_m x} l_x m_x = 1$$

[1]

Where $x$ is the age, $l_x$ is the age-specific survival, $m_x$ is the number of progeny per female per day.

Net replacement rate ($R_0$) was calculated as:

$$\sum l_x m_x$$

[2]

Finite rate of increase ($\lambda$) was calculated as:

$$e^{r_m}$$

[3]

Doubling time ($DT$) was calculated as:

$$\frac{\ln(2)}{r_m}$$

[4]

Mean generation time ($T$) was calculated as:

$$\frac{\ln(R_0)}{r_m}$$

[5]

4.2.4 Aphid Probing Behavior

EPG studies were carried out to monitor the feeding behavior of GPA and CA on SPW-infested vs. uninfested plants. The plants were prepared in the same way as the population dynamic study. The whole plant after 21 days of SPW infestation was used in this bioassay. The testing protocol was described in Davis and Radcliffe (2008). The EPG experiments were performed in a Faraday cage using a Giga8 DC amplifier (Wageningen Agricultural University, The Netherlands) with 1 gigaohm input resistance and an AD conversion rate of 100 Hz running only the first four channels. A DI-710 (DATAQ Instruments, Inc., Akron OH) acquisition card converted the analog signals to digital signals, which were recorded using WinDaq Serial Acquisition software (DATAQ Instruments, Inc., Akron OH). 18-μm gold wire (Semiconductor
Packaging Material, Armonk, NY) was attached to the dorsal tergum of an adult aphid with silver paint (Pelco Colloidal Silver Liquid no. 16034, Ted Pella, INC., Redding, CA), and then placed on the abaxial surface of the upper most leaf for the test and recorded for 30 min or 6 hr. Four aphids were tested at a time with two aphids per plant per treatment. Treatments were randomized within the cage. The experiments were replicated 20 times for a total of 40 aphids per treatment. The percentage of aphids probed on plants of each treatment was recorded for both 30 min and 6 hr recordings. Eleven different behaviors were analyzed for each aphid per 30 min recording: time to the first probe and second probe (sec), time to the first potential drop (cellular puncture, sec), total number of probes, number of potential drops per probe, duration of each potential drop (sec), duration of each probe (sec), number of archlets, duration of cellular puncture phase II-1 (sec), phase II-2 (sec), and phase II-3(sec). For the 6 hr recording, eleven different behaviors were analyzed for each aphid: total probe duration (sec), total number of probes, probe duration (sec), time to the first probe, time to the first E1 (phloem salivation, sec), time to the first E2 (phloem ingestion, sec), time to the first G (xylem ingestion), duration of E1, E2, and G (sec).

4.2.5 Data Analysis

For laboratory and greenhouse bioassay, means and standard error of intrinsic rate of population growth ($r_m$), net replacement rate ($R_0$), finite rate of increase ($\lambda$), doubling time ($DT$), mean generation time ($T$) was calculated for treatment and control respectively, using the Jackknifing procedure described in Meyers et al. (1986). The percentage of aphids probed per treatment was compared in Ch-square test (PROC FREQ, SAS Institute, 2013). For probing behaviors, the values recorded for each treatment were analyzed in ANOVA (PROC GLIMMIX, SAS Institute, 2013). The potential drops w/ and w/out archlets were analyzed separately for the duration of cellular puncture subphase, II-1, II-2, and II-3, and overall duration of each potential
Means and standard errors of each probe behaviors were calculated for each treatment (PROC MEANS, SAS Institute, 2013).

4.3 Results

4.3.1 Population Dynamics

GPA on SPW-infested and uninfested plants performed Type II survivorship curves with essentially constant slopes of death rates with 50% of the nymphs perishing at day 8 (Fig. 4.1). The death rates were slightly sharper when GPA was feeding on SPW-infested plants. Given an initial starting population of 50 aphids, the population of GPA feeding on uninfested plants would be 200 more than the one feeding on SPW-infested plants after 8 days (Fig. 4.2). Intrinsic rate of increase \( r_m \), net reproductive rate \( R_0 \), and finite rate of increase \( \lambda \) of GPA growing on SPW-infested plants were lower compared to uninfested plants (Table 4.1). Mean generation time and doubling time were longer on uninfested plants than SPW-infested plants (Table 4.1).

![Fig. 4.1. Age-specific survivorship \( l_x \) of GPA feeding on SPW-infested vs uninfested sweetpotato plants.](image-url)
Fig. 4.2. Population abundance of GPA growing on SPW-infested vs uninfested sweetpotato plants from day 1 to day 8 given an initial population of 50 females.

Table 4.1. Life-table statistics of GPA (n = 240) on SPW infested vs uninfested sweetpotato.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Infested (mean ± se)</th>
<th>Uninfested (mean ± se)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intrinsic rate of increase ( (r_m) )</td>
<td>0.180 ± 0.016</td>
<td>0.213 ± 0.015</td>
</tr>
<tr>
<td>Net reproductive rate ( (R_0) )</td>
<td>4.25 ± 0.05</td>
<td>5.40 ± 0.05</td>
</tr>
<tr>
<td>Mean generation time ( (T) )</td>
<td>8.05</td>
<td>7.90</td>
</tr>
<tr>
<td>Doubling time ( (DT) )</td>
<td>3.85</td>
<td>3.25</td>
</tr>
<tr>
<td>Finite rate of increase ( (\lambda) )</td>
<td>1.20</td>
<td>1.24</td>
</tr>
</tbody>
</table>

4.3.2 Probing Behaviors

For GPA, the percentage of aphids probed on the SPW-infested plants were lower than on uninfested plants over 30 min \( (\chi^2 = 4.02, P = 0.04, \text{Table 4.2}) \). In contrast, the percentage was lower on the uninfested plants than the SPW-infested plants over 6 hr \( (\chi^2 = 6.65, P = 0.02, \text{Table 4.4}) \). Time to the first potential drop was longer on SPW-infested than uninfested plants \( (F = 4.95, P = 0.03, \text{Table 4.2}) \). GPA feeding on uninfested plants had higher number of archlets per potential drop and total number of archlets \( \text{(per pd: } F = 6.24, P = 0.01; \text{total archlets: } F = 15.33, P < 0.01, \text{Table 4.4}) \).
Table 4.2. Feeding behavior of GPA on SPW infested vs uninfested sweetpotato in 30 min (n = 80). Significant results were marked with asterisk ($P < 0.05$).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Infested (mean ± se)</th>
<th>Uninfested (mean ± se)</th>
<th>$P$-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>% probes</td>
<td>90.00</td>
<td>72.50</td>
<td>0.04 *</td>
</tr>
<tr>
<td>Time to 1st probe (sec)</td>
<td>227.64 ± 50.72</td>
<td>233.47 ± 55.12</td>
<td>0.85</td>
</tr>
<tr>
<td>Time to the next probe (sec)</td>
<td>108.62 ± 18.51</td>
<td>99.75 ± 19.33</td>
<td>0.61</td>
</tr>
<tr>
<td>Time to the next pd (sec)</td>
<td>47.40 ± 2.43</td>
<td>41.13 ± 2.66</td>
<td>0.03 *</td>
</tr>
<tr>
<td>Duration of pd (sec)</td>
<td>3.91 ± 0.12</td>
<td>4.07 ± 0.13</td>
<td>0.34</td>
</tr>
<tr>
<td>Duration of probe (sec)</td>
<td>331.16 ± 42.46</td>
<td>285.67 ± 44.29</td>
<td>0.30</td>
</tr>
<tr>
<td>No. of pd per probe</td>
<td>5.78 ± 0.75</td>
<td>4.86 ± 0.77</td>
<td>0.29</td>
</tr>
<tr>
<td>No. of probes</td>
<td>3.34 ± 0.46</td>
<td>3.35 ± 0.46</td>
<td>0.90</td>
</tr>
<tr>
<td>No. of archlets per pd</td>
<td>0.39 ± 0.07</td>
<td>0.61 ± 0.08</td>
<td>0.01 *</td>
</tr>
<tr>
<td>Total No. of archlets</td>
<td>5.10 ± 1.74</td>
<td>15.67 ± 2.06</td>
<td>&lt; 0.01 *</td>
</tr>
<tr>
<td>Pd w/archlets duration (sec)</td>
<td>5.03 ± 0.19</td>
<td>5.38 ± 0.18</td>
<td>0.13</td>
</tr>
<tr>
<td>II-1 w/ archlets duration (sec)</td>
<td>1.51 ± 0.04</td>
<td>1.46 ± 0.04</td>
<td>0.24</td>
</tr>
<tr>
<td>II-2 w/ archlets duration (sec)</td>
<td>0.96 ± 0.03</td>
<td>0.92 ± 0.03</td>
<td>0.12</td>
</tr>
<tr>
<td>II-3 w/ archlets duration (sec)</td>
<td>2.61 ± 0.17</td>
<td>3.08 ± 0.16</td>
<td>0.04 *</td>
</tr>
<tr>
<td>Pd w/out archlet duration (sec)</td>
<td>3.79 ± 0.10</td>
<td>3.73 ± 0.12</td>
<td>0.69</td>
</tr>
<tr>
<td>II-1 w/out archlets duration (sec)</td>
<td>1.60 ± 0.16</td>
<td>1.91 ± 0.17</td>
<td>0.11</td>
</tr>
<tr>
<td>II-2 w/out archlets duration (sec)</td>
<td>0.90 ± 0.02</td>
<td>0.93 ± 0.02</td>
<td>0.05 *</td>
</tr>
<tr>
<td>II-3 w/out archlets duration (sec)</td>
<td>1.18 ± 0.04</td>
<td>1.21 ± 0.04</td>
<td>0.34</td>
</tr>
</tbody>
</table>
Table 4.2). The duration of cellular puncture phase II-3 was longer on uninfested plant than SPW-infested ones when potential drops had archlets ($F = 6.22, P = 0.04$, Table 4.2). The duration of cellular puncture phase II-2 was long on uninfested plant than SPW infested plants when potential drops did not have archlets ($F = 3.95, P = 0.05$, Table 4.2). Total number of probes was larger during 6 hr on uninfested plant vs. SPW-infested ($F = 6.08, P = 0.02$, Table 4.3). Although the total probe duration was not different, the average duration per probe was lower on uninfested plants vs. SPW-infested plants ($F = 7.54, P = 0.01$, Table 4.4). Time to the first probe was longer for GPA feeding on uninfested vs. SPW-infested plants ($F = 3.92, P = 0.05$, Table 4.3).

Table 4.3. Feeding behavior of GPA on SPW infested vs uninfested sweetpotato in 6 hr (n =80). Significant results were marked with asterisk ($P < 0.05$).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Infested (mean ± se)</th>
<th>Uninfested (mean ± se)</th>
<th>$P$-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>% Aphid w/Probe</td>
<td>70.00</td>
<td>92.50</td>
<td>0.02</td>
</tr>
<tr>
<td>#Probes</td>
<td>6.68 ± 1.20</td>
<td>11.28 ± 1.65</td>
<td>0.02</td>
</tr>
<tr>
<td>Total probe duration (10^3 sec)</td>
<td>14.35 ± 1.29</td>
<td>12.29 ± 1.10</td>
<td>0.23</td>
</tr>
<tr>
<td>Mean probe duration (10^3 sec)</td>
<td>3.39 ± 0.88</td>
<td>1.66 ± 0.32</td>
<td>0.01</td>
</tr>
<tr>
<td>Time to the 1st probe (10^3 sec)</td>
<td>0.24 ± 0.09</td>
<td>0.90 ± 0.28</td>
<td>0.05</td>
</tr>
<tr>
<td>Mean E1 duration (10^3 sec)</td>
<td>0.17 ± 0.05</td>
<td>0.17 ± 0.09</td>
<td>0.99</td>
</tr>
<tr>
<td>Mean E2 duration (10^3 sec)</td>
<td>2.93 ± 0.99</td>
<td>2.40 ± 0.82</td>
<td>0.70</td>
</tr>
<tr>
<td>Mean G duration (10^3 sec)</td>
<td>1.67 ± 0.51</td>
<td>1.98 ± 0.48</td>
<td>0.74</td>
</tr>
<tr>
<td>Time to 1st E1 (10^3 sec)</td>
<td>1.88 ± 0.53</td>
<td>1.46 ± 0.19</td>
<td>0.21</td>
</tr>
<tr>
<td>Time to 1st E2 (10^3 sec)</td>
<td>3.25 ± 0.95</td>
<td>1.92 ± 0.45</td>
<td>0.15</td>
</tr>
<tr>
<td>Time to 1st G (10^3 sec)</td>
<td>2.00 ± 0.84</td>
<td>1.35 ± 0.24</td>
<td>0.49</td>
</tr>
<tr>
<td>% E1 duration</td>
<td>9.53 ± 2.68</td>
<td>5.74 ± 1.55</td>
<td>0.28</td>
</tr>
<tr>
<td>% E2 duration</td>
<td>30.57 ± 7.88</td>
<td>37.35 ± 7.55</td>
<td>0.54</td>
</tr>
<tr>
<td>% G duration</td>
<td>32.92 ± 6.77</td>
<td>42.24 ± 4.88</td>
<td>0.42</td>
</tr>
</tbody>
</table>
For CA, time to the next potential drop was significantly longer on uninfested plants ($F = 4.55, P = 0.03$, Table 4.4). Aphids spent long time in potential drops with archlets on uninfested plants ($F = 5.08, P = 0.03$, Table 4.4). For potential drops with archlets, cellular puncture phase II-2 had longer duration on SPW-infested plant ($F = 5.29, P = 0.03$, Table 4.4), while II-3 had shorter duration on SPW-infested plant ($F = 6.93, P = 0.01$, Table 4.4). For potential drops without archlets, cellular puncture phase II-1 had longer duration on uninfested plants ($F = 25.48, P < 0.01$, Table 4.4). No probing behaviors were found different between SPW-infested and uninfested plants for 6 hr recordings (Table 4.5).

Table 4.5. Feeding behavior of CA on SPW infested vs uninfested sweetpotato in 6 hr (n =80). Significant results were marked with asterisk ($P < 0.05$).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Infested (mean ± se)</th>
<th>Uninfested (mean ± se)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>% Aphid w/Probe</td>
<td>75.00</td>
<td>67.50</td>
<td>0.62</td>
</tr>
<tr>
<td>#Probes</td>
<td>5.73 ± 1.24</td>
<td>4.03 ± 0.73</td>
<td>0.23</td>
</tr>
<tr>
<td>Total probe duration (10^3 sec)</td>
<td>12.26 ± 1.43</td>
<td>12.29 ± 1.10</td>
<td>0.21</td>
</tr>
<tr>
<td>Mean probe duration (10^3 sec)</td>
<td>3.21 ± 0.79</td>
<td>2.32 ± 0.43</td>
<td>0.35</td>
</tr>
<tr>
<td>Time to the 1st probe (10^3 sec)</td>
<td>1.70 ± 0.64</td>
<td>3.20 ± 0.97</td>
<td>0.20</td>
</tr>
<tr>
<td>Mean E1 duration (10^3 sec)</td>
<td>0.05 ± 0.01</td>
<td>0.03 ± 0.01</td>
<td>0.30</td>
</tr>
<tr>
<td>Mean E2 duration (10^3 sec)</td>
<td>3.22 ± 1.60</td>
<td>2.40 ± 0.82</td>
<td>0.07</td>
</tr>
<tr>
<td>Mean G duration (10^3 sec)</td>
<td>1.69 ± 0.51</td>
<td>2.76 ± 1.05</td>
<td>0.74</td>
</tr>
<tr>
<td>Time to 1st E1 (10^3 sec)</td>
<td>1.48 ± 0.42</td>
<td>1.81 ± 0.46</td>
<td>0.60</td>
</tr>
<tr>
<td>Time to 1st E2 (10^3 sec)</td>
<td>1.48 ± 0.27</td>
<td>2.20 ± 0.82</td>
<td>0.13</td>
</tr>
<tr>
<td>Time to 1st G (10^3 sec)</td>
<td>1.50 ± 0.30</td>
<td>1.47 ± 0.28</td>
<td>0.99</td>
</tr>
<tr>
<td>% E1 duration</td>
<td>2.01 ± 1.09</td>
<td>2.00 ± 0.70</td>
<td>0.90</td>
</tr>
<tr>
<td>% E2 duration</td>
<td>49.66 ± 19.40</td>
<td>24.48 ± 14.03</td>
<td>0.33</td>
</tr>
<tr>
<td>% G duration</td>
<td>36.16 ± 4.65</td>
<td>41.64 ± 4.94</td>
<td>0.49</td>
</tr>
</tbody>
</table>
Table 4.4. Feeding behavior of CA on SPW infested vs uninfested sweetpotato in 30 min (n = 80). Significant results were marked with asterisk ($P < 0.05$).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Infested (mean ± se)</th>
<th>Uninfested (mean ± se)</th>
<th>$P$-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>% probes</td>
<td>50.00</td>
<td>47.50</td>
<td>0.82</td>
</tr>
<tr>
<td>Time to 1st probe (sec)</td>
<td>556.78 ± 120.21</td>
<td>597.63 ± 122.84</td>
<td>0.77</td>
</tr>
<tr>
<td>Time to the next probe (sec)</td>
<td>76.17 ± 21.77</td>
<td>116.61 ± 21.09</td>
<td>0.18</td>
</tr>
<tr>
<td>Time to the next pd (sec)</td>
<td>48.41 ± 4.90</td>
<td>59.68 ± 5.20</td>
<td>0.03 *</td>
</tr>
<tr>
<td>Duration of pd (sec)</td>
<td>4.38 ± 0.15</td>
<td>4.43 ± 0.16</td>
<td>0.79</td>
</tr>
<tr>
<td>Duration of probe (sec)</td>
<td>429.02 ± 62.23</td>
<td>361.24 ± 62.23</td>
<td>0.44</td>
</tr>
<tr>
<td>No. of pd per probe</td>
<td>6.16 ± 0.98</td>
<td>4.86 ± 0.98</td>
<td>0.35</td>
</tr>
<tr>
<td>No. of probes</td>
<td>1.23 ± 0.28</td>
<td>1.26 ± 0.28</td>
<td>0.90</td>
</tr>
<tr>
<td>No. of archlets per pd</td>
<td>0.71 ± 0.18</td>
<td>0.71 ± 0.19</td>
<td>0.98</td>
</tr>
<tr>
<td>Total No. of archlets</td>
<td>10.54 ± 4.32</td>
<td>9.78 ± 4.50</td>
<td>0.89</td>
</tr>
<tr>
<td>Pd w/ archlets duration (sec)</td>
<td>7.19 ± 0.43</td>
<td>8.37 ± 0.44</td>
<td>0.03 *</td>
</tr>
<tr>
<td>II-1 w/ archlets duration (sec)</td>
<td>1.62 ± 0.06</td>
<td>1.68 ± 0.06</td>
<td>0.37</td>
</tr>
<tr>
<td>II-2 w/ archlets duration (sec)</td>
<td>1.15 ± 0.07</td>
<td>0.98 ± 0.07</td>
<td>0.03 *</td>
</tr>
<tr>
<td>II-3 w/ archlets duration (sec)</td>
<td>4.41 ± 0.43</td>
<td>5.80 ± 0.44</td>
<td>0.01 *</td>
</tr>
<tr>
<td>Pd w/out archlet duration (sec)</td>
<td>3.88 ± 0.10</td>
<td>3.94 ± 0.10</td>
<td>0.47</td>
</tr>
<tr>
<td>II-1 w/out archlets duration (sec)</td>
<td>1.60 ± 0.03</td>
<td>1.76 ± 0.03</td>
<td>&lt; 0.01 *</td>
</tr>
<tr>
<td>II-2 w/out archlets duration (sec)</td>
<td>0.91 ± 0.02</td>
<td>0.88 ± 0.02</td>
<td>0.20</td>
</tr>
<tr>
<td>II-3 w/out archlets duration (sec)</td>
<td>1.42 ± 0.09</td>
<td>1.35 ± 0.09</td>
<td>0.23</td>
</tr>
</tbody>
</table>
4.4 Discussion

Our study had similar results to the previous studies on life-table statistics of GPA on sweetpotato plant (cv. Beauregard). Wosula et al. (2013b) estimated the life-table statistics of GPA on whole plant of Beauregard. The intrinsic rate was 0.338 compared to 0.213 in our study. The mean generation time was 9 days compared to 5 days here. The net reproductive rate was 19.7 compares to 7.9 in our study. The higher statistics in the study of Wosula’s were expected since the study used whole plants instead of leaf punches, which entailed a mechanic wound to the plant. The doubling time and finite rate of increase were similar in both studies. Life history performance can be influenced by the virus-infection status of the plant (Wosula et al. 2013b). GPA performed better with higher values of intrinsic rate of population increase and net reproductive rate, and shorter generation time on Beauregard plant infected with a mixture of three potyviruses: *Sweet potato feathery mottle virus*, *Sweet potato virus G*, and *Sweet potato virus 2*. However, in our study, the GPA performed better on the plant without SPW infestation. Thus, it indicates that herbivory infestation in the root part created different sources of stress or changes in the plant compares to virus-infection. The plant-mediated response from herbivory infestation decreased the fitness of the GPA. Given an initial number of 50 individuals (Fig. 4.2), the GPA population can reach over $1,000 \times 10^3$ on uninfested plants over one generation without disturbance, which is 25% more than growing on SPW-infested plant. Thus, the field population of GPA can be reduced sharply when the field is infested with SPW.

GPA and CA are both efficient vectors of potyviruses on sweetpotato (Wosula et al. 2012). The inoculation efficiency range of GPA is 0 - 39%, which is wider than that of CA as 0 - 22% (Wosula et al. 2012). From the probing behaviors in our study, GPA and CA had different feeding patterns between SPW-infested and uninfested plant. The probe frequencies of GPA during 30
min and 6 hr were between the treatments. However, such differences were not detected on CA for either 30 min or 6 hr. Additionally, the percentage of aphid probed on the plant ranged from 70 - 92% for GPA, while the percentage of that for CA ranged from 48 to 75%. This indicates that as a virus vector, GPA is more competent than CA since GPA is more likely to perform feeding activity on sweetpotato than CA. During the first 30 min, GPA and CA had different virus-acquisition related behaviors, and they were between the infested and uninfested plant, such as time between the first probe and the first intracellular puncture (pd), number of archlets per pd, total number of archlets, and duration of pd. The duration of intracellular puncture pd subphase II-1 and II-2 were also different between the aphid species. However, the duration of subphase II-3 with archlets during the potential drop was longer on uninfested plant for both aphid species. During the 6 hr recording, virus-acquisition related behaviors such as total number of probes, mean probe duration, and time to the first probe were different between infested and uninfested plant for GPA. However, CA performed the same between the treatments for all recorded behaviors. Thus, the feeding behaviors of GPA and CA were different even though they are both efficient vectors of potyviruses.

GPA probed more often in the 30 min on SPW-infested plant but switched to probe more on the uninfested plant during the 6 hours. It indicates that the feeding behavior during the short term is different from the behavior in the longer period. For the first 30 min, behaviors related to virus acquisition, such as subphase II-3 with archlets were inhibited on SPW-infested plant. Reduced duration of subphase II-3 could limit the ingestion of cytosolic fluid and impede virus acquisition (Powell et al. 1995, Martín et al. 1997). A longer duration between first probe initiation and first pd was observed on infested plant, indicating a slower initiation of the intracellular puncture on infested plant. Although GPA had significantly longer pd subphase II-2 on SPW-infested plant
than uninfested, the implication of subphase II-2 remains unkown (Powell 2005). For 6 hr recording, time to the first probe on infested plant was shorter compares to uninfested plant. The reduced pre-probing duration indicates a faster initiation of probing behavior on infested plant. The number of probes on infested plant was only half of that on uninfested plant during the 6 hr, indicating GPA incline to accept uninfested plants as host.

In contrast, CA took a shorter period between the first probe to the first pd on SPW-infested plant, indicating a faster initiation on intracellular puncture on infested plant. The pd subphase II-1, II-2, and II-3 was affected by SPW infestation. The feeding duration for virus inoculation (subphase II-1) and acquisition (subphase II-3) were decreased on SPW-infested plant, indicating a negative effect of SPW infestation on aphid feeding.

Sweetpotato larval infestation within the storage roots induces plant secondary compounds, furanoterpenoids, in the plant (Uritani et al. 1975). Several of the terpenes have been identified as toxic to herbivores or related to reduce insect injury. Resin glycosides is lethal to the diamondback moth, *Plutella xylostella* (L.). Caffeic acid is a phenolic stress metabolite and is associated with insect survivorship and oviposition behavior (Stange et al. 2001, Harrison et al. 2003, Harrison et al. 2008). Octadecyl and hexadecyl esters of hydroxycinnamic acids affect the survivorship and development of insects on sweetpotato (Stevenson et al. 2009). Trans-β-farnesence emanated from the glandular hairs on the foliage of the wild potato (*Solanum berthaultil* Hawkes) serves as a repellent to GPA (Avé et al. 1987, Gibson and Pickett 1983). Although the quantity of secondary compounds in the foliage induced by root-infestation of SPW is still unknown, it is possible that the induced secondary compounds provided feeding cues for GPA. Additionally, the feeding activity of the SPW larvae reduce the biomass of the root, which result in reduced above-ground biomass. The reduced above-ground plant quality could inhibit the fitness and development of the
above-ground herbivores. Although our study did not reveal the underlying changes in the host plant from SPW infestation, future studies could focus on identifying the mechanisms of plant-mediated responses and utilized the reduced fitness and feeding behavior from SPW infestation.

In conclusion, below-ground SPW infestation had negative impacts on the fitness, population increase, and feeding behavior of above-ground virus vector, GPA and CA. The plant-mediated response between herbivores was found in our study. The reduced population combined with inhibited feeding behaviors could largely decrease the virus infection on sweetpotato. Although releasing SPW in the field to decrease virus infection is unfeasible, the underlying mechanisms could be used potentially for management purposes. Future studies should focus on identifying the mechanism and further incorporate it into the field for management of virus infection.

4.5 References


CHAPTER 5. BASELINE SUSCEPTIBILITY OF SWEETPOTATO WEEVIL 
(COLEOPTERA: CURCULIONIDAE) POPULATIONS IN LOUISIANA TO SELECTED 
INSECTICIDES

5.1 Introduction

Sweetpotato, Ipomoea batatas L. (Lam), is in the family Convolvulaceae and has been identified as the crop that can alleviate food security (Mukhopadhyay et al. 2011). Sweetpotato is one of the major staple foods worldwide, with over 95% of production in the developing countries of Asia and Africa (FAO 2013). In the U. S., sweetpotato production had an economic value of $706 million in 2016 (USDA 2016). North Carolina, Mississippi, California, and Louisiana are the four leading sweetpotato producing states (USDA 2016). In Louisiana, sweetpotato is attacked by numerous insect pests including root-feeders, foliage feeders, and virus vectors (Smith and Beuzelin 2015). Among these insect pests, sweetpotato weevil (SPW), Cylas formicarius, is one of the major threats to sweetpotato production (Chalfant 1990). Both larvae and adults can cause direct damage to the harvestable tissue. SPW is a multivoltine insect capable of completing its life cycle on a single root. Larvae develop inside of the root and induce secondary metabolites that render the root unpalatable (Uritani 1975).

All commercial sweetpotato fields in Louisiana are under a mandatory monitoring program for the presence of SPW using pheromone traps provided by the Louisiana Department of Agriculture and Forestry (LDAF) (Smith and Beuzelin 2015). Commercial fields with previous detection of SPW are required, by law, to spray the field with rotated insecticides. Thus, the management of SPW in Louisiana relies heavily on chemical control. Currently, the labeled insecticides for the management of SPW in Louisiana include beta-cyfluthrin, bifenthrin, carbaryl, phosmet, and a pre-mix of beta-cyfluthrin and imidaclopid (Smith and Beuzelin 2015). The listed chemicals are targeted against SPW adults and affect the insect Central Nervous System.
Imidacloprid, carbaryl, and phosmet are synaptic poisons and bind to insect nicotinic receptors, resulting in disruption in the nicotinergic neuronal pathway and, ultimately, inhibiting the production of acetylcholinesterase (Ware 2004). Insects display symptoms such as paralysis and eventually die. Beta-cyfluthrin and bifenthrin are in the class of pyrethroids which are considered axonic poisons (Ware 2004). Pyrethroids are synthetic derivatives of flower extracts of *Chrysanthemum* species, which have insecticidal properties (Elliott et al. 1978). Pyrethroids act as sodium channel modulators and inhibit channel deactivation, leaving the sodium channel open which results in death (Vijverberg and vanden Bercken 1990).

The labeled insecticides include several classes of insecticides, including neonicotinoids, pyrethroids and organophosphate, based on different modes of action. Although the spraying program seems to be working in maintaining low SPW population levels and in slowing SPW range expansion in Louisiana, there are concerns over intensive insecticide usage. Chemical control is also questionable for the control efficacy against SPW, due to the cryptic living habitat of SPW and limited the access of insecticides to SPW.

Another major disadvantage of continuous insecticide applications is the potential development of insecticide resistance (Roush and Tabashnik 2012). Initially, insecticide resistance is “the development of an ability in some individuals of a given organism to tolerate doses of a toxicant which would prove lethal to a majority of individuals in a normal population of the same organism” (World Health Organization, 1957). This definition has been modified with small changes (National Research Council 1986). Tabashinik et al. (2014) defined resistance in a more general concept as the “genetically based decrease in susceptibility to a pesticide”. Gene regulatory changes in insects, such as mutation, gene duplication and amplification, often lead to the increased efficiency of physiological processes used for detoxification, such as oxidation,
conjugation and excretion, as well as reducing target-site sensitivity (Liu et al. 2006). The increased production of detoxifying enzymes could be achieved through constitutive up-regulation and gene duplication/amplification in coding regions (Heckel 2012). Insecticide resistance has been reported in many insects, such as the maize weevil (*Sitophilus zeamais*), red flour beetle (*Tribolium castaneum*), lesser grain borer (*Rhyzopertha dominica*), and Colorado potato beetle (*Leptinotarsa decemlineata*) (Ribeiro et al. 2003, Alyokhin et al. 2008, Zettler and Cuperus 1990). Resistance to pyrethoids and organophosphate insecticides was reported in field-collected populations of *S. zeamais* (Ribeiro et al. 2003). *L. decemlineata* has been reported to be resistant to all major insecticide classes (Alyokhin et al. 2008).

Monitoring for field-developed increases in insecticide tolerance can help to alert and possibly prevent insecticide failures. According to the Arthropod Pesticide Resistance Database, SPW had been found resistant to three chemicals; aldrin, DDT, and dieldrin in 1974 (https://www.pesticideresistance.org). However, no incidents of insecticide resistance have been reported since 1974. A few studies have been conducted on monitoring the insecticide susceptibility of SPW to selected insecticides. Smith and Hammond (2006) evaluated the baseline susceptibility of SPW populations collected from Louisiana and Texas to insecticides using adult vial tests (AVT). The toxicity ranking of the formulated chemicals tested was methyl parathion > bifenthrin > cyfluthrin > phosmet > carbaryl. This study also reported the differences in insecticide susceptibility between populations in Louisiana and Texas. Mason et al. (1991) also evaluated the baseline of SPW to the technical grade of five insecticides; parathion, carbamate methomyl, chlorpyrifos, endosulfan, and carbaryl using topical applications. Hwang and Hung (1994) tested the efficacy of two formulated organophosphates, chlorpyrifos and terbufos in the field before and after planting. Both studies reported that chlorpyrifos had the highest toxicity and recommended
chlorpyrifos for field management of SPW. However, chlorpyrifos is not registered for SPW control. An updated study is needed due to the intensity of the mandatory spraying schedule and the change in registered products for SPW control. The objective of this study was to evaluate the susceptibility of SPW populations to five insecticides labeled for SPW management.

5.2 Materials and Methods

5.2.1 Insect Source

SPW adults used in this study included a laboratory colony (maintained > 4 yrs.) and field-collected colonies. For the laboratory colony, weevils were originally collected from a field population in south Louisiana and maintained under dark conditions at 27.0 ± 1ºC with 65.0 ± 5% RH in the laboratory (Mao et al. 2001). The colony has been reared on whole sweetpotato roots for over four years and has not been exposed to any insecticides since colony initiation. The adults of the colony were kept in 14 L plastic containers (Sterilite®, Townsend, MA). A section (21.0 by 25.0 cm) of the top lid was cut and replaced with screen wire mesh (Saint-Gobain ADFORS, Grand Island, NY) to allow airflow. The inside edge (approx. 2.5 cm) of the bottom container was coated with Vaseline Petroleum Jelly (Vaseline®, Trumbull, CT) mixed with mineral oil (Top Care®, Skokie, IL) at a 3:1 ratio to prevent the escape of adult SPW. Storage roots of cultivar Beauregard were provided by the Louisiana State Agricultural Center (LSU AgCenter) Sweetpotato Research Station located at Chase, LA, planted and harvested under the production guidelines (Smith et al. 2012). To maintain the colony, two to three fresh storage roots were added to the colony on a weekly basis, providing a food source and oviposition site for the adults. Infested roots with SPW eggs were incubated at 27.0 ± 1ºC in one-quart, cylindrical paper containers (Ridgid Paper Tube Corporation, Wayne, NJ), 17.5 cm in depth and 8.6 cm in diam., until adults of the next generation emerged
Field colonies were initiated with field-collected SPW adults using pheromone traps and infested roots placed inside and underneath the traps. Field trapping was conducted from May to October in 2015 and from July to October in 2016. Universal funnel traps (IPM-SPWK-12) and pheromone lures (IPM-SPW-25) of sweetpotato weevil were purchased from Great Lakes IPM (Vestaburg, MI). Each funnel trap was attached to an iron pole pegged in the soil with the top of the trap adjusted to stand 50 cm above the soil surface. Each funnel trap was baited with one pheromone lure and replaced every two weeks. One storage root of cv. Beauregard was placed inside of the bucket as a food source for the trapped males. Below the bucket, three storage roots of cv. Beauregard were placed to provide oviposition sites for females in 2015. In 2016, these storage roots underneath the funnel trap were secured in a hand-crafted cage (30 cm in length, 15 cm in height, 15 cm in width) built using mesh hardware cloth (Weitech, Inc., Sisters, OR). The cage was attached to the iron pole that was used to secure the trap.

Trapping locations were selected on commercial farms where SPW had been observed in past growing season and to represent geographically diverse sweetpotato production areas. In 2015, the trapping locations included four commercial fields located in Mansura, Evergreen, Grand Prairie, and Iota, Louisiana. In 2016, the trapping locations were on the same farms but in different field. However, the Mansura location was dropped due to the lack of SPW presence in 2015. The selected commercial fields were managed under the mandatory SPW spray program. Thus, SPW captured from commercial fields would have experienced intensive insecticide exposure (estimated 10 to 14 applications per year). In each field, 7 to 10 traps were set up 50 m apart along field edges. All traps were checked and replaced with fresh roots once every other week from mid-May to late-September in 2015 and from late-July to early-September in 2016. The field-collected adults were reared separately by location under laboratory conditions. The infested roots
of all traps were collected and retained in cylinder paper containers until adults emerged and were placed with field-collected adults. In 2015, two commercial-field colonies, ‘Evergreen’ and ‘Prairie’, were set up with SPW collected from Evergreen and Grand Prairie, respectively. In 2016, one commercial field colony, ‘Iota’, was set up from SPW collected from Iota. In addition to commercial farms, another trapping location included the Burden Research Station in Baton Rouge where two traps were placed for one month in 2015 and 2016. A colony, ‘Burden’, with SPW collected from the Research Station was set up in 2015 and 2016. Sweetpotato plots at the research station receive applications of bifenthrin once pre-planting and of phosmet five times post-planting. However, sweetpotato is not produced commercially in the region.

5.2.2 Insecticides

Analytical technical grade chemicals, beta-cyfluthrin (PESTANAL®, Sigma-Aldrich, St. Louis, MO), bifenthrin (Supelco, Sigma-Aldrich, St. Louis, MO), carbaryl (PESTANAL®, Sigma-Aldrich, St. Louis, MO), imidacloprid (PESTANAL®, Sigma-Aldrich, St. Louis, MO), and phosmet (PESTANAL®, Sigma-Aldrich, St. Louis, MO) were diluted in serial concentrations in acetone (Sigma-Aldrich, St. Louis, MO). The concentrations (µg/ml) of all chemicals are listed in Table 5.1. The listed ranges of concentrations were developed from pilot experiments conducted prior to the study. A control concentration of pure acetone was included for all chemicals.

5.2.3 Adult Vial Test

The adult vial test (AVT) method was used to evaluate the efficacy of SPW to beta-cyfluthrin, bifenthrin, carbaryl, and phosmet. The bioassay protocol was developed from Miller et al. (2010), using 20-ml scintillation glass vials. This bioassay contained three trials. In each trial, the diluted concentrations of each chemical were tested with adults emerged within two to four weeks. For
Table 5.1. The diluted concentrations (µg/ml) of the analytical-grade insecticides, including beta-cyfluthrin, bifenthrin, carbaryl, imidacloprid and phosmet.

<table>
<thead>
<tr>
<th>Year</th>
<th>Insecticide</th>
<th>Concentrations (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2015</td>
<td>Beta-cyfluthrin</td>
<td>0.25  0.50  1.00  2.00  4.00  -</td>
</tr>
<tr>
<td></td>
<td>Bifenthrin</td>
<td>0.03  0.06  0.13  0.25  0.50  -</td>
</tr>
<tr>
<td></td>
<td>Carbaryl</td>
<td>8.06 16.12 32.24 64.48 128.96 -</td>
</tr>
<tr>
<td></td>
<td>Imidacloprid</td>
<td>0.02  0.03  0.07  0.14  0.28  -</td>
</tr>
<tr>
<td></td>
<td>Phosmet</td>
<td>3.20  6.30 12.50 25.00 50.00  -</td>
</tr>
<tr>
<td>2016</td>
<td>Beta-cyfluthrin</td>
<td>20   40   80   160   800   -</td>
</tr>
<tr>
<td></td>
<td>Bifenthrin</td>
<td>0.01  0.06  0.07  0.13  0.70  -</td>
</tr>
<tr>
<td></td>
<td>Carbaryl</td>
<td>20   40   80   400   1550  -</td>
</tr>
<tr>
<td></td>
<td>Imidacloprid</td>
<td>0.01  0.02  0.03  0.09  0.17  1.67</td>
</tr>
<tr>
<td></td>
<td>Phosmet</td>
<td>7    14   57   284   -    -</td>
</tr>
</tbody>
</table>

One concentration of a chemical, 20 vials were prepared, 10 for males and 10 for females. A half milliliter of the prepared concentration was added to the interior surface of the vials using an Eppendorf micropipette (Eppendorf North America Inc., New York, NY). The vials were then air dried by being rotated on a commercial hot dog roller without heat under the fume hood. In this way, the acetone that was used to dilute the analytical grade would be evaporated, leaving only the chemical on the surface of the vials. One adult was added to a single vial and secured with the plastic cap. The vials were kept under dark conditions at 22.0 ± 1°C in the laboratory for 24 h. According to another study of SPW using AVT, mortality was defined as the uncoordinated movement and inability of maintaining an upright posture after being exposed to warm air flow (Smith and Hammond 2006). The total number of dead weevils were recorded at each concentration using the same standard to define mortality.
5.2.4 Root-core Bioassay

The root-core bioassay was a modified version of AVT to test the susceptibility of adults to the systemic chemical imidacloprid. The set-up of experimental design was the same as AVT, except the application of the chemical. Root cores (diam. 1.4 cm, depth 1 cm) were cut using a No. 9 cork borer (diam. 1.4 cm, depth 2.0 cm) (Humboldt, Raleigh, NC). A single root core was then immersed in the imidacloprid solutions for 10 s and air dried for 30 min under a fume hood. After the acetone evaporated, one root core was kept with one adult in a vial for 24 h. For one concentration per trial, 20 vials were prepared and tested with 20 adults (10 males and 10 females). Mortality was defined using the same standard in AVT. Mortality per concentration per trial was recorded.

5.2.5 Discriminating-dose Bioassay

In 2015, all colonies were tested at LC$_{50}$ and LC$_{90}$ of each insecticide developed from the baseline of the laboratory colony. The testing methods were AVT for non-systemic insecticides and root-core method for imidacloprid as described above. The mortality at both concentrations was recorded. In 2016, the field colonies were only tested with the LC$_{90}$ of each insecticide developed from the baseline of the laboratory colony. If a colony had survivorship at the LC$_{90}$, a probit line was then developed. Otherwise, the colony was marked as susceptible to the insecticide.

5.2.6 Data Analysis

Mortality was adjusted by Abbott’s formula (1925). For the laboratory colony, lethal concentrations (LC) of each insecticide killing 50% and 90% of the exposed adults were calculated by probit analysis (PROC PROBIT, SAS Institute, 2016). In 2015, the mortality among colonies at LC$_{50}$ and LC$_{90}$ of the insecticides were compared using ANOVA (PROC GLIMMIX, SAS Institute, 2016). In 2016, the probit line was developed when survivorship was detected from the
diagnostic concentration. The resistance ratio of LC$_{50}$ and LC$_{90}$ of the tested population(s) according to the method of Robertson and Preisler (1992) using the LC$_{50}$ and LC$_{90}$ of the lab colony as the ratio divisor.

5.3 Results

The baseline susceptibility varied among chemicals. Probit lines of susceptibility to beta-cyfluthrin (2015), carbaryl (2015), imidacloprid (2015), and bifenthrin (2016) were detected with non-normal distribution on the residual errors from the Chi-square test of Lack-Of-Fit (Table 5.2). No control mortality occurred in this study. From the LC$_{50}$ values, the toxicity rank of the five chemicals was imidacloprid > bifenthrin > phosmet > beta-cyfluthrin > carbaryl. The slopes of most probit lines are within the range of 1 and 2, except bifenthrin (2015) and beta-cyfluthrin (2016). The larger slope value indicates a more sensitive response to the insecticide within the range of diluted concentrations. Comparing the results across years, the confidence intervals of the LC$_{50}$s only overlapped when SPW were tested with imidacloprid and phosmet, indicating different susceptibilities of SPW to beta-cyfluthrin, bifenthrin and carbaryl between years. However, the confidence intervals of the LC$_{90}$s overlapped for all insecticides but bifenthrin between 2015 and 2016, which indicates the susceptibility was only different for bifenthrin between the two years. Such inconsistent overlaps at different lethal concentrations were expected as the chi-square test reported non-homogeneity in the lab colony. The non-homogeneity indicated that the variation at each concentration did not all follow normal distribution with the same variance.

In 2015, all colonies had no difference in mortality at LC$_{50}$ of each insecticide (Table 5.4). However, the mortality among colonies was different when tested on carbaryl and phosmet at LC$_{90}$ (Table 5.5; Carbaryl: $F = 9.83$, d.f. = 3, 6, $P = 0.01$; Phosmet: $F = 12.0$, d.f. = 3, 6, $P = 0.006$). From
the pairwise comparison, the colony ‘Prairie’ had significantly lower mortality than other colonies. In 2016, the colony ‘Burden’ had no survivorship at the LC\textsubscript{90}s of all chemicals. Thus, no probit lines were developed for the colony ‘Burden’. The colony ‘Iota’ had survivors at the LC\textsubscript{90}s of imidacloprid and bifenthrin. The probit lines of Colony ‘Iota’ to imidacloprid and bifenthrin were listed in Table 5.3. The Chi-square test indicated non-homogeneity in the probit line of bifenthrin and homogeneity in that of imidacloprid. For bifenthrin, the confidence intervals of both LC\textsubscript{50} and LC\textsubscript{90} overlap between the colony ‘Iota’ and the laboratory colony, indicating no difference in the ranges of LC\textsubscript{50} and LC\textsubscript{90} respectively. Similar overlap was found comparing LC\textsubscript{50} ranges of imidacloprid between laboratory colony and the colony ‘Iota’. However, the confidence interval of LC\textsubscript{90} did not overlap between the laboratory colony and the colony ‘Iota’. The LC\textsubscript{90} of imidacloprid was higher in the colony ‘Iota’ than the laboratory colony. From the resistance ratios, the colony ‘Iota’ was one-fold more resistant to bifenthrin and two-fold more resistant to imidacloprid at the LC\textsubscript{90}s.

5.4 Discussion

From our study, bifenthrin and imidacloprid have the highest toxicity to SPW, while carbaryl is the least toxic. Our result is consistent with previous studies. Carbaryl was found to be the least toxic to SPW using both AVT and topical application (Smith and Hammond 2006, Mason et al. 1991). In the previous studies, the insecticide with the highest toxicity would be recommended to use for chemical control (Mason et al. 1991). In our study, no large resistance ratios (> 10 folds) were found. Colony ‘Iota’ and ‘Prairie’ were found to be slightly resistant to bifenthrin and imidacloprid, which indicates the potential of resistance development of SPW in the commercial field. Field-evolved resistance is the inheritable increase in susceptibility of a population to an insecticide or a toxin caused by insecticide application in the field (Tabashnik et al. 2014).
Table 5.2. Baseline susceptibility of SPW adults of the laboratory colony to selected insecticides in a laboratory adult vial test in 2015 and 2016.

<table>
<thead>
<tr>
<th>Year</th>
<th>Insecticides</th>
<th>N</th>
<th>Slope (SE)</th>
<th>LC\textsubscript{50} (95% CL)</th>
<th>LC\textsubscript{90} (95% CL)</th>
<th>$\chi^2$ (df)\textsuperscript{a}</th>
</tr>
</thead>
<tbody>
<tr>
<td>2015</td>
<td>Beta-cyfluthrin</td>
<td>450</td>
<td>1.06 (0.22)</td>
<td>0.95 (0.61, 1.46)</td>
<td>15.35 (6.43, 116.09)</td>
<td>59.41 (28)*</td>
</tr>
<tr>
<td></td>
<td>Bifenthrin</td>
<td>450</td>
<td>2.69 (0.22)</td>
<td>0.07 (0.07, 0.08)</td>
<td>0.22 (0.19, 0.28)</td>
<td>35.79 (28)</td>
</tr>
<tr>
<td></td>
<td>Carbaryl</td>
<td>450</td>
<td>1.53 (0.25)</td>
<td>46.66 (34.23, 68.32)</td>
<td>632.23 (420.87, &gt;1000)</td>
<td>69.44 (28)*</td>
</tr>
<tr>
<td></td>
<td>Imidacloprid</td>
<td>450</td>
<td>1.34 (0.23)</td>
<td>0.03 (0.02, 0.04)</td>
<td>0.28 (0.17, 0.70)</td>
<td>55.11 (28)*</td>
</tr>
<tr>
<td></td>
<td>Phosmet</td>
<td>450</td>
<td>0.84 (0.18)</td>
<td>21.80 (14.43, 41.29)</td>
<td>55.32 (39.71, 92.83)</td>
<td>40.85 (28)</td>
</tr>
<tr>
<td>2016</td>
<td>Beta-cyfluthrin</td>
<td>300</td>
<td>3.90 (0.47)</td>
<td>31.30 (27.10, 35.55)</td>
<td>66.69 (56.53, 84.54)</td>
<td>20.86 (28)</td>
</tr>
<tr>
<td></td>
<td>Bifenthrin</td>
<td>300</td>
<td>1.83 (0.26)</td>
<td>0.12 (0.09, 0.17)</td>
<td>0.61 (0.37, 1.39)</td>
<td>47.03 (28)*</td>
</tr>
<tr>
<td></td>
<td>Carbaryl</td>
<td>300</td>
<td>1.54 (0.16)</td>
<td>92.83 (71.90, 120.56)</td>
<td>321.37 (173.38, &gt;1000)</td>
<td>20.67 (28)</td>
</tr>
<tr>
<td></td>
<td>Imidacloprid</td>
<td>360</td>
<td>1.53 (1.66)</td>
<td>0.05 (0.03, 0.07)</td>
<td>0.31 (0.21, 0.54)</td>
<td>20.01 (34)</td>
</tr>
<tr>
<td></td>
<td>Phosmet</td>
<td>240</td>
<td>2.10 (0.28)</td>
<td>13.59 (10.66, 16.99)</td>
<td>55.32 (39.71, 92.83)</td>
<td>23.00 (22)</td>
</tr>
</tbody>
</table>

\textsuperscript{a} Asterisk (*) indicates a lack of fit of the data to the probit model is significant ($P < 0.05$).

Table 5.3. Baseline susceptibility of SPW adults collected on the commercial farm site in Iota, LA to bifenthrin and imidacloprid in 2016.

<table>
<thead>
<tr>
<th>Insecticides</th>
<th>N</th>
<th>Slope (SE)</th>
<th>LC\textsubscript{50} (95% CL)</th>
<th>LC\textsubscript{90} (95% CL)</th>
<th>$\chi^2$ (df)\textsuperscript{a}</th>
<th>RR\textsubscript{50}\textsuperscript{b}</th>
<th>RR\textsubscript{90}\textsuperscript{c}</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bifenthrin</td>
<td>300</td>
<td>1.17 (0.19)</td>
<td>0.11 (0.07, 0.16)</td>
<td>1.32 (0.64, 5.15)</td>
<td>41.74 (28)*</td>
<td>0.92</td>
<td>2.16</td>
</tr>
<tr>
<td>Imidacloprid</td>
<td>300</td>
<td>1.17 (0.14)</td>
<td>0.08 (0.06, 0.11)</td>
<td>1.02 (0.58, 2.45)</td>
<td>26.95 (28)</td>
<td>1.60</td>
<td>3.29</td>
</tr>
</tbody>
</table>

\textsuperscript{a} Asterisk (*) indicates a lack of fit of the data to the probit model is significant ($P < 0.05$).

\textsuperscript{b} Resistance ratios of LC\textsubscript{50} and LC\textsubscript{90} were calculated by the method of Robertson and Preisler (1992) by using the LC\textsubscript{50} and LC\textsubscript{90} developed from the lab colony as the ratio divisor.
Table 5.4. Proportion of mortality of SPW adults from different colonies collected from commercial farms and research station tested on LC$_{50}$ of the selected insecticides developed from laboratory baseline.

<table>
<thead>
<tr>
<th>Insecticide</th>
<th>Colony</th>
<th>Concentration</th>
<th>%Mortality</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>mean</td>
<td>sd</td>
</tr>
<tr>
<td>Beta-cyfluthrin</td>
<td>Laboratory</td>
<td>LC$_{50}$</td>
<td>46.67</td>
<td>8.43</td>
</tr>
<tr>
<td></td>
<td>Evergreen</td>
<td>LC$_{50}$</td>
<td>44.44</td>
<td>13.77</td>
</tr>
<tr>
<td></td>
<td>Prairie</td>
<td>LC$_{50}$</td>
<td>35.56</td>
<td>23.73</td>
</tr>
<tr>
<td></td>
<td>Burden</td>
<td>LC$_{50}$</td>
<td>37.78</td>
<td>13.11</td>
</tr>
<tr>
<td>Bifenthrin</td>
<td>Laboratory</td>
<td>LC$_{50}$</td>
<td>39.33</td>
<td>21.19</td>
</tr>
<tr>
<td></td>
<td>Evergreen</td>
<td>LC$_{50}$</td>
<td>35.33</td>
<td>23.32</td>
</tr>
<tr>
<td></td>
<td>Prairie</td>
<td>LC$_{50}$</td>
<td>22.00</td>
<td>20.38</td>
</tr>
<tr>
<td></td>
<td>Burden</td>
<td>LC$_{50}$</td>
<td>19.33</td>
<td>13.86</td>
</tr>
<tr>
<td>Carbaryl</td>
<td>Laboratory</td>
<td>LC$_{50}$</td>
<td>30.00</td>
<td>0.00</td>
</tr>
<tr>
<td></td>
<td>Evergreen</td>
<td>LC$_{50}$</td>
<td>5.57</td>
<td>10.33</td>
</tr>
<tr>
<td></td>
<td>Prairie</td>
<td>LC$_{50}$</td>
<td>13.33</td>
<td>15.06</td>
</tr>
<tr>
<td></td>
<td>Burden</td>
<td>LC$_{50}$</td>
<td>20.00</td>
<td>14.14</td>
</tr>
<tr>
<td>Imidacloprid</td>
<td>Laboratory</td>
<td>LC$_{50}$</td>
<td>23.33</td>
<td>22.51</td>
</tr>
<tr>
<td></td>
<td>Evergreen</td>
<td>LC$_{50}$</td>
<td>5.00</td>
<td>8.37</td>
</tr>
<tr>
<td></td>
<td>Prairie</td>
<td>LC$_{50}$</td>
<td>11.67</td>
<td>11.69</td>
</tr>
<tr>
<td></td>
<td>Burden</td>
<td>LC$_{50}$</td>
<td>8.33</td>
<td>4.08</td>
</tr>
<tr>
<td>Phosmet</td>
<td>Laboratory</td>
<td>LC$_{50}$</td>
<td>1.67</td>
<td>4.08</td>
</tr>
<tr>
<td></td>
<td>Evergreen</td>
<td>LC$_{50}$</td>
<td>3.33</td>
<td>8.16</td>
</tr>
<tr>
<td></td>
<td>Prairie</td>
<td>LC$_{50}$</td>
<td>1.67</td>
<td>4.08</td>
</tr>
<tr>
<td></td>
<td>Burden</td>
<td>LC$_{50}$</td>
<td>1.67</td>
<td>4.08</td>
</tr>
</tbody>
</table>
Table 5.5. Proportion of mortality of SPW adults from different colonies collected from commercial farms and research station tested on LC\textsubscript{90} of the selected insecticides developed from laboratory baseline.

<table>
<thead>
<tr>
<th>Insecticide</th>
<th>Colony</th>
<th>Concentration</th>
<th>%Mortality</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>mean</td>
<td>sd</td>
</tr>
<tr>
<td>Beta-cyfluthrin</td>
<td>Laboratory</td>
<td>LC\textsubscript{90}</td>
<td>100.00</td>
<td>0.00</td>
</tr>
<tr>
<td></td>
<td>Evergreen</td>
<td>LC\textsubscript{90}</td>
<td>100.00</td>
<td>0.00</td>
</tr>
<tr>
<td></td>
<td>Prairie</td>
<td>LC\textsubscript{90}</td>
<td>100.00</td>
<td>0.00</td>
</tr>
<tr>
<td></td>
<td>Burden</td>
<td>LC\textsubscript{90}</td>
<td>100.00</td>
<td>0.00</td>
</tr>
<tr>
<td>Bifenthrin</td>
<td>Laboratory</td>
<td>LC\textsubscript{90}</td>
<td>96.00</td>
<td>7.17</td>
</tr>
<tr>
<td></td>
<td>Evergreen</td>
<td>LC\textsubscript{90}</td>
<td>86.00</td>
<td>25.81</td>
</tr>
<tr>
<td></td>
<td>Prairie</td>
<td>LC\textsubscript{90}</td>
<td>82.00</td>
<td>14.42</td>
</tr>
<tr>
<td></td>
<td>Burden</td>
<td>LC\textsubscript{90}</td>
<td>84.67</td>
<td>18.87</td>
</tr>
<tr>
<td>Carbaryl</td>
<td>Laboratory</td>
<td>LC\textsubscript{90}</td>
<td>53.33</td>
<td>5.16</td>
</tr>
<tr>
<td></td>
<td>Evergreen</td>
<td>LC\textsubscript{90}</td>
<td>43.44</td>
<td>27.33</td>
</tr>
<tr>
<td></td>
<td>Prairie</td>
<td>LC\textsubscript{90}</td>
<td>18.33</td>
<td>14.72</td>
</tr>
<tr>
<td></td>
<td>Burden</td>
<td>LC\textsubscript{90}</td>
<td>71.67</td>
<td>14.72</td>
</tr>
<tr>
<td>Imidacloprid</td>
<td>Laboratory</td>
<td>LC\textsubscript{90}</td>
<td>53.33</td>
<td>15.06</td>
</tr>
<tr>
<td></td>
<td>Evergreen</td>
<td>LC\textsubscript{90}</td>
<td>35.00</td>
<td>16.43</td>
</tr>
<tr>
<td></td>
<td>Prairie</td>
<td>LC\textsubscript{90}</td>
<td>35.00</td>
<td>24.29</td>
</tr>
<tr>
<td></td>
<td>Burden</td>
<td>LC\textsubscript{90}</td>
<td>13.33</td>
<td>16.33</td>
</tr>
<tr>
<td>Phosmet</td>
<td>Laboratory</td>
<td>LC\textsubscript{90}</td>
<td>100.00</td>
<td>0.00</td>
</tr>
<tr>
<td></td>
<td>Evergreen</td>
<td>LC\textsubscript{90}</td>
<td>100.00</td>
<td>0.00</td>
</tr>
<tr>
<td></td>
<td>Prairie</td>
<td>LC\textsubscript{90}</td>
<td>91.11</td>
<td>6.89</td>
</tr>
<tr>
<td></td>
<td>Burden</td>
<td>LC\textsubscript{90}</td>
<td>100.00</td>
<td>0.00</td>
</tr>
</tbody>
</table>
In our sampling locations, all commercial fields had experience intensive insecticide applications. However, we did not observe significant resistance development. The genetic property of the insecticide resistance in SPW could have slowed down the development process. For a closed population, the initial allele frequency plays an important role in the development of insecticide resistance (Gould et al. 1997). According to the mutation-selection equilibrium theory, the frequency of any allele prior to the favorable selection is kept at equilibrium between the mutation and selection forces (Haldane 1927). When selection alone acts as pressure on the generation of new alleles, mutation would act against selection and maintain the frequency at balance inclusively. By this mathematically developed theory, the resistance allele frequency exists at no more than one percent (Roush and McKenzie 1987). In insects, the resistance allele frequency does not always follow the mathematical estimation and does not guarantee a positive correlation with resistance development (Roush and McKenzie 1987, Tabashnik et al. 2014). The dominance of resistance genes(s) is another factor that could affect the time of resistance development (Roush and McKenzie 1987). When resistance is completely recessive, the resistance development could take longer than when it is partially dominant (Tabashnik and Carrière 2013). Even if resistance develops, insects need to overcome the challenges of fitness costs and incomplete resistance (Tabashnik and Carrière 2013). Thus, the farmers would not need to consider the resistance development of SPW in the field.

Insect populations, including SPW, are rarely closed in nature. SPW is known to be capable of feeding and surviving on alternative host plants, including many plant species in the family of Convolvulaceae., such as such as I. pes-caprae, I. hederacea var. integriuscula, I. hederifolia, I. triloba, I. horsfalliae and I. obscura (Chittenden 1919, Cockerham 1943, Muruvanda et al. 1986, Jansson et al. 1989, Reddy and Chi 2015). These Ipomoea species commonly exist in and around
sweetpotato fields. Other species, including carrot (*Dacus carota* L.), radish (*Raphanus sativus* L.), and rhubarb (*Rheum rhaponticum* L.) can also serve as alternative hosts for SPW (Muruvanda et al. 1986). The presence of alternative hosts could provide shelter for SPW and allow SPW to avoid contact with insecticides. The alternative hosts could also inhibit the resistance development. SPW feeding on alternative hosts without exposure to insecticide could mate with individuals in the sweetpotato field and slow down the resistance development (Caprio and Tabashnik 1992).

Major insecticide studies depend on probit analysis and developing lethal concentrations of certain proportions of mortality. Although this methodology has been widely adopted in many studies of insecticide resistance monitoring, the probit analysis is unable to detect differences in insecticide susceptibility when the resistance frequency is below 4% (Roush and Miller 1986). However, not all insect species maintain a resistance frequency above 4% (Roush and Tabashnik 1987). Thus, the probit analysis stands a chance of being incapable of differentiating susceptibilities of insect populations. Our found the lack of fit in several probit lines and indicated the probit line was not a good estimation of the mortality rate in response to different concentrations. The significant of the *P*-value for Chi-square test indicates a large heterogeneity and different level of insecticide resistance in the population (Hagle and Mitchell 1992). The probit analysis is not powerful when the heterogeneity in the population is large.

In general, our study established baseline susceptibility of SPW to five labeled insecticides for SPW management. The current insecticide spraying schedule in commercial fields has not lead to severe resistance or failure of insecticides.

5.5 References


CHAPTER 6. EFFECT OF HOST PLANT ON THE INSECTICIDE SUSCEPTIBILITY OF SWEETPOTATO WEEVIL (COLEOPTERA: CURCULIONIDAE)

6.1 Introduction

Sweetpotato weevil (SPW), *Cylas formicarius elegantulus* (Summers) is one of the most severe pests of sweetpotato, *Ipomoea batatas* L. (Lam), internationally (Sorensen 2009). The damage caused by larval and adult feeding can lead to severe yield losses both in the field and in storage (Sutherland, 1986). Additionally, the feeding behavior of adult and larvae can induce plant secondary compounds, such as terpenoids and phenols, rendering the roots unpalatable. Even at low densities, SPW can cause a significant economic loss due to the multicomponent damage of reduced yield, cosmetic concerns, and unmarketable taste of the roots (Sorensen 2009). In the U.S., SPW occurs throughout the Southern region of Texas to the coastal regions of North Carolina (Chalfant 1990). In Louisiana, SPW is found in the southern parishes and currently is under a quarantine management plan (LDAF 2014). Any commercial fields identified with SPW must, by law, follow a mandatory spray schedule to slow down SPW range expansion. Currently, the insecticides labeled for SPW control include beta-cyfluthrin, bifenthrin, carbaryl, imidacloprid, and phosmet (Smith and Beuzelin 2015). Although the mandatory spray program is working, the disadvantages of chemical control still exist.

Aside from low environmental compatibility and non-target effects, insecticide efficacy is questionable. Since the larvae develop within the root, insecticide applications focus on reducing adult populations. Due to the cryptic living condition of the adults, direct exposure to insecticide applications is difficult to achieve. Additionally, intensive applications of insecticides can lead to the development of insecticide resistance and ultimately a failure in chemical control (Roush and McKenzie 1987).
Insecticide resistance has been found in over 500 insect species (Whalon et al. 2012). The impact of insecticide resistance could reach over $4 billion annually in the U. S. and substantially more worldwide (Pimentel et al. 1992). To reduce the reliance on insecticides, integrated pest management (IPM) was developed in the 1950s and expanded to integrate multiple control tactics including biological, cultural, chemical tactics and host plant resistance. The idea is to manage insect populations to reduce economic losses while alleviating environmental contamination and health concerns (Smith et al. 1976).

Host plant resistance is the purposeful exploitation of intraspecific variation in plant resistance (Eigenbrode and Trumble 1994). Insect-resistant cultivars have been identified in many crops and vegetables (Stout and Davis 2009). Sweetpotato cultivars are under recurrent selection for resistance to pest injury and profitable production. For the management of SPW, host plant resistance has great potential and could reduce the intensity and number of insecticide applications. Previous studies have shown that cv. Murasaki could reduce the adult emergence of SPW in the laboratory (Story et al. 2000). From chapter 2 and 3 of this dissertation, cv. Murasaki is both antibiotic and antixenotic to SPW. Thus, cv. Murasaki has the potential to be deployed as a resistant cultivar and integrated into a SPW management plan. However, the compatibility of host plant resistance and insecticide against SPW has not been evaluated.

The effect of host plants at species and cultivar level on insecticide susceptibility has been tested on several insects in the orders Hemiptera, Coleoptera, and Lepidoptera (Kea et al. 1978, Campbell and Wynne 1985). The larvae of soybean looper, *Pseudoplusia includens* (Walker), exhibited different susceptibility to methyl parathion when feeding on different cultivars of soybean, *Glycine max* (Kea et al. 1978). Giustolin et al. (2001) evaluated the mortality of tomato leafminer, *Tuta absoluta* (Meyrick), when feeding on two species of the genus *Lycopersicon, L.*
*Hirsutum* f. *glabratum* (insect resistant) and *L. esculentum* (insect susceptible) treated with *Bacillus thuringiensis* var. *kurstaki* (Btk). Btk susceptibility was found to differ when *T. absoluta* was reared on different species. Larvae of diamondback moth, *Plutella xylostella* (L.), respond differently to abamectin and cypermethrin when reared on different cultivars of cabbage, *Brassica oleracea* L. (Abro and Wright 1989).

The types of interaction between resistant host plants and insecticide efficacy are characterized as additive, synergistic, and antagonistic (Abro and Wright 1989, Giustolin et al. 2001). An additive effect between a resistant cultivar and an insecticide occurs when no interference between the two control tactics is found. A synergistic effect between a resistant cultivar and an insecticide occurs when both tactics are additive while an antagonistic effect occurs when one tactic interferes with the other tactic. If a synergistic or additive effect were identified, utilization of resistant cultivars could reduce insecticide application rates and concentrations (Eigenbrode and Trumble 1994, Teete 1994). Nevertheless, if a resistant cultivar is reducing the insecticide efficacy, the resistant cultivar is not compatible with chemical control and ultimately jeopardize the effectiveness of the overall management.

In this study, our objectives were 1) to establish baseline susceptibilities of SPW fed on three commercial cultivars of sweetpotato, Beauregard, Evangeline, and Murasaki, to the five labeled insecticides for the management of SPW in Louisiana and 2) to categorize the interactions of host plant on insecticide efficacy.

**6.2 Materials and Methods**

**6.2.1 Insect Sources**

Sweetpotato weevils were collected from a field population in south Louisiana and maintained under dark conditions at 27.0 ± 1°C with 65.0 ± 5% RH in the laboratory (Mao et al.
The colony has been reared on the whole root of sweetpotato for over four years and has never been exposed to any insecticides. The adults of the colony were kept in 14 L plastic containers (Sterilite®, Townsend, MA). A section (21.0 by 25.0 cm) of the top lid was cut and replaced with screen wire mesh (Saint-Gobain ADFORS, Grand Island, NY) to allow airflow. The inside edge (approx. 2.5 cm) of the bottom container was coated with Vaseline Petroleum Jelly (Vaseline®, Trumbull, CT) mixed with mineral oil (Top Care®, Skokie, IL) at a 3:1 ratio to prevent the escape of adult SPW. Storage roots were provided by the Louisiana State Agricultural Center (LSU AgCenter) Sweetpotato Research Station located at Chase, LA, planted and harvested under recommended production guidelines (Smith et al. 2012). To maintain the colony, two to three fresh storage roots were added to the colony on a weekly basis, providing a food source and oviposition site for the adults. Adult males and females were randomly selected from the lab colony and fed cvs. Evangeline or Murasaki for two weeks.

6.2.2 Insecticides

Analytical standards, beta-cyfluthrin (PESTANAL®, Sigma-Aldrich, St. Louis, MO), bifenthrin (Supelco, Sigma-Aldrich, St. Louis, MO), carbaryl (PESTANAL®, Sigma-Aldrich, St. Louis, MO), imidacloprid (PESTANAL®, Sigma-Aldrich, St. Louis, MO), and phosmet (PESTANAL®, Sigma-Aldrich, St. Louis, MO) were diluted in serial concentrations in acetone (Sigma-Aldrich, St. Louis, MO). Insecticides and concentrations (µg/ml) are listed in Table 6.1. A control concentration of pure acetone was included for all chemicals.

6.2.3 Adult Vial Test

The adult vial test (AVT) was used to evaluate the efficacy of SPW to beta-cyfluthrin, bifenthrin, carbaryl, and phosmet (Miller et al. 2010). Scintillation glass vials (20 ml) were used in this study. This bioassay contained three trials. In each trial, the diluted concentrations of each
Table 6.1. The diluted concentrations of the analytical-grade insecticides.

<table>
<thead>
<tr>
<th>Insecticide</th>
<th>Concentrations (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Beta-cyfluthrin</td>
<td>20  40  80  160  800</td>
</tr>
<tr>
<td>Carbaryl</td>
<td>20  40  80  400  1550</td>
</tr>
<tr>
<td>Imidacloprid</td>
<td>0.01 0.02 0.04 0.1 0.17</td>
</tr>
<tr>
<td>Bifenthrin</td>
<td>0.01 0.06 0.07 0.13 0.7</td>
</tr>
<tr>
<td>Phosmet</td>
<td>7   14   57  284 -</td>
</tr>
</tbody>
</table>

chemical were tested with adults feeding on each cultivar. For one concentration of a chemical, 20 vials were prepared, 10 for males and 10 for females. A half milliliter of the prepared concentration was added to the interior surface of the vials using Eppendorf micropipette (Eppendorf North America Inc., New York, NY). The vials were then air dried under a fume hood and rotated on a commercial hot dog roller without heat. In this way, the acetone that was used to dilute the analytical grade would be evaporated, leaving only the chemical on the surface of the vials. One adult was added to a single vial and secured with the plastic cap. The vials were kept under dark conditions at 22.0 ± 1°C in the laboratory for 24 h. Mortality was defined and recorded as the uncoordinated movement and inability of maintaining an upright posture after being exposed to warm airflow.

6.2.4 Root-core Bioassay

The root-core bioassay was a modified version of AVT to test the susceptibility of adults to the systemic chemical imidacloprid. The set-up of experimental design was the same as AVT, except the application of the chemical. Root cores (diam. 2 cm, depth 1 cm) were cut using No. 9 cork borer (diam. 1.4 cm, depth 2.0 cm) (Humboldt, Raleigh, NC). A single root core was then immersed in the solutions of imidacloprid for 10 s and air dried for 30 min under the fume hood. After the acetone was evaporated, one root core was placed in one vial and kept with one adult for 24 h. Mortality was defined and recorded using the same standards above.
6.2.5 Data Analysis

Mortality was adjusted by Abbott’s formula (1925). Lethal concentrations (LC) of each chemical killing 50% and 90% of the exposed adults (LC\(_{50}\) and LC\(_{90}\)) were calculated by probit analysis (PROC PROBIT, SAS Institute, 2016). Confidence intervals of both LC\(_{50}\) and LC\(_{90}\) values were also calculated. Resistance ratios of LC\(_{50}\) and LC\(_{90}\) of each chemical feeding on cvs. Evangeline and Murasaki were calculated according to the method of Robertson and Preisler (1992) by using the LC\(_{50}\) and LC\(_{90}\) from SPW feeding on cv. Beauregard as the ratio divisor.

6.3 Results

The susceptibility of SPW was different among insecticides (Table 6.2). No control mortality was observed in this study. Bifenthrin and imidacloprid had a comparable toxicity with similar LC\(_{50}\) and LC\(_{90}\) values. Beta-cyfluthrin and phosmet were less toxic to SPW adults (Table 6.2). When comparing LC\(_{50}\)s between beta-cyfluthrin and phosmet, SPW adults were more susceptible to phosmet. However, the LC\(_{90}\)s between the two insecticides were overlapping, indicating no difference in toxicity at the LC\(_{90}\). Carbaryl is the least toxic insecticide to SPW adults (Table 6.2). A lack of fit was found in several chemicals tested with various cultivars: beta-cyfluthrin on cv. Murasaki, bifenthrin on cvs. Beauregard and Murasaki, carbaryl on cv. Murasaki, imidacloprid on cvs. Evangeline and Murasaki (Table 6.2). The heterogeneity of SPW in these treatments was large, indicating the dose-response curve was not an efficient model. The response at each log-transformed concentration did not follow a normal distribution with a random variance.

Based on the confidence intervals of LC\(_{50}\) by insecticides, SPW adults feeding on cv. Murasaki were the least susceptible to carbaryl, followed by SPW feeding on cv. Evangeline, and the most susceptible on cv. Murasaki. SPW on cv. Murasaki were 2.9- and 2.3- fold more resistant compared to SPW on cv. Beauregard. In contrast, SPW adults were more susceptible to
imidacloprid on cv. Murasaki than on cvs. Beauregard and Evangeline. SPW were 0.8- and 0.2-fold more susceptible on cvs. Evangeline and Murasaki respectively compared to SPW on cv. Beauregard. Using the reciprocal of resistance ratios, SPW adults were 5-fold more resistant to imidacloprid on cv. Beauregard compared to cv. Murasaki. This indicates a synergistic effect of cv. Murasaki on imidacloprid efficacy at low doses. However, no difference in mortality was found among cultivars at LC$_{90}$ of all insecticides except carbaryl based on resistance ratios. SPW feeding on cv. Murasaki is 2.3-fold more resistant compared to feeding on cv. Beauregard. However, the confidence intervals of LC$_{90}$ by carbaryl were not different between cv. Murasaki and cv. Beauregard, showing no significance in SPW mortality.

6.4 Discussion

A syneristic effect of cv. Murasaki on imidacloprid efficacy was found in this study, as SPW feeding on cv. Murasaki was slightly more susceptible to imidacloprid at a low dose. Similar results were found in peach-potato aphid, *Myzus persicae* (Sulzer), to foliage insecticides on resistant potato cv. Cardinal (Saljoqi and van Emden 2003). The low doses of fenitrothion, oxydemeton-methyl, and methamidophos reached the same level of control of the aphids feeding on cv. Cardinal as with high dose of the insecticides on a susceptible cv. Desiree. A insect-resistant cv. NC6 of peanut, *Arachis hypogaea* L., reduced insecticide dose by 20% and still sufficiently controlled the southern corn rootworm, *Diabrotica undecimpunctata howardt* Barber (Campbell and Wynne 1985). A synergistic effect can be utilized in many ways to optimize IPM. Insecticide application rates and action thresholds can be modified to be cultivar-specific (Eigenbrode and Trumble 1994). Murasaki has been identified as both antibiotic and antixenotic to SPW. Thus, the SPW population would develop at a slower growth rate and is less likely to reach an economic injury level. In this way, the number of insecticide applications can be reduced. Additionally,
Table 6.2. The susceptibility of SPW adults feeding on different sweetpotato cultivars, Beauregard, Evangeline, and Murasaki, to selected insecticides (µg/ml) in a laboratory test.

<table>
<thead>
<tr>
<th>Insecticides</th>
<th>Cultivars</th>
<th>N</th>
<th>Slope (SE)</th>
<th>LC&lt;sub&gt;50&lt;/sub&gt; (95% CL)</th>
<th>LC&lt;sub&gt;90&lt;/sub&gt; (95% CL)</th>
<th>χ² (df)&lt;sup&gt;a&lt;/sup&gt;</th>
<th>RR&lt;sub&gt;50&lt;/sub&gt;&lt;sup&gt;b&lt;/sup&gt;</th>
<th>RR&lt;sub&gt;90&lt;/sub&gt;&lt;sup&gt;c&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Beta-cyfluthrin</td>
<td>Beauregard</td>
<td>300</td>
<td>3.90 (0.47)</td>
<td>31.30 (27.10, 35.55)</td>
<td>66.69 (56.53, 84.54)</td>
<td>20.86 (28)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Evangeline</td>
<td>300</td>
<td>4.55 (0.52)</td>
<td>35.40 (31.34, 39.69)</td>
<td>67.67 (58.30, 83.50)</td>
<td>28.62 (28)</td>
<td>1.13</td>
<td>1.01</td>
</tr>
<tr>
<td></td>
<td>Murasaki</td>
<td>300</td>
<td>3.31 (0.44)</td>
<td>41.14 (33.69, 49.15)</td>
<td>100.35 (79.93, 141.93)</td>
<td>42.72 (28)*</td>
<td>1.31</td>
<td>1.50</td>
</tr>
<tr>
<td>Bifenthrin</td>
<td>Beauregard</td>
<td>300</td>
<td>1.83 (0.26)</td>
<td>0.12 (0.09, 0.17)</td>
<td>0.61 (0.37, 1.39)</td>
<td>47.03 (28)*</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Evangeline</td>
<td>300</td>
<td>1.95 (0.21)</td>
<td>0.11 (0.09, 0.14)</td>
<td>0.50 (0.35, 0.86)</td>
<td>31.25 (28)</td>
<td>0.92</td>
<td>0.82</td>
</tr>
<tr>
<td></td>
<td>Murasaki</td>
<td>300</td>
<td>1.52 (0.22)</td>
<td>0.15 (0.11, 0.22)</td>
<td>1.02 (0.55, 2.96)</td>
<td>44.88 (28)*</td>
<td>1.25</td>
<td>1.67</td>
</tr>
<tr>
<td>Carbaryl</td>
<td>Beauregard</td>
<td>300</td>
<td>1.54 (0.16)</td>
<td>92.83 (71.90, 120.56)</td>
<td>632.23 (420.87, &gt;1000)</td>
<td>20.67 (28)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Evangeline</td>
<td>300</td>
<td>1.81 (0.17)</td>
<td>165.73 (131.31, 213.63)</td>
<td>847.55 (586.67, &gt;1000)</td>
<td>20.06 (28)</td>
<td>1.79</td>
<td>1.36</td>
</tr>
<tr>
<td></td>
<td>Murasaki</td>
<td>300</td>
<td>1.74 (0.20)</td>
<td>270.02 (195.34, 393.19)</td>
<td>&gt;1000</td>
<td>44.14 (28)*</td>
<td>2.91</td>
<td>2.31</td>
</tr>
<tr>
<td>Imidacloprid</td>
<td>Beauregard</td>
<td>360</td>
<td>1.53 (1.66)</td>
<td>0.05 (0.03, 0.07)</td>
<td>0.31 (0.21, 0.54)</td>
<td>20.01 (34)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Evangeline</td>
<td>360</td>
<td>1.09 (0.15)</td>
<td>0.04 (0.02, 0.06)</td>
<td>0.59 (0.31, 1.72)</td>
<td>49.00 (34)*</td>
<td>0.80</td>
<td>1.90</td>
</tr>
<tr>
<td></td>
<td>Murasaki</td>
<td>360</td>
<td>0.76 (0.17)</td>
<td>0.01 (&lt;0.01, 0.02)</td>
<td>0.50 (0.18, 5.47)</td>
<td>89.50 (34)*</td>
<td>0.20</td>
<td>1.61</td>
</tr>
<tr>
<td>Phosmet</td>
<td>Beauregard</td>
<td>240</td>
<td>2.10 (0.28)</td>
<td>13.59 (10.66, 16.99)</td>
<td>55.32 (39.71, 92.83)</td>
<td>23.00 (22)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Evangeline</td>
<td>240</td>
<td>1.89 (0.34)</td>
<td>11.81 (7.54, 16.70)</td>
<td>56.15 (35.60, 132.96)</td>
<td>39.50 (22)*</td>
<td>0.87</td>
<td>1.02</td>
</tr>
<tr>
<td></td>
<td>Murasaki</td>
<td>240</td>
<td>2.13 (0.26)</td>
<td>16.45 (13.11, 20.60)</td>
<td>65.94 (47.18, 109.66)</td>
<td>12.41 (22)</td>
<td>1.21</td>
<td>1.19</td>
</tr>
</tbody>
</table>

- Asterisk (*) indicates a lack of fit of the data to the probit model is significant (P < 0.05).
- Resistance ratio of LC<sub>50</sub> was calculated by the method of Robertson and Preisler (1992) by using the LC<sub>50</sub> when feeding on cv. Beauregard as the ratio divisor.
- Resistance ratio of LC<sub>90</sub> was calculated using the same method of calculating RR<sub>50</sub>.

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imidacloprid could be applied at a lower rate but still control the population effectively when combined with cv. Murasaki. This would reduce the cost of control. More importantly, reduced insecticide use can alleviate environmental and health concerns as well as protecting natural enemies and beneficial insects. The control of insect pests through tritrophic interactions between plant, insect and natural enemies would be facilitated. The heterogeneity of the SPW in response to different insecticides was high at several treatments as indicated by Chi-square test. This indicates that the model accuracy of dose-response curves was compromised by the internal heterogeneity of the insect population itself (Hoskins and Craig 1962). In Chapter 5, the heterogeneity also existed in the field populations. Smith and Hammond (2006) also reported the lack-of-fit in insect populations collected from Louisiana and Texas on selected chemicals. The dose-response curve is developed based on the assumption of random distribution of the residual errors at each log-transformed dose. The lack-of-fit is a violation of the model assumption. Although the calculation of the dose-response curve is still valid, the calculation was built on a pre-assigned marginal heterogeneity parameter. Thus, an alternative model with better model accuracy is needed to address the variance between the insect individuals of SPW. Using a diagnostic dose of the insecticides is one example of an alternative model (Roush and Miller 1986). Usually, two to three times the \( LC_{99} \) is developed as the discriminating concentration/dose (Roush and Miller 1986). If survivorship is detected at the discriminating concentration, the insects can be marked as resistant. However, using diagnostic concentrations would not provide quantitative evaluations of insecticide resistance.
As the pressure of reducing the reliance on insecticide application is increasing, the importance of incorporating resistant cultivars into insect management will increase (Eigenbrode and Trumble 1994). Compatibility between resistant cultivars and insecticides has been identified in soybean, rice, peanut, cabbage, and potato (Kea et al. 1978, Heinrichs et al. 1984, Campbell and Wynne 1985, Abro and Wright 1989, Saljoqi and van Emden 2003). Cultivar-specific recommendations with strongly increased control efficacy on resistant cultivars will guarantee the growers a profitable yet environmentally friendly management plan.

In general, sweetpotato cultivars did not reduce the efficacy of insecticide. Only cv. Murasaki slightly increased the efficacy of imidacloprid at low doses. Murasaki is a compatible cultivar that can reduce the fitness and preference of SPW as well as increase insecticide efficacy. Future management of sweetpotato fields could consider cultivar-specific recommendations. For fields planted with cv. Murasaki, imidacloprid could be the primary choice for controlling SPW. In addition, a reduced concentration of imidacloprid should be considered to maximize the exploitation of cv. Murasaki.

6.5 References


CHAPTER 7. SUMMARY AND CONCLUSIONS

Sweetpotato weevil (SPW), *Cylas formicarius elegantulus* (Summers), is the most damaging root-feeding insect of sweetpotato, *Ipomoea batatas* L. (Lam), worldwide. Larval feeding on storage roots reduces yield and induces terpene production, rendering roots inedible. Selection of sweetpotato cultivars with resistance to insect pests has been carried out for over a century but no high yielding, production acceptable varieties are currently available that are resistant to SPW. Previous studies have compared cultivar effect on the behaviors of SPW but have not considered the effect of pre-imaginal experience. Hopkins’ Host-plant Selection Principle (Hopkins’ HSP) states that phytophagous insects have an oviposition preference for the host that they were reared on. In this study, we tested cultivar effect on oviposition preference of SPW reared on different cultivars for a minimum of two generations. For adults reared on cvs. Beauregard and Evangeline, adult oviposition preference followed their pre-imaginal experience. Thus, our results indicate a strong effect of host fidelity, supporting Hopkins’ HSP. Our results also confirm that cv. Murasaki is a resistant cultivar, resulting in reduced oviposition but not oviposition capacity. It is possible that the reduced oviposition is due to the stress-triggered oosorption from the females feeding on cv. Murasaki.

According to the Preference-Performance Hypothesis (PPH), females oviposit on the host that maximizes offspring fitness. To test PPH and previous experience on this linkage, we placed SPW eggs collected from all colonies on sweetpotato root cores of cvs. Beauregard, Evangeline, and Murasaki, and performed daily observations on offspring developmental time, egg-hatching percentage, immature survivorship, adult weight, and sex ratio. Overall, our data suggest cv. Beauregard is the most suitable host for larval performance, resulting in short SPW
developmental time and high survivorship. In contrast, cvs. Evangeline and Murasaki expressed antibiosis. Our study did not support PPH; the offspring fitness was not optimized on the preferred host by adult oviposition. Interestingly, the adult weight observed on cvs. Evangeline and Murasaki for both sexes was higher than on cv. Beauregard. This indicates that offspring adaptation occurred during immature stages on the inferior host. It is likely that the increased adult weight is due to increased egg capacity of adults to counter host unsuitability.

In Louisiana, sweetpotato is frequently infected with potyviruses which can be transmitted efficiently by green peach aphid (GPA), *Myzus persicae* (Sulzer), and cotton aphid (CA), *Aphis gossypii* Glover. Virus epidemiology depends on the fitness and feeding behaviors of the virus vector which is also influenced by host plant. In the field, sweetpotato is under attack by SPW. Host plant responds to herbivory injury by reallocating resources and inducing secondary plant compounds. Little is known on how plant disease vectors respond to plant-mediated injury from root herbivory. In this study, population dynamics of GPA were studied on sweetpotato plants grown from either SPW-infested or uninfested storage roots in the laboratory. GPA had a lower intrinsic rate of population increase, decreased longevity, and reduced net reproductive rate on plants grown from SPW-infested storage roots compared to plants from uninfested roots. Thus, GPA fitness was negatively influenced by SPW infestation. The stylet probing behavior of GPA and CA on SPW-infested vs. uninfested plants was monitored by electrical penetration graph (EPG) with direct current as the voltage source. The probing behaviors were recorded for 30 min (behaviors related to potyvirus acquisition and inoculation) and 6 hr (behaviors related to host acceptance and colonization). GPA probed more often during the first 30 min
on SPW-infested plants, but probed less often on infested plants during 6 hr recording periods. GPA feeding behavior was affected by SPW infestation, including duration between time to the first probe and time to the first potential drop, number of archlets, number of probes, duration of pd subphase II-2, and II-3 (virus acquisition). In contrast to GPA, CA did not exhibit difference in probing frequency between infested and uninfested plant. The feeding duration for virus inoculation (subphase II-1) and acquisition (subphase II-3) of CA were decreased on SPW-infested plant. This study indicates that below-ground herbivory can influence virus vector epidemiology by affecting the population dynamics and feeding behavior.

In Louisiana, SPW is a quarantinable pest; sweetpotato fields are under a mandatory spraying program if the presence of SPW is detected. The spray program involves applying insecticides every 10 to 14 days, rotating chemistries to reduce insecticide resistance. Such intensive insecticide applications can lead to the development of insecticide resistance and failures in chemical control. In our study, the baseline susceptibility of SPW to five chemicals recommended for SPW control was developed using dose response curves and a laboratory colony. Wild populations of SPW were collected from commercial sweetpotato fields and a research station in Louisiana using insect pheromone traps. SPW collected from different locations were maintained under laboratory conditions as different colonies and were evaluated for insecticide susceptibility in 2015 and 2016. Adult vial test (AVT) was used for all non-systemic insecticides. A new method was designed for testing a systemic insecticide, imidaclorpid, with modifications of AVT. In 2015, adults (males and females) of all colonies were tested using discriminating doses at the LC$_{50}$ and LC$_{90}$ of the insecticides from the laboratory baseline. In 2016, only a LC$_{90}$ diagnostic concentration was tested on field colonies.
In 2015, Colony ‘Prairie’ was found to be more resistant to carbaryl and phosmet at the LC$_{90}$. In 2016, Colony ‘Iota’ was found to be 1 to 2 folds more resistant to bifenthrin and imidacloprid compared to the laboratory colony. From our study, no high levels of resistance were detected, suggesting that the current spraying program is not building resistance of SPW to rotational insecticides.

Insecticide efficacy could also be affected by host plant. The current study showed the effect of sweetpotato cultivars Beauregard, Evangeline, and Murasaki on insecticide efficacy of five labeled insecticides for SPW management was evaluated. Adults from a laboratory colony of SPW were fed on the three separate cultivars for two weeks. Dose-response curves were developed for each insecticide on each cultivar. Synergistic effects between cultivar and host plant were found on cv. Murasaki which increased the susceptibility of SPW to imidacloriprid at a low dose. A weak antagonistic effect was also found on cv. Murasaki with decreased susceptibility of SPW to carbaryl. The resistance ratios on cvs. Evangeline and Murasaki were all less than 3 fold compared to SPW susceptible cv. Beauregard. Our study indicates that host plant resistance in sweetpotato is compatible with chemical control for SPW.
VITA

Jie Chen was born in Dazhou, Sichuan Province, China. After graduating from high school, she attended Southwest University in Chongqing, China and graduated in 2010 with a Bachelor’s degree in Plant Protection. After graduation, Jie was accepted into the graduate program in the School of Biology and Ecology in the University of Maine, Orono, where she received her Master degree in Entomology in 2013. Her master’s research focused on the insecticide susceptibility and heat-induced mortality of Colorado potato beetle (*Leptinotarsa decemlineata* L.).

In August 2013, Jie began her doctoral studies in the Department of Entomology at Louisiana State University (LSU), Baton Rouge. She joined Dr. Jeffrey A. Davis’s lab in 2014. Her doctorate research has focused on evaluation of the control tactics for the management of sweetpotato weevil, *Cylas formicarius elegantulus* (Summers). She received her Master degree of Applied Statistics in May 2017, from the Department of Experimental Statistics at LSU. She is currently completing the requirements for the degree of Doctor of Philosophy and plans to pursue her career as a biostatistician.

Jie is married to Brent Matthews and has two lovely fur-babies, Teemo and Poppy.