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Determining the Presence or Absence of Euscepes postfasciatus in Haiti and use of Molecular Markers for the Distinction of Cylas formicarius elangantulus Biotypes

Marie Rachele Lexidort
Louisiana State University and Agricultural and Mechanical College

DETERMINING THE PRESENCE OR ABSENCE OF *EUSCEPES POSTFASCIATUS* IN HAITI AND USE OF MOLECULAR MARKERS FOR THE DISTINCTION OF *CYLAS FORMICARIUS ELEGANTULUS* BIOTYPES

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ABSTRACT

Cylas formicarius elegantulus (Summers) and *Euscepes postfaciatus* (Fairmaire) are the most destructive sweetpotato weevils in the Caribean Basin. However, damage occurring in sweetpotato production in Haiti is attributed to *C. formicarius* (S), since it is known to be the most prevalent species while, conflicting information is available pertaining to the occurrence of the *E. postfaciatus* (F). This study was conducted in the summer of 2018 to determine the presence or absence of *E. postfaciatus* (F) in Haiti. 98 weevil samples from field (70%) and infested sweetpotato roots from field and regional markets were collected (30%) in the most relevant sweetpotato production regions in Haiti. Based on morphological characters, 100% of the weevils collected were identified as *C. formicarius* (S) suggesting that *E. postfaciatus* (F) is not present in Haiti.

Internal transcriptase spacer-1 (ITS-1) region of *C. formicarius* (S) samples collected in Haiti and Louisiana were used to determine the relatedness among *C. formicarius* (Summers) biotypes. Cluster analysis displayed high rate of similarities among the ITS-1 regions. The findings also show significant homology between biotypes of Haiti and Hawaii while Louisiana biotypes have no relatedness to those of Georgia, and Hawaii suggesting that updating identification upon *C. formicarius* biotypes is of high importance for enhancing sanitation and preventing infestation.

C. formicarius larvae are the stage at which the insect causes the highest economic loss in sweetpotato production. Due to cryptic feeding habits of the larvae, insecticide use is limited. Thus, host plant resistance is of high importance as an IPM component for the management of this pest. Resistance traits were introgressed in seventy-one sweetpotato lines and were assessed to determine their resistance level to *C. formicarius*. Murasaki, 17-221, 16-205 w, 16-209w, 18-194w, 16-213w, and 18-178w are more resistance to *C. formicarius* than susceptible checks.

CHAPTER 1. INTRODUCTION

1.1. General Introduction

Sweetpotato is grown both in developed and developing countries however, production in developing countries represents more than 70% of the world total (FAOSTAT, 2016). Sweetpotato can be grown in tropical and temperate climates (Bouwkamp, 1985), from sea level and up to 2500 m (Collins, 1995). It is a resilient crop, having several agricultural advantages due to its perennial ability, drought-tolerance, high-yield potential, and ability to develop even under 5,000 mm annual rainfall (Pole, 1989). Sweetpotato is used as a vegetable, snack food, animal feed, and for industrial starch production (Bowell-Benjamin, 2007; Wang et al, 2016).

Sweetpotato is the second most cultivated tuber worldwide and the 7th most important food crop with an annual production over 112.5 million metric/year (FAOSTAT, 2019). Most production is shared between Asia (70.5%) and Africa (24.5%). From 2007 to 2017, United States sweetpotato acreage increased over 64% (USDA/NASS, 2017), raising the United States production to the eighth largest sweetpotato producer worldwide and first in the Americas (FAOSTAT, 2019). Sweetpotato is also a very important crop in the Caribbean region despite production accounting for only 1.1% worldwide. In this region, Haiti (50%) and Cuba (41%) produce the most sweetpotato, the second most important root crop after cassava. Moreover, this staple food plays a valuable role in food security with an annual production of 630,000 MT harvested from nearly 105,000 hectares (FAOSTAT, 2019). Ironically, sweetpotato yield harvested in Haiti is more than one and a half times the United States yield; however, the United States production yielded more than 250 metric/ha versus 60 metric/ha. In addition, there is a lack of innovation and facilities for research to improve varieties traditionally used by growers. Moreover, the support provided by the Ministry of Agriculture, the State faculty of Agriculture, and non-governmental organizations are minor.

1.2. Sweetpotato production and major pests

Developing countries are not the only regions facing sweetpotato production issues. Despite agricultural advantages, sweetpotato production is associated with many worldwide pests including nematodes, insects, weeds, and diseases, all of which affect quality and yield. However, most economic damage to sweetpotato is ascribed to insects.

1.2.1. Sweetpotato major diseases

Sweetpotato diseases are a major issue both in field and storage (Edmunds et al, 2008) and include virus, nematodes, and fungi (Kokoa, 2001; Clark et al. 2012; Hare, 2019).

1.2.1.1. Viral diseases

More than 30 types of virus have been reported to attack sweetpotato (Brunt et al., 1996; Clark and Moyer, 1988). The most important ones worldwide are *Sweetpotato feathery mottle virus* (SPFMV), S*weetpotato chlorotic stunt virus* (SPCSV) , and *Sweetpotato latent virus* (SPLV) which synergistically interact to cause sweetpotato virus disease (SPVD) (Loebenstein, 2014; Wang et al., 2009). In Jamaica, of 420 sweetpotato roots assessed for SPVD, 23 to 43% were infected with *Sweetpotato collusive virus* (SPCV) and *Cucumber mosaic virus* (CMV), ranking them the most common viral diseases present in this area (Llyod, 2012).

Viral diseases are mainly characterized in the field by foliage discoloration; chlorosis, vein clearing, chlorotic spots, mottle, mosaic, and leaf curling (Gibson et al., 1998). Losses are recorded only when there is co-infection of the plant by several viruses (Schaefers and Terry, 1976; Ames et al., 1997). Reduction in yield in China from viral diseases can reach up to 78% (Shang et al., 1996; Gao et al., 2000). In the United States, failure to control SPVD can reduce yields up to 50% (Clark and Hoy, 2006). African countries are threatened by SPVD and losses can range from 50 to 90% (Barkesa, 2018). In Peru, SPVD is reported to be the major disease constraint of sweetpotato (Gutiérrez et al., 2003). Once virus diseases infect one field, their

expansion is unavoidable since sweetpotato is vegetatively propagated (Loebenstein, 2014). The most efficient method for viral disease control is the use of virus resistant cultivars and the use of virus free seeds, vines, and roots (Yada et al., 2017).

1.2.1.2. Nematodes

Nematodes can also affect sweetpotato yield. More recently a new nematode species, Guava root knot nematode, *Meloidogyne enterolobii,* was identified in North Carolina sweetpotato fields. This nematode originated in China and appears to be the most destructive nematode species (Hare, 2019). This new nematode has recently been detected in Louisiana and infected fields were immediately placed under quarantine (LDAF, 2018). These pests attack all parts of the plants however, the most important damage occurs to storage roots (Coleman, 2009). Damage caused by nematodes can exceed 10% (Clark et al., 2013).

1.2.1.3. Fungi

Among the most commonly found fungal pathogens in sweetpotato field are *Fusarium* root rot, *Fusarium* surface rot, black rot, and soft rot (Hugues et al., 2009; Clark et al., 2013). Generally, harvested fresh sweetpotato roots are symptomless and display diseases only in storage (Edmunds, 2008). Therefore, appropriate harvest practices are of primary importance in controlling these pests. Thus, in countries with poor infrastructure or absence of formal seed systems, production losses by fungi due to decreased yield and quality range from 19% and 34% respectively (Coleman, 2009). Finally, Nur Aida et al. (2017) reported *Fusarium oxysporum* had severe pathogenicity on healthy sweetpotato roots following attack by *C. formicarius*, indicating the need for disease and insect free sweetpotatoes.

1.2.2. Weeds

Any undesirable plant in a cropping system that can affect yield is considered a weed (Schonbeck, 2013). Sweetpotato production is threatened by many early and late-season weeds

including Bermuda grass, *Cynodon dactylon* (L.) (Pers), alligator weed, *Alternanthera philoxeroides* (Mart.) (Griseb.), yellow and purple nutsedge, *Cyperus esculentus* L., and *Cyperus roduntus L.*), Barnyardgrass (*Echinochloa crus-galli* (L.) (Beauv.), morning glory (*Ipomea spp*), prickly sida (*Sida alba* L.), common cocklebur (*Anthium strumarium* L.**),** Italian ryegrass (*Lolium perenne* L. *ssp. multiflorum* (Lam.) (Husnot), itchgrass, *Rottboellia cochinchinensis* (Lour.), Johnson grass, *Sorghum halepense* (L.) (Pers.), rice flatsedge, *Cyperus iria* L, pigweed :*Amaranthus palmer*, and tall waterhemp, *Amaranthus tuberculatus* L. (Stephenson, 2019; WSSA, 2019). *Amaranthus palmer* is reported to be the most common weed species targeting United States sweetpotato production (Webster and Cole, 1997; Ward et al., 2013; WSSA, 2019). This herbaceous weed grows year-long and can compete with sweetpotato for water and nutrients the entire production season. *Amaranthus palmer* can decrease sweetpotato yield from 36 to 81% (Smith, 2007; Meyers et al., 2010a). Weeds are more problematic at early sweetpotato growth stages (Leigh and Gugerty, 2013) and thus, weed management of this crop early on in production is of high importance.

Weed control involves the combination of many methods including cultivation practices, herbicides, mowing, and hand removal (Meyers at al., 2010a; Stephenson, 2019). However, the most common management tactic used is herbicides and accounts for more than a half of the total pesticides used in the USA (USDA/ERS, 1990-1997). Glyphosate is the herbicide most widely used in the United States, including in sweetpotato crop production, due its efficacy (Fernandez-Cornejo et al., 2014). However, excessive use of glyphosate by growers has led to weed resistance (Livingston et al. 2015).

Due to the low number of herbicides registered in the United States, other IPM tools have been assessed for more environmental friendly weed management. Harrison and Peterson (1986 and 1991) reported sweetpotato allelopathic effects on *Cyperus esculentus* and *Medicago sativa*,

and phenotyping studies have also been conducted on enhancing sweetpotato competitiveness to weeds by comparing the canopy characteristics (LaBonte et al,. 1999).

In the Asian sweetpotato growing countries, weed management was concentrated on the screening of varieties more competitive to weeds and cultural control such as intercropping, rotation, and planting date (William and Chiang, 1980). In South Asia, herbicides are now more affordable for large holder sweetpotato growers and are widely used, however, small holder sweetpotato producers use hand weeding and mechanical strategies as primarily weed control (Devendra and Thomas, 2002). Furthermore, many studies have been carried out to select for sweetpotato varieties with highest allelopathy capacity to compete with weeds species such as *Mikania micrantha* and *Lactuca sativa* (Shen et al., 2018; Shen et al., 2014).

In Africa, hand weeding has been the primary control until recently where herbicides are gaining in popularity (Iyagba, 2010). In Kenya, the combination of hand weeding, intercropping, and herbicides (glyphosate) are used for control of *Digitaria abbysinica* (Momanyi et al., 2016). The pattern of weed control is not so different in the Caribbean Basin. Hand weeding, rotation, and intercropping sweetpotato with corn, sorghum, sugarcane are the primary methods used (CARDI, 2010; Jean-Baptiste, 2018).

1.2.3. Sweetpotato insect pests

Talekar (19887a) reported over 270 insect species feed on sweetpotato worldwide, causing injury in field and/or storage (Ray and Ravi, 2005; Kitinoja and Kader, 2002). These insects are mostly species in the Orders Lepidoptera, Hemiptera, and Coleoptera (Ames et al., 1997), and are categorized as foliage feeders, vine and stem borers, and root feeders, however the latter are the most destructive (Sherman and Tamashiro, 1954). For foliage feeders, the most problematic are the sweetpotato butterfly, *Acraea acerata* (Hew) (Lepidoptera: Nymphalidae), and three armyworms: *Spodoptera eridania* (Stoll), *Spodoptera exigua* (Hübner), and

Spodoptera litura (Fabricius) (Lepidoptera: Noctuidae) (Smit et al, 1997b). These foliage feeders can compromise sweetpotato growth if leaves are fed upon in the early stages of the crop (Ames et al, 1997; Skoglund and Smit, 1994; Okonya et al., 2013). *Agrius convolvuli* (Linnaeus) (Lepidoptera: Sphingidae) is also a sweetpotato leaf feeder and can cause high defoliation rates leading to significant losses (Clark et al., 2013). *Aphis gossypii* (Glover) and *Bemisia tabaci* (Gennadius) are also major pests since these species feed on the plant tissue, reducing photosynthate and transmitting viral diseases simultaneously (Waterhouse, 1997; Gibson et al., 2004).

The major sweetpotato vine borers are reported to be *Omphisa anastomasalis* (Guenée); *Megastes grandalis* (Guenée), and *Megastes pucialis* (Snell) (Lepidopteran insects of the Family Pyralidae) that bore into sweetpotato stems until they reach the first part of the roots, preventing early plant growth (Ames et al., 1997; Clark et al., 2013). These vine borers are predominantly found in the Pacific, Asia, Hawaii, and some regions in South America (Yan et al., 2014) and are reported as severe pests in some regions in the Caribbean Basin, resulting in yield losses of 30 to 50% (Ames et al., 1997; Wakamura et al., 2010; Clark et al., 2013).

The sweetpotato root feeders are classified as pre-harvest and post-harvest pests (Edmunds, 2008; Ray and Ravi, 2005). Although the adults feed on the epidermis of the roots, the larvae are the most damaging stage, tunneling throughout the roots (Uritani, 1975). Larvae of the three *Cylas* species: *C. formicarius elegantulus* (Summers)*, C. puncticollis* (Boheman)*, and C. brunneus* (Fabricius) are reported to be the most destructive root feeders (Chalfant et al. 1990; Jansson and Raman 1991, Ames et al., 1997), and are referred to as sweetpotato weevils. *Euscepes postfasciatus* (Fairmaire) is also a serious sweetpotato root feeder and is considered a weevil as well (Alleyne, 1982).

Other harmful root feeders include sugarcane beetles *Euetheola humilis* (Burmeister) (Smith, 2006), wireworms *Agrypnus* spp. and *Gonocephalum* spp. (Chalfant et al., 1990; O'Sullivan, 2005), and *Blosyrus* spp. (Ames et al.,1997); rootworms *Diabrotica* spp., flea beetles *Systena* spp., spotted cucumber beetle, *Diabrotica undecimpunctata* (Barber), and the banded cucumber beetle, *Diabrotica balteata* (LeConte) (Cuthbert, 1967). Although the similarities found in damage caused by these root feeders, the major economic losses are caused by the sweetpotato weevil group (Chalfant et al. 1990; Jansson and Raman 1991; Ames et al., 1997; Alleyne, 1982). Injury and tunneling of weevils in sweetpotato roots elicit terpene formation and render these roots unmarketable and unfit for human (Uritani et al., 1975; Sato et al., 1977) and animal (Sharman and Tamashiro, 1954; Uritani et al., 1975) consumption. According to Sutherland (1986), worldwide yield loss caused by *C. formicarius* and *E. postfasciatus* can range from 5 to 80%, and post-harvest loss can reach 10 to 50%.

1.3. Major Sweetpotato weevils: Geographic Origin and Distribution

Twenty-five *Cylas* spp. have been identified throughout the world, however, only *C. brunneus*, *C. puncticollis*, and *C. formicarius* are recorded as threats to sweetpotato (Wolfe, 1991). The first two species are found only in Africa (Wolfe, 1991) while *C. formicarius* is present worldwide with prevalence in the Western Hemisphere (Jansson, 1990a). *Euscepes postfasciatus* (F.) is present in Central and South America and in the South Pacific and Caribbean basin (Sherman and Tamashiro, 1954; Raman and Alleyne, 1991).

Weevils, though having well developed wings, primarily walk and thus, their expansion occurs mainly by human transportation of infested slips and roots (Cockerham, 1954). *Cylas formicarius* is reported to have originated in the Indian subcontinent 90 millions years ago (Wolfe, 1991) and fed on all Convolvulaceae, excluding sweetpotato, since this plant species was not present on this continent until it was brought by the Europeans (Yen, 1982) from South America

(Austin, 1988). Since then, sweetpotato has become *C. formicarius'* preferred host and this weevil is widespread in more than 50 countries (C.A.B International/ Institute of Entomology, 1996), due to the expansion of this crop to Europe, Africa, Asia (LeConte and Horn 1883; Wolfe 1991), and India (Austin, 1991). *Cylas formicarius* was discovered for the first time in the United States in Louisiana in 1875 (Summers, 1875) and is thought to have been introduced through the importation of infested storage roots from the Caribbean basin (Newell, 1917). This weevil spread quickly to Florida in 1878, Hawaii in 1887 (Blackburn and Sharp 1887), Mississippi and Georgia in 1917, Alabama in 1918, and Oklahoma in 1922 (Cockerham, 1954).

Studies conducted revealed the dispersal pattern of *C. formicarius* was mainly due to international trade. Phylogeny analysis of the genetic variation of *C. formicarius* revealed similarities of populations from Georgia, Hawaii (USA), and St. Kitts (West Indies) and those from Guangdong (China) and Hanoi (Vietnam). Also, weevil populations from the Ogasawara Islands (Japan) are from the same subclade (Kawamura et al. 2007). Moreover, intra-specific similarities of *C. formicaricus* were found among samples from Florida, Alabama, and those from the Caribbean basin while those from Mississippi showed differences (Louis, 2006). This indicates there is potential for biotypes or sub-populations amongst sweetpotato weevils within the United States. No reports are available pertaining to *E. postfasciatus* detection in the United States despite sweetpotato exportation from the Dominican Republic to Puerto Rico, and commodity exchange with countries where *E. postfasciatus* occurs especially China (USDA/NASS, 2017).

Even though *E. postfasciatus* is endemic to the Caribbean Basin, this species seems not to occur in all regions of this area (Bonfils and Bart, 1950). Haiti and Dominican Republic are two countries sharing the Hispaniola Island in the West Indies. Suah (1981) and Raman (1988b) reported the occurrence of *E. postfasciatus* in these two countries. Conversely, Sherman and

Tamashiro (1954), and Raman and Alleyne (1991) stated that *E. postfasciatus* is found only in Dominican Republic. A more recent study listed the occurrence of *E. postfasciatus* on the Island of Hispaniola with no specification about its accurate location (Perez-Gelabert, 2008). Furthermore, methods for these studies have not been described and are not available. Hence, the first objective of this research aims to remove uncertainties surrounding the occurrence of *E. postfasciatus* in Haiti.

1.4. Sweetpotato weevil Biology

Cylas formicarius and *E. postfasciatus* are taxonomically and morphologically different. However, their life cycle is approximately the same. For both species, developmental growth, fecundity, and longevity are temperature dependent (Hahn and Leuschner, 1982), and they complete their entire life cycle in a range of 24 to 37^{o} C (Mullen, 1980; Alleyne, 1982). The two species are holometabolous and, lay eggs both in stems and roots. However, the roots are the female preffered sites to oviposit (Lebot, 2009). For *C. formicarius,* the mean number of eggs oviposited is 56 to 256 eggs a year (Jansson and Raman, 1991). For *E. postfasciatus*, Alleyne (1982) reported 106 eggs per month for a length of 4 to 6 months. Eggs of the two species can be discriminated by their shape, color and size. Eggs of *C. formicarius* are broadly oval, creamy-white and 0.50 to 0.79 mm in length, while for *E. postfasciatus*, eggs are globular, greyish yellow, and 0.34 to 0.38 mm in length (Cockerham, 1954; Sherman and Tamashiro,1954; Alleyne, 1982). There are conflicting reports pertaining to the number of larval instars for *C. formicarius.* It has been suggested that there are 4 or 5 instars by Fukuda (1933) and by Gonzales (1925), whereas, Sharman and Tamashiro (1954) and Alleyne (1980) reported 5 larval instars. Nevertheless, the larval stage is dependent on food availability and temperature, ranging from 10 to 36 days (Reinhard, 1923) versus 20 to 30 days for *E. postfasciatus* (Tucker,1937).

The larvae are distinguishable by the placement of hairs on their frontal head (Sherman and Tamashiro,1954). The two weevil species have a non-feeding pre-pupal stage which lasts 1 to 2 days (Alleyne, 1982). The pupal stage typically lasts from 7 to 9 days (Tucker, 1937). Adults of both species are obviously discernable; *C. formicarius* have red and dark coloration and are longer (5.5 to 7.0mm) compared to adult *E. postfasciatus* that are very small (3.2 to 4.0 mm) and yellow to brown (Sherman and Tamashiro, 1954).

1.5. Sweetpotato weevil Ecology

Both species are active at night (Howard, 1982; Proshold, 1983; Shimoji and Kohama, 1994) and *E. postfasciatus* exhibits positive phototactic behavior (Nakamoto and Takushi, 2001). The two species are multivoltine and spend their whole life on all parts of their preferred host plant, sweetpotato (Jansson and Raman, 1991). The most destructive attacks occur late in the sweetpotato growing season precisely after root development. However, the level of injury is genotype and environmental factors dependent. Wilson and al. (1989) reported the presence of the chemical compound boehmeryl acetate in susceptible sweetpotato variety which is very attractive for female *C. formicarius* oviposition. Mao et al. (2001), shown difference in adult *C. formicarius* behavior among roots for feeding and oviposition in the same sweetpotato genotype cultivated in different locations sites. Also, findings of study carried out by Okada et al. (2014) on *E. postfasciatus* and thirty-three sweetpotato cultivars interactions show significant different between time eclosion and body length of adults suggesting adult preference for some cultivars for food or oviposition or no preference for others. These results also show the effects of certain cultivars on preventing *E. postfasciatus* immature performance to complete development.

In addition, since *C. formicarius* and *E. postfasciatus* are oligophagous they can survive on other *Ipomea* spp. as hosts in absence of sweetpotato production including *Ipomea indica* and *Ipomea pes-caprae* (Sato et al. 2009). However, sweetpotato remains their prefered host. Reddy

and Chi (2015) reported that adult emergence and egg production on sweetpotato roots, *Ipomea batatas* (L.) Lamarck, occured 42 days earlier than there are on *Ipomea tribola* and, significant difference are found in eggs number.

1.6. Sweetpotato Weevil Dispersal

One of the most important differences between *C. formicarius* and *E. postfasciatus* resides in their dispersal ability. Both adult species have elytra however only *C. formicarius* can fly (Reddy et al., 2012b, 2014a) even if it is for short distances (Moriya and Hiroyoshi, 1998). The male of this species was observed flying up to 2 km (Miyateka et al., 1997). *Euscepes postfasciatus* is flightless and ambulation is its only means of dispersal (Alleyne, 1982). Mean dispersal of *E. postfasciatus* in laboratory experiments was reported to be 178 m and 218 m for male and female respectively in a 7-day period (Shimizu and Moriya, 1996c), while in the field, this species can only reach 33 m in a 5-day period (Kinjo et al, 1995). Moreover, *E. postfasciatus* is more active in summer and can disperse at a maximum distance of 0.33 m/day compared to the other three seasons; 0.13 m, 0.20 m, and 0.20 m for winter, spring, and autumn respectively (Ichinose et al., 2016).

1.7. Sweetpotato weevil management

1.7.1. Chemical control

Synthetic insecticides were developed following World War II to enhance insect control (USDA/ERS, 1990-1997) and have become the primary IPM tool to control insects (Schalk et al. 1991). However, the expansion and reliance on insecticides has caused environmental damage and insect resistance (Mason, 1991). Insecticides registered currently in use for foliage feeding sweetpotato weevil management in Louisiana include, bifenthrin, phosmet, carbaryl, and baythroid; and those for soil and storage insects feeding include chlorpyrifos and thiamethoxam (LSUAgCenter, 2019).

1.7.2. Pheromones

The active ingredient of the synthetic *C. formicarius* female pheromone is (Z)-3-dodecen-1-ol (E)-2-butenoate (Heat, 1989). Traps baited with sex pheromones have been used in the field to both monitor and control *C. formicarius* populations, especially in highly infested-areas (Braun and Van De Fliert, 1999). Use of pheromones has been tested in mating disruption (Yasuda, 1995; Smit et al, 2001). However, the effectiveness largely depends on the concentration of the pheromone, trap placement, and trap densities in the field (Korada, 2012). Combinations of sex pheromone with other IPM tools such as insecticides have also been tested. Use of pheromone traps can significantly lower the cost of inputs in a field by saving three applications of insecticides (Hwang and Hung, 1991). For *E. postfasciatus*, no sex pheromones have been isolated (Nakamoto and Katsuki, 2002; Nakamoto and Kuba, 2004; Katsuki et al., 2012). This may be due to the very low rate of sex volatiles emitted by females to attract males for mating (Raman and Moriya, 1997). Nevertheless, traps have been tested using sweetpotato slices or ultraviolet light as bait to control *E. postfasciatus* (Katsuki et al., 2012).

1.7.3. Biological control

Biological control is considered one of the main IPM tools to control insects (Stern et al, 1959). This method consists of the release of natural enemies to prevent the outbreak of pest insect species in a given area (Altieri, 2014). Biological control is divided into three categories: classical involving the introduction of the natural enemies from its native habitat to control the exotic pests; augmentative by rearing and releasing gradually an amount of natural enemy when the pest is in very low number, and conservation consisting in managing appropriate environment to conserve the augmented natural enemies already present in the area (Altieri et al, 2014). Natural enemies include entomopathogenic fungi or nematodes and parasitoids (Yasuda 1999, Reddy et al. 2014a).

There are some reports on biological control for sweetpotato weevil. Palaniswami and

Rajamma (1986) first identified and described braconids *Rhaconocus* spp. and *Bracon* spp. on sweetpotato weevils and two decades later Maeto and Uesato (2007) reported 19.9 to 40.7% of the ectoparasitoids *Bracon yasudai* on *E. postfasciatus* and *C. formicarius* in field. In addition, *Euderus purpureas* was found parasitizing *C.* f*ormicarius* in southern Florida (Palaniswami, 2002; Jansson et al. 1990b). However, little effort has been placed on using biological control in commercial production.

Many studies revealed that the species *Heterorhabditis* is the most effective nematode on *C. formicarius elegantulus*. Ekenayake et al (2001) found under laboratory conditions 80 to 90% mortality of all stages of *C. formicarius* infested with *Heterorhabditis megidis* in Sri Lanka. Further positive results revealed that *Heterorhabditis indica* induced mortality on *C. formicarius* (Banu and Rajendran, 2002). Nderitu et al. (2009) reported 23.3% larval mortality of *C. formicaricus* in sweetpotato vines 14 days after entomopathogenic induction under semi-field conditions in Kenya and 90% indirect mortality of larval exposed to soil infested *Heterorhabditis indica*. However, the short life span both in transportation and field after release, are the main constraints to using entomopathogenic nematodes in biological control of sweetpotato weevil (Georgis et al., 2006).

Valuable work has also been conducted on entomopathogenic fungi as biological control agents for sweetpotato weevil management (Sherman and Tamashiro, 1954). The most extensively studied has been *Beauveria bassiana* and *Metarhizium brunneum* isolated from *C. formicarius* (Cockerham et al, 1954; Jansson, 1954). Yasuda (1999) reported that the effectiveness on adult *C. formicarius* depends on fungal cornidia concentrations. Since Castineiras et al. (1984a) first isolated four isolates of *B. bassiana* from adult *C. formicarius* in Cuba this method is continuously used in IPM programs. Five different fungal cornidia concentration (1 x 10^8 ; 1 x 10^7; 1 x 10^6; 1 x 10^5, and 1 x 10^4 ml^{-1}) have been tested in Cuba on *C.*

formicarius under laboratory conditions and the highest mortality occurred at the two highest concentrations (Nicholls et al. 1995). A preliminary result of a study on the use of *B. bassiana* in sweetpotato weevil control shown no difference between vines dipped in insecticides and those sprayed with *B. bassiana* cornidia (Hlerema et al, 2017). Also, repellence of *C. formicarius* was evident when exposed to a virulent strain of *Metarhizium anisopliae* (Donata et al., 2017). Biological control agents including *B. bassiana* are industrially and traditionally mass-reared in Cuba and widely adopted by farmers in the field for *C. formicarius* control (Lagaoui, 2000). At least nine centers of *B. bassiana* have been established in Cuba (Luareta et al., 2003).

1.7.4. Cultural control

In traditional agriculture, growers have always used cultural methods to control pest in their fields. These include crop rotation, alternating planting dates, intercropping, orientation of slips while planting, sanitation by the removal of crop debris and wild hosts, soil management, and planting density (Glass and Thurston, 1978; Abate et al. 2000). These practices disrupt the pest life cycle and subsequently decrease populations (Korada et al. 2010). Crop rotation is one of the most effective cultural controls of sweetpotato weevil (Talekar, 1987c), and has long been recommended (Holdaway, 1941). A study over 6 years in Ghana on cultural practices reported more than two fold sweetpotato yield (4.5 MT/acre) when followed by two years of bahia grass (*Papsalum notatum* Flüggé). A two-year study in Cuba showed sweetpotato root damage decreased to 14.7% in sweetpotato plots intercropped with maize when compared to a sweetpotato monoculture (Suris, 1995).

1.7.5. Irradiation

Sterile Insect Technique (SIT) is one of the tactics tested to control sweetpotato weevils. SIT is an area-wide based approach consisting of mass rearing and the release of irradiated-

sterile males in order to decrease reproduction rates, and therefore suppress or eradicate the target insect populations (Kumano et al., 2018). According to Knipling (1979), the effectiveness of SIT depends on the mating performance and fitness of the released sterile males. A major constraint to the technique is the fact that irradiation limits the locomotive abilities of *C. formicarius*, and thus, decreases the expected mating rates (Reddy et al., 2012b, 2014a). Furthermore, spermiogenesis is lessened (Korada, 2012). However, Hiroyoshi (2018) recently reported that irradiation do not decrease spermiogenesis but increased the ejaculated time.

1.7.6. Host plant resistance (HPR)

Plants do not passively suffer from insect herbivory but possess natural heritable traits for their defenses. Traditionally, resistance of a plant to herbivory is categorized by the capacity of the plant to negatively affect the insect fitness; the capacity of the plant to elicit changes in insect behavior in finding, feeding or oviposition, and the capacity of the plant to tolerate and compensate from injury (Painter, 1951). More recently, host plant resistance has been viewed as complex plants-insect interactions based on ecological and evolutionary approaches (Stout, 2014). Plant defenses against insects are governed mostly by biochemical compounds (War et al. 2012), and detecting, identifying, understanding, and manipulating plants defense traits can reduce insect pest populations and therefore, reduce yield loss (Barlow and Holston, 1981).

Since sweetpotato is a highly heterozygous haploid hybrid and has the ability to rapidly develop genetic diversity (Grüneberg et al, 2015), making host plant resistance an attractive approach that fits well into sweetpotato crop (Collins et al., 1991; Lawrence et al., 1997). However, identification of the resistance types is the primary step towards creating an efficient HPR program (Barlow and Holston, 1981). Wang and Krays (2002) found thirty-three different types of chemical compounds in four different clones of sweetpotato roots and foliage which can attract or repel female *C. formicarius*, implying resistance and susceptibility of a sweetpotato

variety is related to the types and concentrations of the compounds present in the variety. Korada et al. (2010) found that female oviposition preference is due to the counterbalance of the chemicals emitted between above and below parts of sweetpotato and stated this method could be utilized in HPR. For instance, Anyanga et al. (2013) reported that application of ocradecyl coumarate and octadecyl caffeate reduced *C. puncticullus* and *C. brunneus* oviposition.

Jackson and Bohac (2006) reported twenty-seven sweetpotato genotypes that expressed significantly less weevil damage than “Beauregard” which is the main commercial moist orange-flesh genotype in the United States. Moreover, most of these dry-flesh sweetpotato genotypes had high levels of resistance to soil insect pests and are therefore part of the United States sweetpotato germplasm for use in sweetpotato breeding program. In Louisiana, an effective sweetpotato propagation pathway has been set up including a breeding nursery for continuous variety evaluation for resistance to pests (Clark and Smith, 2016).

1.7.8. Sanitation

Each US State has established specific laws respective to *C. formicarius* control. In North Carolina, any sweetpotato growing county in which *C. formicarius* is detected even in wild morning glory is immediately declared a quarantine area (Adams, 2018). In Louisiana, quarantine regulations are also established for all sweetpotato industries including production, storage, and marketing, requiring weekly field spraying for weevils, trapping during the entire growing season, and fumigation of sweetpotato storage facilities (LDAF, 2014).

Sweetpotato production throughout the world faces weevils and thus, gives rise to the need to update information about sweetpotato weevil species and biotypes present in Haiti and the need for identifying resistant varieties in order to decrease risk of infestation from weevil infested-areas to un-infested areas. This thesis will begin to elucidate which weevil species exist in Haiti, if biotypes exist which may affect control tactics, and exploring different sweetpotato varieties for

resistance.

1.8. References

Abate, T. et al. 2000. Pest management strategies in traditional agriculture: an African perspective. *Annual Review of Entomology*, 45:631–659.

Adams, R. 2018. NCDA&CS Declares Sweetpotato Weevil Quarantine https://ipm.ces.ncsu.edu/2018/08/ncdacs-declares-sweet-potato-weevil-quarantine/ Accessed on May 2019.

Alleyne, E. H. 1982. Studies on the biology and behavior of the West Indian sweetpotato weevil, Euscepes postfasciatus (Fairmaire) (Coleoptera: Curculionidae). *Procedures Caribbean Food Crops Society*, 18:236–43.

Altieri, M. A. et al. 2014. Manage Insects on your Farm: A guide to Ecological Strategies. *Sustainable Agriculture Research and Education (SARE).*

Ames, T. et al. 1997. Sweetpatato: Major Pest, Diseases, Nutritional Disorders. *International Potato Center* (CIP), Lima, Peru, 1-148.

Anyanga, M. O. et al. 2013. Resistance to the Weevils *Cylas puncticollis* and *Cylas brunneus* Conferred by Sweetpotato Root Surface Compounds. *Journal of Agriculture and Food Chemistry,* 61 (34):8141-8147.

Austin, D. F. 1991. Associations between the plant family Convolvulaceae and Cylas weevils. In Sweetpotato Pest Management, a Global Perspective (R. K. Jansson and K. V. Raman eds.). *Westview Press, Boulder,* 45–57.

Banu, J. G. an Rajendran, G. 2002. Host Records on Entomopathogenic Nematode, *Heterorhabditis indica*. *Insect Environment*, 8(2):61–63.

Barkesa, M. K. E. 2018. A Review on Sweetpotato (*Ipomea batatas*) Viruses and Associated Diseases. *International Journal of Research in Agriculture and Forestry*, 5 (9):1–10.

Barlow, T. and Rolston, L. H. 1981. Types of Host Plant Resistance to the Sweetpotato Weevil Found in Sweetpotato Roots. *Journal of the Kansas Entomological Society,* 54 (3):649–657.

Blackburn, T. and Sharp, D.1887. Memoirs on the Coleoptera of the Hawaiian Islands. *Royal Dublin Society and Science Transs*., 2-253.

Bonfils, J. and Bart, J. A.1950. Distribution of Sweetpotato Weevils in the French West Indies. Root Crops Symposium, Guadaloupe.

Bouwkamp, J.C. 1985. Introduction-part 1, pp.3-7. In J.C. Bouwkamp (ed.) Sweetpotato products: a natural resource for the tropics. *CRC Press*, Boca Raton, Florida.

Bowell-Benjamin, A. C. 2007. Sweetpotato: A Review of its Past, Present, and Future Role in Human Nutrition. *PubMed*, 52:1-59.

Braun, A. R., and Van De Fliert. 1999. Evaluation of the impact of sweetpotato weevil (Cylas formicarius) and of the effectiveness of Cylas sex pheromone traps at the farm level in Indonesia. *International Journal of Pest Management*, 45:101–110.

Brunt, A. A., et al. 1996. Viruses of Plants. Descriptions and Lists from the VIDE Database. *CAB International*, Kew, England.

CABI, 1986-1996. Distribution maps of plant pests. C.A.B. *International, Institute of Entomology*, London: The Institute, Series A. No. 278.

CARDI, 2010. Technical manual of Sweetpotato. *Caribbean Agricultural Research and Development Institute* (CARDI).

Castiñeiras, A., T., Cabrera, A. Calderón, and O. Obregón. 1984. Virulencia de cuatro cepas de *Beauveria bassiana* sobre adultos del Cylas formicarius elegantulus (Coleoptera: Curculionidae). *Ciencia y Técnica en la Agricultura*. Serie: Protección de Plantas (Cuba). 7 (1): 65–74.

Chalfant, et al. 1990. Ecology and management of sweetpotato insects. *Annual Review Entomology*, 35:157–180.

Clark, C. A., and Moyer, J. W. 1988. Compendium of SweetPotato Diseases. *The American Phytopathological Society*, St. Paul, MN.

Clark, C.A. and Hoy, M.W., 2006. Effects of common viruses on yield and quality of Beauregard sweetpotato in Louisiana. *Plant Disease*, 90(1):83–88.

Clark, C. A. et al., 2012. Sweetpotato viruses: 15 years of progress on understanding and managing complex diseases. *Plant Diseases*, 96:168–185.

Clark, C.A. et al. 2013. Compendium of Sweetpotato Diseases, Pests and Disorders. Minnesota, MN, USA: *APS Press*.

Clark, C.A., Ferrin D.M., Smith T.P., Holmes G.J. (Eds) (2013). Compendium of Sweetpotato Diseases, Pests and Disorders. Minnesota, MN, USA: *APS Press*.

Clark, C. A and Smith, T. P. 2016. Sweet Potato Foundation Seed Production and the National Clean Plant Network Support. https://www.lsuagcenter.com/. Accessed on May 2019.

Cockerham, K. L. et al. 1954. The biology of the sweetpotato weevil. *Louisiana Technical Bulletin*, (483): 3.

Coleman, E.A. et al. 2009. Genetics and disease as factors in the yield decline of sweetpotato in the Papua New Guinea high lands. *In* Soil Fertility in Sweetpotato based Cropping Systems in the Highlands of Papua New Guinea, pp. 33–42. Ed. G. Kirchhof. Canberra, Australia:*Australian Centre for International Agricultural Research*.

Collins, W. W. et al, 1991. Breeding sweetpotato for insect resistance: a global overview, 379 - 397. In R. K. Jansson and K. V. Raman [eds.], Sweetpotato pest management: a global perspective. *Westview Press, Boulder*, CO.

Collins, W. 1995. Sweetpotato. https://hort.purdue.edu/newcrop/CropFactSheets/sweetpotato.html. Accessed on May 2019.

Cuthbert, F.P. Jr. 1967. Insects affecting sweetpotatoes. *USDA Agricultural Handbook*, 28:329. Washington, D.C.

Devendra, C. and Thomas, D. 2002. Smallholder farming systems in Asia. *Agricultural Systems*, 71(1): 17–25.

Dotona, R. et al. 2017. Sweetpotato weevil, Cylas formicarius (Fab.) (Coleoptera: Brentidae) avoids its host plant when a virulent *Metarhizium anisopliae* isolate is present. *Journal of Invertabrate Pathology*, 148:67–72.

Edmunds, B.A. et al. 2008. Postharvest Handling of Sweetpotatoes. Raleigh, NC, USA: North *Carolina Cooperative Extension Service*.

Ekenayake, H.M.R.K. et al. 2001. Potential of entomopathogenic nematodes as bio-control agents of sweetpotato weevil, *Cylas formicarius* (Fabricus) (Coleoptera: Brenthidae). *Japan Journal of Nematology*, 31:19–25.

FAOSTAT, 2016. http://www.fao.org/faostat/en/#data. Accessed on February 2018.

FAOSTAT, 2019. http://www.fao.org/faostat/en/#data/QC. Accessed on May 2019.

Fernandez-Cornejo et al. 2014. Pesticide Use in U.S. Agriculture: 21 Selected Crops, 1960-2008. *Economic Information Bulletin*/USDA, (24):1-86.

Fukuda,1933. Insect pests of Sweetpotato in Formosa. Part 1. The Sweetpotato weevil. *Formosa Government Resources Institute Report*, 62:1–35.

Gao, F., Gong, Y. P., and Zhang, P. B. 2000. Production and employment of virus-free sweetpatato in China. *Crops Protection*, (19):105–111.

Georgis, R. et al. 2006. Successes and failures in the use of parasitic nematodes for pest control. *Biological Control*, 38:103–123.

Gibson, R.W. et al. 1998. Symptoms, aetiology and serological analysis of sweetpotato virus disease in Uganda. *Plant Pathology*, 47:95–102.

Gibson, R.W., et al. 2004. Control strategies for sweet potato virus disease in Africa. *Virus Research*, 100:115–122.

Glass, E.H. and Thurston, H.D. 1978 Traditional and modern crop protection in perspective. *BioScience*, 28: 109–115.

Gonzales, S. S. 1925. The Sweetpotato Weevil (*Cylas formicarius fabricus*). *Philippines Agriculture*,14(5): 257–282.

Grüneberg, W. J. et al. 2015. Advances in Sweetpotato Breeding from 1992 to 2012, 66pp. *In* Potato and Sweetpotato in Africa: Transforming the Value Chains for Food and Nutrition Security (eds. J. Low et al.). *CAB International*.

Gutiérrez, D. L. et al., 2003. Sweetpotato Virus Disease (SPVD): Distribution, Incidence, and Effect on Sweetpotato Yield in Peru. CIP, Peru. *The American Phytopathological Society*, 87(3):297–302.

Hager, A. 2015. Weed Resistance and new technologies. https://www.farmprogress.com/management/weed-resistance-and-new-technologies. Accessed on May 2019.

Hahn, S. K. and Leuschner, J. 1982. Breeding sweetpotato for weevil resistance, pp 331–336. In Villareal and Griggs. Proceedings of the first International Symposium. *Asian Vegetable Research and Development Center*, (82):172, Tainan, Taiwan.

Hare, R. 2019. The Guava root-knot nematode- A new pest in Louisiana. https://www.lsuagcenter.com/profiles/coverstreet/articles/page1531770181050. Accessed on May 2019.

Harrison, H. F. and Peterson, J. K. 1986. Allelopathic effects of sweetpotatoes (Ipomoea batatas) on yellow nutsedge (*Cyperus esculentus*) and alfalfa (*Medicago sativa*), *Weed Science Society of the Americas*, 34(4):623–627.

Harrison, H. F. and Peterson, J. K. 1991. Evidence That Sweetpotato (Ipomoea batatas) Is Allelopathic to Yellow Nutsedge (*Cyperus esculentus*). *Weed Science Society of the Americas*, 39(2):308–312.

Heat, R. R. et al. 1986. Identification of sex pheromone produced by female sweetpotato weevil, *Cylas formicarius* (Summers). *Journal of chemical Ecology*, 12(6): 1–15.

Hiroyoshi, S, et al. 2018. Effects of Irradiation Dose on Sperm Production, Insemination, and Male Mating Possible Period in the Sweetpotato Weevil (Coleoptera: Brentidae) *Journal of Economic Entomology*, 111(3):1151–1156.

Hlerema, I. et al. 2017. Preliminary observations on use of *Beauveria bassiana* for the control of the sweetpotato weevil (*Cylas* spp.) in South Africa. *Open Agriculture*, 2:595–599.

Holdaway, F.G. 1941. Insects of sweetpotato and their control. *Hawaii Agricultural Experiment Station,* Progress Notes. No. 2.

Howard, F.W. 1982. Diurnal rhythm in *Cylas formicarius elegantulus* and some other arthropods in a *sweetpotato* field. *Florida Entomologist*, 65: 194–195.

Hwang, J.S., Hung, C.C. (1991) Evaluation of the effect of integrated control of sweetpotato weevil, *Cylas fomicarius Fabricius*, with sex pheromone and insecticide. *Chinese Journal of Entomology*, 11:140–146

Howard, F. W. 1982. Howard, F. W. 1982. Diurnal rhythm in Cylas formicarius elegantulus and some other arthropods in a sweetpotato field. *Florida Entomologist,* 65:194–9.

Hughes, M. 2013. Identifying appropriate strategies for reducing virus and weevil losses in sweetpotato production systems in Papua New Guinea and Australia. In Final Report HORT/2011/053. Canberra, Australia: *Australian Centre of International Agricultural Research*.

Ichinose, K. et al. 2016. Reduced dispersal and survival in the sweet potato weevil (*Euscepes postfasciatus*) after irradiation. *Agricultural and Forest Entomology*, 18:157–166.

Iyagba, A.G. 2010. A review on root and tuber crop production and their weed management among small scale farmers in Nigeria. *Journal of Agricultural and Biological Science*, 5(4): 52–58.

Jackson, D.M., Bohac J.R. 2006. Evaluation of pheromone traps for monitoring sweetpotato weevils. *Journal of Agricultural and Urban Entomology*, 23:141–158.

Jansson, R.K. and Raman, K.V. 1991. Sweetpotato pest management: a global overview. Sweet Potato Pest Management: A Global Perspective (ed. by Jansson, R. K. and Raman, K. P.), 1–12 pp. *Westview Press*, *Boulder*, CO, USA.

Jansson, R. K. et al. 1990a. Seasonal abundance, population growth, and within plant distribution of sweetpotato weevil (Coleoptera: Curculionidae) on sweetpotato in southern Florida. *Environmental Entomology*, 19:313-321.

Jansson, R.K. et al. 1990b. Potential of entomopathogenic nematodes as biological control agents of sweetpotato weevil Coleoptera: Curculionidae). *Journal of Economic Entomology,* 83: 1818–1826.

Jean-Baptiste, P. L. O. 2018. Caractérisation agro-morphologique de seize (16) variétés de patate douce (*Ipomoea batatas* Lam.) sur la ferme de Damien. *State University of Haiti*, 83.

Kawamura, K. et al. 2007. Genetic variation of sweet potato weevils, Cylas formicarius (Fabricius) (Coleoptera: Brentidae), in main infested areas in the world based upon the internal transcribed spacer-1 (ITS-1) region. *Applied Entomoly and Zoology,* 42 (1): 89–96.

Katsuki, M., et al. 2012. Ultraviolet light-emitting diode (UV LED) trap the West Indian sweet potato weevil, *Euscepes postfasciatus* (Coleoptera: Curculionidae). *Applied Entomology and Zoology*, 47(3):285–290.

Kinjo, K. et al. 1995. Estimation of population Density, Survival and Dispersal Rates of the West Indian Weevil, *Euscepes postfasciatus* Fairmaire (Coleoptera: Curculionidae), with Mark and Re-capture Methods. *Applied Entomology and Zoology*, 30(2): 313–318.

Kitinoja, L. and Kader, A.A. 2002. Small-scale postharvest handling practices: a manual for horticultural crops. Fourth edition. Postharvest Technology Center, Postharvest Horticulture Series 8E. *University of California, Davis*.

Knipling, E.F., 1979. The basic principles of insect population suppression and management. *Science and Education Administration, U.S. Department of Agriculture.*, pp.512.

Kokoa, P. 2001. Review of sweetpotato diseases in PNG. In Food Security for Papua New Guinea. Proceedings of the Papua New Guinea Food and Nutrition 2000 Conference. Eds. R.M. Bourke, M.G. Allen and J.G. Salisbury. *Canberra*, 730–735.

Korada, R. R. et al. 2010. Differential volatile emission from sweetpotato plant: mechanism of resistance in sweetpotato for weevil *Cylas formicarius* (Fab.). *Current Science*, 99(9): 1597-1601.

Korada, R. R. et al. 2012. Management of Sweetpotato Weevil: *Cylas formicarius*. A World Review. *Fruit, Vegetable, and Cereal Science and Biotechnology*, 6(1):79 – 92.

Kumano, N. et al., 2018. Effects of X-ray irradiation on male sperm transfer ability and fertility in the sweetpotato weevils Euscepes postfasciatus (Coleoptera: Curculionidae) and Cylas formicarius (Coleoptera: Brentidae). *Applied Entomology and Zoology*, 53(4):485– 492.

LaBonte, D. R. et al. 1999. Sweetpotato Clone Tolerance to Weed Interference. *Hort Science*, 34(2):229–232.

Lagaoui, A. 2000. et al. A Sustainable Pest Management Strategy for Sweetpotato weevil in Cuba: A success story. *International Centre of Potato* (CIP), No.1558, Lima, Peru.

Lawrence, J. et al. 1997. Sweetpotato weevil integrated pest management, 37-45. In Progress in IPM CRSP Research. Proceedings of the Second IPM CRSP Symposium, 16-20 May 1997, Guatemala City, Guatemala. *Virginia Tech University*, Blacksburg, VA.

LDAF, 2018. Quarantine Emergency. Guava Root Knot Quarantine (LAC 7:XV.171). Louisiana Department of Agriculture and Forestry. *Office of Agriculture and Environmental Sciences Horticulture and Quarantine*. https://www.doa.la.gov/osr/EMR/2018/1810EMR070.pdf. Accessed on May 2019.

LDAF, 2014. Louisiana Department of Agriculture and Forestry. http://www.ldaf.state.la.us/wp-content/uploads/2014/04/OrnamentalSweetPotatoPolicy.pdf. Accessed May 2019.

LeConte, J. L. and Horn, G. H. 1883. Classification of Coleoptera of North America. *Smithonian Miscellaneous Collection,* (507): 503.

Leigh, A. and Gugerty, M. K. 2013. Agriculture-Environment Series – Sweetpotato and Yam Systems At-A-Glance. *Evans School Policy Analysis and Research* (EPAR), (225):2–15.

Llyod, J. and Tennant, P. 2012. Viruses infecting Sweetpotato in Jamaica. Conference: 16th Triennial Symposium ISTRC. West Indian University, 1-11. *ResearchGate*: https://www.researchgate.net/publication/301488797_Viruses_infecting_Sweet_Potato_in_Jamaica. Accessed on May 2019.

Lebot, V. 2009. Tropical Root and Tuber Crops: Cassava, Sweet Potato, Yams and Aroids. Crop Production Science in Horticulture Series, Centre de Coopération Internationale en

Recherche Agronomique pour le Développement (CIRAD). *Crop Production Science in Horticulture*, 17:91–177.

Livingston, M. et al. 2015. The Economics of Glyphosate Resistance Management in Corn and Soybean Production. United States Department of Agriculture 9USDA), *Economic ResearchService*, (184):1– 43.

Loebenstein. 2014. Control of Sweetpotato Virus Diseases. *Advances in Virus Research*, 91:34–42.

Louis, J. 2006. Occurrence and Intra-specific Variation of Sweetpotato Weevil (Brentidae: Coleoptera) in Relation to Its Potential Spread in SouthernUnited States of America and the Caribbean. Procedures l IS on Sweetpotato and Cassava. Ed. S.L. Tan. *Act Hort*, 703, ISFIS, 197– 203.

LSUAgCenter, 2019. Louisiana insect Pest Management Guide. Publication No.1838 https://www.lsuagcenter.com . Accessed on May 2019.

Luareta, C. I. and Ines, M. C. 1995. Dosage mortality of three different strains of *Beauvaria bassiana* against sweetpotato weevil (Cylas formicarius Fabricus). *International Centre of Potato* (CIP), No. 1558, Lima, Peru, 20:21.

Maeto, K. and Uesato, J. 2007. A new species of Bracon (Hymenoptera: Braconidae) parasitic on alien sweetpotato weevils in the south-west islands of Japan: Original article. *Entomological Science,* 10(1):55–63.

Mao, L. et al. 2001. Effect of Sweetpotato Genotypes, Storage Sime and Production Site on Feeding an Oviposition Behavior of the Sweetpotato Weevil, *Cylas formicarius* (Coleoptera:Apoinidae), *Florida Entomologists*, 84(2):254 – 264.

Mason, J. L. et al. 1991. Response of Sweetpotato Weevil (Coleoptera: Apionidae) to Select insecticides. *Florida Entomologist,* 74(2):350 – 355.

Meyers, S. L. et al. 2010a. Interference of Palmer amaranth (*Amaranthus palmer*) in sweetpotato. *Weed Sciences Society of Americas*, 58:119–203.

Miyatake, T., et al. 1997. Dispersal Potential of Male *Cylas formicarius* (Coleoptera: Brentidae) Over Land and Water. *Environmental Entomology*, 26 (2):271–276.

Momanyi, V. N., Ruth, A., and Wakoli, E. 2016. Evaluation of Weed Management Options to Enhance Sweetpotato Production in Kenya. *IJISET - International Journal of Innovative Science, Engineering and Technology,* 3(2):286–289.

Moriya, S. and Hiroyoshi, S. 1998. Flight and Locomotion Activity of the Sweetpotato Weevil (Coleoptera: Brentidae) in Relation to Adult Age, Mating Status, and Starvation. *Journal of Economic Entomology*, 9 (2):39–443.

Nakamoto, N. Y., and Kuba, H. 2004. The effectiveness of a green light emitting diode (LED) trap at capturing the West Indian sweetpotato weevil, *Euscepes postfasciatus* (Fairmaire)

(Coleoptera: Curculionidae) in a sweetpotato field. *Applied Entomology and Zoology,* 39(3): 491–495.

Nakamoto, N. Y., and Takushi, J. 2001. Positive phototactic response of the West Indian sweetpotato weevil, *Euscepes postfasciatus* (Fairmaire) (Coleoptera: Curculionidae) to chemiluminescent light and effectiveness of the light source for attracting weevils in the field. *Japan Journal of Applied Entomology and Zoology,* 45:212–214 (in Japanese with English summary).

Nakamoto, N. Y., and Takushi, J. 2002. A newly developed LED (light emitting diode) trap for the West Indian sweetpotato weevil, *Euscepes postfasciatus* (Fairmaire) (Coleoptera: Curculionidae). *Japan Journal of Applied Entomology and Zoology,* 46:145–151 (in Japanese with English summary).

Nderitu, J. et al. 2009. Effectiveness of Entomopathogenic Nematodes against Sweetpotato Weevil (*Cylas puncticollis* Boheman (Coleoptera: Apionidae) under Semi-Field Conditions in Kenya. *Journal of Entomology*, 6:145–154.

Newell, W. 1917. Sweetpotato root weevil. Fla. *State Plant Bd. Quarterly Bulletin,* 2:81– 100.

Nicholls, C. I. et al. 2003. The development and status of biologically based integrated pest management in Cuba. *Integrated Pest Management Reviews,* 7: 1–16.

Nur Aida, H. et al. 2017. Morphological Identification of Weevil and Fungal Pathogen Associated with Sweetpotato tuber during Storage. *Malaysian Application Biology*, 46(3): 81–88.

Okada, Y. et al. 2014. Sweetpotato Resistance to *Euscepes postfasciatus* (Coleoptera: Curculionidae): Larval Performance Adversely Effected by Adult's Preference to Tuber for Food and Oviposition. *Jourrnal of Economical Entomology*, 107(4):1662–1673.

Okonya, J.S., and Kroschel, J. 2013. Incidence, abundance and damage by the sweetpotato butterfly *Acraea acerata* Hew. and the African sweetpotato weevils (Cylas spp.) across an altitude gradient in Kabale district, Uganda. International. *Journal of Agriculture and Science*, 3:814–824.

O'Sullivan, J. 2005. *Wireworms and WDS complex* [Online]. https://keys.lucidcentral.org/keys/sweetpotato/key/. Accessed May 2019.

Painter, R.H. 1951. Insect resistance in crop plants. *LWW*, 72(6):481 .

Palaniswani, M. S. and Rajamma, P. 1986. Biological Control of sweetpotato weevil and Cassava Spider mites. Annual Progress Report 1986 for the Period January - December 1986, *Central Tuber Crops Research Institute, Trivandrum*, India, 90–92.

Palaniswami, M.S. et al. 2002. Evaluation of soil application method of insecticides against *Cylas formicarius* (F.) and its impact on soil microflora in sweetpotato ecosystem. *Journal of Root Crops*, 28: 55– 60.

Perez-Colbert, D. E. 2008. Arthropods of Hispaniola (Dominican Republic and Haiti): A checklist and bibliography. Zootaxa 1831, *Magnolia Press*, 4–76.

Pole, F. S. 1988. Vine Thickness in sweetpotato (Ipomea batatas): Its Inheritance and Relationship to Weevil Damage. University of Agriculture (South Pacific), 1–58.

Proshold, F. I. 1983. Mating activity and movement of *Cylas formicarius elegantulus* (Coleoptera: Curculionidae) on sweetpotato. *Proceedings of the American Society of Horticultural Science Tropical*, Section 27(B): 81–92.

Raman, K. V. 1988b. Strategies to Develop Sweet Potato with Weevil Resistance in Developing Countries. *International Potato Center* (CIP), Lima, Perou.

Raman, K. V. and Alleyne, E. H. 1991. Biology and Management of the West Indian Sweet Potato Weevil. *International Potato Center* (CIP), Lima Peru, 263–281.

Ray, R.C. and Ravi, V. 2005. Postharvest spoilage of sweetpotato in tropics and control measures. *Food Sciences and Nutrition*, 45:623–644.

Reddy, G.V.P., McConnel J., Badilles A. (2012b) Estimation of the population density of the sweetpotato weevils on the Mariana Islands. *Journal of Entomological and Acarological Research*, 44:20–23.

Reddy, G.V.P., Wu S., Mendi R.C., Miller R.H. (2014a) Efficacy of pheromone trapping of the sweetpotato weevil (Coleoptera: Brentidae): based on dose, septum age, attractive radius, and mass trapping. *Environmental Entomology*, 43:767–773.

Reddy, G.V.P. and Chi, H. 2015. Demographic comparison of sweetpotato weevil reared on a major host, *Ipomoea batatas*, and an alternative host, *I. triloba*. *Nature*, *Scientific Reports,* 5(11871):1–9 .

Reinhard, 1923. The Sweetpotato weevil. *Texas Agricultural Experiment Station Bulletin*, 308: 90.

Sato, K. et al., 1977. Isolation of the Sweetpotato Weevil Factor Causing Terpene Induction in Sweetpotato Roots. *Agricultural and Biological Chemistry*, 41(8):1419–1423.

Sato, Y. et al. 2009. Population dynamics of the West Indian sweetpotato weevil Euscepes postfasciatus (Fairmaire): a simulation analysis. *Journal of Applied Entomology*, 134(4):303–312.

Schaefers, G. A. and Terry, E. R. 1976. Insect Transmission of Sweetpotato Disease Agents in Nigeria. *Phytopathology,* 66:642–645.

Schalk, J.M. et al. 1991. Approaches to the control of multiple insect problems in sweet potato in the southern United States. p. 283-301. In: R.K. Jansson and K.V. Raman (eds.), Sweetpotato Pest Management: A Global Perspective. *Westview Press, Boulder*, CO, USA.

Schonbeck, M. 2013. An Ecological Understanding of Weeds. Virginia Association for Biological Farming. https://articles.extension.org/pages/18529/an-ecological-understanding-of-weeds. Accessed on May 2019.

Shang, Y. et al. 1996. Techniques of sweetpotato virus-free by meristem tip culture. *Plant Protection*, 22(5):14–16 (in Chinese, English abstract).

Shen, S. C. et al. 2018. Allelopathic potential of sweetpotato (Ipomoea batatas) germplasm resources of Yunnan Province in southwest China. *Acta Ecologica Sinica*, 38:444–449.

Shen, S. C. et al. 2014. Effects of sweetpotato (Ipomoea batatas) cultivation on the community composition and diversity of weeds in arid fields, *Biodiversity and Science*, 22:485-491.

Sherman, M. and Tamashiro, M. 1954. The sweetpotato weevil in Hawaii, their biology and control. *Hawaii Agricultural Experiment Station, Technical Bulletin*, (23): 36.

Shimizu, T. and Moriya, S. 1996c. Daily locomotor activity in the Indian sweetpotato weevil, *Euscepes postfasciatus* (Farmaire) (Coleoptera: Curculionidae) and sweetpotato weevil, *Cylas formicarius* (Fabricius) (Coleoptera: Brentidae) monitored by an actograph system. *Applied Entomology and Zoology,* 31: 626–628.

Shimoji, Y. and T. Kohama. 1994. Oviposition behavior of the West Indian Potato Weevil, *Euscepes postfasciatus* (Fairmaire). *Japanese Journal of Applied Entomology and Zoology,* 38(1):43–46.

Skoglund, L.G. and Smit, N.E.J.M. 1994. Major diseases and pests of sweetpotato in Eastern Africa. Lima, Peru: *International Potato Center* (CIP).

Smit, N.E.J.M. et al., 2001. Mass-trapping male *Cylas* spp. with sex pheromones: a potential IPM component in sweetpotato production in Uganda. *Crop Protection*, 20:643–651.

Smith, T. P. 2006. Biology and chemical ecology of the sugarcane beetle and integrated pest management of sweet potato soil insects in Louisiana. PhD dissertation. *Louisiana State University*, Baton Rouge, LA.

Smith, T. P. 2007. Weed Management in Sweetpotato. *Louisiana State University Agricultural Center,* Publication No. 3007.

Stephenson, D. 2019. Louisiana Suggested Chemical Weed Management Guide. *Louisiana State University Agricultural Center,* Publication No.1565.

Stern, V. M. et al. 1959. The Integration of Chemical and Biological Control of the Spotted Alfalfa Aphid. *California Agricultural Experiment Station*, 29(2):83–101.

Stout, M. J. 2014. Host-Plant Resistance in Pest Management. Louisiana State University, LA, USA. D. P. Abrol. (Ed): *Integrated Pest Management*, 1–21.

Suah, J. R. R. 1981. Majors Pests of roots crops in the Caribbean: in short course on integrated Pest management of tropical crops. *Faculty of Agriculture, University of The West Indies,* Sainte Augustine, Trinidad.

Summers, S. V. 1875. The sweetpotato root borer. *Home and Journal*, 10(26):401.

Suris, M. et al. 1995. Evaluation of the damage caused by *Cylas formicarius* (Coleoptera: Curculionideae) and *Typophorus nigritus* (Coleoptera: Chrysomelidae) in sweetpotato intercropped with *maize. Revista de la Proteccion Vegetal*, 10(2):181–84.

Sutherland, J. A. 1986. A review of the biology and control of the sweetpotato weevil *Cylas formicarius* (Fab). *International Journal of Pest Management*, 32(4):304–315.

Talekar, N.S. 1987a. Influence of cultural pest management techniques on infestation of sweet potato weevil. *Insect Science and application*, 8(4/5/6): 809–814.

Tucker, 1937. The Control of Scarbe (*Euscepes batatae*) in Barbados. Barbados Department of Sciences and Agriculture, *Agricultural Journal,* 6(4):133.

Uritani, I. T. et al. 1975. Induction of furano-terpenoids in sweetpotato roots by the larval components of the sweetpotato weevils. *Agricultural Biology and Chemistry*, 37:1857–1862.

USDA/ERS, 1990-1997. Pest Management Practices in Crop Production. Production Practices for Major Crops in U.S. Agriculture. *United States Department of Agriculture, Economic Research Services*, 17–52.

USDA/NASS, 2017. National Agricultural Statistics Service. United States Department of Agriculture. https://www.nass.usda.gov/Publications/AgCensus/2017
Accessed on May 2019

Wakamura, S. et al. 2010. Identification and field activity of the sex pheromone component of the sweetpotato vine borer moth *Omphisa anastomosalis* (Lepidoptera: Crambidae). *Applied Entomology and Zoology*, 45:635–640.

Wang, Y. and Kays, J. 2002 Sweetpotato Volatile Chemistry in Relation to Sweetpotato Weevil (C. formicarius) Behavior. *Journal American Society and Hort Science,* 127(4):656–662.

Wang et al., 2009. Sweetpotato virus in China. *Crop Protection*, 29(2):1–15.

Wang, F. et al. 2016. An environmentally friendly and productive process for bioethanol production from potato waste. *Biotechnology for Biofuels*, 9(50):1–10.

War, A.B. et al. 2012. Mechanisms of Plant Defense against Insect Herbivores. *Plant Signaling & Behavior*, 7(10):1306–1320.

Ward, S. M., Webster, T. M., and Steckel, L. E. 2013. Palmer Amaranth (Amaranthus palmer): A Review. *Weed Technology*, 27:12–27.

Waterhouse, D.F. 1997. The Major Invertebrate Pests and Weeds of Agriculture and Plantation Forestry in the Southern and Western Pacific. ACIAR, Monograph No. 44. Camberra, Australia, *The Australian Centre for International Agricultural Research.*

Webster, T. M. and Coble, H. D. 1997. Changes in the weed species composition of the southern United States: 1974 to 1995. *Weed Technology*, 11:308–317.

William, R. D. and Chiang, M. Y. 1980. Weed Management in Asian Vegetable Cropping Systems. *Weed Science Society of America,* 28 (4):445–451.

Wilson, D. D. et al. 1988. Oviposition Stimulant in Sweet Potato Periderm for the Sweetpotato Weevil, *Cylas formicarius elegantulus* (Coleoptera: Cureulionidae). *Environmental Entomology*, 17(4):691–693.

Wolfe, G. W. 1991. The origin and dispersal of the pest species of Cylas with a key to the pest species groups of the world. p. 13-43. In Sweetpotato Pest Management, a Global Perspective (R. K. Jansson and K. V. Raman eds.). *Westview Press, Boulder*, CO, USA.

WSSA, 2019. Fighting Back Against Pigweed. http://wssa.net/2019/05/fighting-back-against-pigweed/ Accessed May 2019.

Yada, B. 2017. Selection of Simple Sequence Repeat Markers Associated with Inheritance of Sweetpotato Virus Disease Resistance in Sweetpotato. *Crop Sciences,* 12:1421–1430.

Yan, Q. et al. 2014.Reexamination of the female sex pheromone of the sweetpotato vine borer moth: identification and field evaluation of a tricosatriene. *Journal of Chemical Ecology*, 40:590–598.

Yasuda, K. 1995. Mass trapping of the sweet potato weevil *Cylas formicarius* (Fabricius) (Coleoptera: Brentidae) with a synthetic sex pheromone. *Applied Entomology and Zoology*, 30:31–36.

Yasuda, K. 1999. Auto-infection system for the sweetpotato weevil, *Cylas formicarius* (Fab.) (Coleoptera: Curculionidae) with entomopathogenic fungi, *Beauveria bassiana* using a modified sex pheromone trap in the field. *Applied Entomology and Zoology*, 34: 501–505

Yen, D.E. 1982. Sweetpotato in historical perspective. In: Villareal, R.L. and T.D. Griggs (eds.). Sweetpotato, Proceedings of First International Symposium. *AVRDC Publ*. No. 82-172, Tainan, Taiwan, 17–30.

CHAPTER 2. DETECTION OF THE WEST INDIAN SWEETPOTATO WEEVIL, *EUSCEPES POSTFASCIATUS*, IN HAITI

2.1. Introduction

Identification of the sex pheromone of *C. formicarius* revolutionized sweetpotato weevil management worldwide. Pheromone traps are used in IPM programs in US sweetpotato producing states to assess weevil numbers. Haiti is one of the countries in the Caribbean basin that had benefited from IPM programs funded by the USDA and implemented by the International Center of Potato in collaboration with the University of Florida via the Ministry of Agriculture of Haiti from 1989 to 2002 (Alvarez et al.1996; Oscar, 2009) that has used pheromone traps. The project designed and assessed the effectiveness of sex pheromone trap models that were adaptable and economically accessible to farmers (Jackson and Bohac, 2006). This program was, until recently, the only major work that had been conducted on sweetpotato weevil control in Haiti. From this work, sweetpotato yield increased from 4.5 MT/ha to 14.9 MT/ha (Athis, 2014). Since then, most collaboration on sweetpotato with international institutions emphasized finding *C. formicarius* resistant sweetpotato varieties or varieties with orange flesh to help people in Haiti who are facing vitamin A deficiency (CIAT, 2018). Studies in Haiti have characterized weevil resistance in different sweetpotato varieties available in Haiti and determined the incidence of *C. formicarius* (Joseph, 1993; Jean-Baptiste, 2018).

Raman and Alleyne (1991) reported that *C. formicarius* and *E. postfasciatus* are often sympatric in the Caribbean basin and the two species can cause significant damage where they co-occur (Suah, 1981). Although *C. formicarius* is the prevalent weevil in Haiti, all damage recorded in sweetpotato should not be attributed to just this one insect, but also to *E. postfasciatus* since it is reported to be also present. Okada et al. (2014) stated in areas where both species of weevils occur sympatricly, the damage to sweetpotato should be evaluated for both species.

Despite conflicting information about the occurrence of *E. postfasciatus* in Haiti, no detailed information had been provided relating to studies conducted and methods used to confirm these results. Jackson and Bohac (2006) stated that the capacity to identify a pest is the first step to an appropriate and effective control plan. Thus, we conducted a survey to investigate the occurrence of the West Indian sweet potato weevil, *E. postfasciatus,* in Haiti in order to clear the controversial information which will be helpful before new effective IPM program is initiated.

2.2. Materials and Methods

2.2.1. Study locations

This study was carried out from June 6th, 2018 to August 8th, 2018 in Haiti, located in the West Indies/Caribbean Basin (N 19^{o}.18'24.36" and W 72^{o}43'45.15") and bordered to the east by the Dominican Republic. Haiti's climate is characterized by two main seasons, a rainy season which extends from May to November with a short intermittent dry period (June and July) and a dry season from December to April (Singh and Cohen, 2014) with an average annual rainful of 1440 mm (World Bank, 2014).

Sweetpotato is grown year-round in Haiti however, most of the production is done during the rainy season (70%) (CNSA, 2013-2014). Sweetpotato is produced in almost all eight agro-ecological areas represented by the Haiti's agricultural land: dry agro-pastoral, agro-pastoral plateau, agro-pastoral semi-humid, humid mountains area, dry area of agriculture and fishing, plains area in monoculture, sea salt area production (MARNDR, 2012). However, most sweetpotato production areas are concentrated in the humid mountains, agro-pastoral plateau, and irrigated plains areas (FEWSNET, 2018; MARNDR, 2011). Site selection for insect sampling was based on the main sweetpotato growing regions, agro-ecological conditions, ontogeny of sweetpotato in field, and distance between collection sites (Table 2.1). Sweetpotato

fields ranged from 50 m^2 to 5000 m^2. One trap was used per field if the field was less than 5000m^2 and two traps per field in fields greater than 5000 m^2.

Table 2.1. Collection site coordinates of trapped weevil and infested sweetpotato roots

Latitude	Longitude	Elevation	Collection Date	Locality	Sites	Department
18.94811	-72.382516	207.541183	2018-06-08T15:13:40Z	Tamarin1	Lachapelle	Artibonite
18.94914	-72.38218	205.428482	2018-06-08T15:05:15Z	Marin2	Lachapelle	
18.8338	-72.105131	52.934696	2018-06-08T13:23:49Z	Bossou	Lachapelle	
19.08337	-72.615334	291.764923	2018-07-24T17:54:29Z	Chiquette	Saint-Marc	
19.06799	-72.611331	279.476959	2018-07-24T15:36:23Z	Corbier/Gilbert	Saint-Marc	
19.53398	-71.729704	166.681442	2018-07-24T15:17:31Z	Ti Plaine/Gilbert	Saint-Marc	
18.78499	-72.179234	202.341934	2018-07-27T15:36:52Z	Madame Jack	Saut/d'Eau	
19.05522	-72.473323	56.072899	2018-06-19T16:28:35Z	Bassin Vincent	Verrettes	
19.13012	-72.459247	47.453407	2018-06-19T21:44:29Z	Laverdure	Petite Riviere	
19.057	-72.468037	58.934326	2018-06-19T16:43:54Z	Desjardins	Verrettes	
19.16438	-72.603167	32.238338	2018-06-20T18:56:16Z	Marche Pont-Sonde	Liancourt	
19.24264	-72.490766	35.469963	2018-06-20T17:37:26Z	Boen	Marchand	
19.03366	-72.436304	28.105	2018-06-20T13:50:49Z	Laroque	Verrettes	
18.38306	-72.589735	765.108948	2018-06-21T16:10:03Z	Bas-Tonnelle	Leogane	Ouest
18.45447	-72.622891	39.056126	2018-06-21T19:12:18Z	Mat Duford	Leogane	
18.36671	-72.585209	869.362915	2018-06-21T15:44:44Z	Fond Droit	Leogane	
18.78077	-72.265687	377.718353	2018-06-29T18:25:11Z	Cas-Michel	Croix des Bouquets	
18.5973	-72.291361	6.942551	2018-06-06T15:03:56Z	Ferme de Damien	Croix des Bouquets	
18.91351	-72.131683	204.282806	2018-06-26T17:42:50Z	Morne Pintade	Boucan Carre	Central Plateau
19.14693	-71.977235	267.717834	2018-06-27T22:09:42Z	Papaye	Hinche	
18.91352	-72.131646	314.628357	2018-06-27T21:13:41Z	Ceramon	Hinche	
19.15963	-71.942852	248.975204	2018-06-28T13:59:33Z	Londrain	Hinche	
18.80626	-72.235223	366.609711	2018-06-29T17:01:18Z	Grand Rack	Saut-d'Eau	
18.83686	-72.297736	583.584961	2018-06-29T15:14:23Z	Lekete	Saut-d'Eau	
18.78369	-72.248479	173.554596	2018-06-29T18:06:15Z	Cas-Turpin	Saut-d'Eau	
18.94608	-72.175889	227.729004	2018-07-03T19:58:58Z	Ferobien	Boucan Carre	
18.9339	-72.162217	228.568573	2018-07-03T21:38:19Z	Pepin	Boucan Carre	
18.84075	-71.815621	316.068054	2018-07-04T14:57:08Z	Cas Laroche1	Belladere	
18.84156	-71.822078	305.588776	2018-07-04T15:34:36Z	Cas Laroche2	Belladere	
18.86798	-71.762712	378.94574	2018-07-04T17:19:22Z	Marche Belladere	Belladere	
18.84979	-71.811785	311.103241	2018-07-05T16:55:38Z	Marche Belladere	Belladere	
18.85767	-71.824032	277.487976	2018-07-05T16:16:42Z	Moliere/Cx-Fer	Belladere	
19.15328	-71.781492	198.552979	2018-07-13T12:39:58Z	Los Posos1	Cerca La Source	
19.14218	-71.767475	164.256561	2018-07-13T13:04:09Z	Los Posos3	Cerca La Source	

(Table cont'd)

Latitude	Longitude	Elevation	Collection Date	Locality	Sites	Department
19.08364	-72.617168	155.85733	2018-07-27T15:31:16Z	Noyeau	Mirebalais	Central Plateau
18.80224	-72.1506	187.715591	2018-07-27T17:08:30Z	Noyeau	Mirebalais	
18.81386	-72.128924	145.663361	2018-07-27T18:04:28Z	Plotier	Mirebalais	
18.82282	-72.117481	157.538223	2018-07-27T18:33:15Z	Zilia	Mirebalais	
18.21144	-73.888287	63.393425	2018-06-15T19:53:03Z	Veille/Chantal	Les Cayes	Sud
18.22042	-73.892007	7.993615	2018-06-15T20:44:47Z	Duperon/Chantal	Cayes	
18.26434	-73.807534	-112.099686	2018-06-15T15:21:12Z	Laval1	Cayes	
18.25742	-73.807927	68.776787	2018-06-15T15:44:59Z	Laval2	Cayes	
19.09201	-72.6349	212.294525	2018-06-15T13:58:40Z	Camp Perrin	Cayes	
18.25121	-73.797367	-40.274017	2018-06-15T16:23:56Z	Vyezo Laval	Cayes	
18.18583	-73.881072	14.942139	2018-06-15T21:17:17Z	Leetang Chantal	Cayes	
19.57989	-71.775606	14.510427	2018-07-18T22:54:21Z	Auvois	Ouanaminthe	Nord'Est
19.55679	-71.774733	37.229206	2018-07-18T22:24:18Z	Dilaire	Ouanaminthe	
19.55928	-71.759221	46.965611	2018-07-18T21:40:19Z	Makemara	Ouanaminthe	

The distance between collection sites varied among localities within a commune, from 300 m to over 1000 m, and between communes, from 10 km to more than 30 km. Based on topographic level, overall traps were placed in plains, plateau, and semi-humid mountains (Fig. 2.1). The sweetpotato fields selected were at least 45 days after planting (DAP).

Fig. 2.1. Trap weevil placement and infested sweetpotato roots collection in the five most sweetpotato production regions in Haiti.

2.2.2. Data Collection

Two methods were used for weevil collection following the five main sweeetpotato growing departments (Table 2.2.): sampling in the fields and from infested sweetpotatoes.

2.2.2.1. Field Sampling

Synthetic sex pheromone is not available for *E. postfasciatus* (Raman and Alleyne, 1991). Instead, traps baited with sweetpotato slices or light traps including light emitting diodes (LED), and ultra violet light are the most common models used for *E. postfasciatus* (Nakamoto and Takushi, 2002 ; Nakamoko and Kuba, 2004). In this study, traps baited with sweetpotato slices were used as lures for weevil collection. The traps (Great Lakes IPM®, Vestaburg, Michigan, USA) were left for twelve to fourteen hours in the field. They were placed from 4:00 to 5:00 pm in the afternoon and retrieved from 6:00 am to 8:00 am in the morning depending on the sites. The traps were 20cm height and placed on the ground in a way to be higher than the sweetpotato canopy as recommanded by the trap manufacturer (Great Lakes IPM, 2018). The insects collected from the traps were emptied into brown paper bags for observation and brought back for processing and identification.

A B C

Fig. 2.2. Trap placement and retrieval for insect observation. A: Trap placement in afternoon. B: Trap collection with few weevils. C: Trap with sweetpotato slices almost completetly covered by weevils.

2.2.2.2. Root Sampling

Fourteen mature sweetpotato fields were selected and four infested roots were collected in each field using the Jansson and McSorley (1990) method. This consisted of dividing the field

into four quadrats and collecting one infested root in each quadrat. In locations where sweetpotato were harvested prior to the study, roots were collected in the regional markets. A total of thirty roots representing fifteen locations (1 to 3 roots/locality) were collected in the regional markets and preserved in paper bags for incubation. From these roots, five were from the Dominican Republic collected in three regional markets in Central Plateau. All collection sites (field and markets) were georeferenced using a Garmin GPSMAP® 78s Handheld Device.

Each root was placed in an 886 ml plastic container covered with tile and held with a rubber band. Weevil rearing was conducted in a room at ambient temperature. Weather conditions at the site of incubation were between 75-99ºF, 79-100ºF, and 89-100ºF for June, July, and August respectively as per CustomWeather© 2018.
https://www.timeanddate.com/weather/haiti/port-au http://agriculture.gouv.ht prince/.
The relative humidity ranged from 32-94%. Containers were observed on a weekly basis for six weeks and emerged adults were collected. At the end of the incubation period, each root was split for detection of immature stages. Immature insects were preserved in 95% EtOH for further identification.

2.2.3. Insect Identification

Adult *E. postfasiciatus* identification can be done using the elytra. The latter is reddish almost black and completely covered with hard hairs and a white spot at the apex of the abdomen (Alleyne, 1982; McCaffrey et al, 2005). A magnifier model Fancii Large LED Lighted Handheld 2X with 3.5X Zoom was used in field for insect identification.

The immatures of the two weevil species are similar at first view. Thus, observation was done in the Soybean Lab of Louisiana State University under a Leica EZ4 D at 35X magnification and where discriminated based on the characters described by Sherman and Tamashiro (1954). These authors stated that the main difference is found in the hair position on the frontal head of

the two weevils and reported that the 2nd hair in the frontal head of the *E. postfasciatus* is ventrally located to hair 1 while it is dorsally placed for the *C. formicarius.* The 3rd hair is almost parallel to the 4th whereas, this is not the case for *C. formicarius*, and the three lateral hairs are longer than those of *C. formicarius*. In this study, eleven weevil larvae and fifteen pupae were collected from the incubated roots and observed under microscope. The difference between the pupal of the two species was studied mainly based on the development color throughout the development time. However, their morphological characters are obviously distinguishable at the last duration day (Sherman and Tamashiro, 1954). Since the samples were collected at once they were stored in EtOH 95% and, only observed based on morphological characters .

2.2.4. Data Analysis

Data was recorded on a weekly-basis in excel table for the adult species emergence. At the end of the root incubation, the data analysis was performed using functions of the same statistic tool (Excel) and GraphPad Prism8© Software Inc. to display the weevils species emergence.

2.3. Results

2.3.1. Trapped weevil species in Field

A total of 69 traps were placed in 18 communes and 48 localities throughout the five departments (Table 2.2). The number of trap placement was unbalanced with Central Plateau and Artibonite department representing 47.8% and 23.3% respectively of the total traps. Artibonite produces more sweetpotato than the other departments (MARNDR, 2016) and the largest number of traps placed in Central Plateau reflects the fact that emphasis was put on the localities closest to the Dominican Republic to enhance the probability of finding *E. postfasciatus* in these areas. All adults weevils trapped in field were identified as *C. formicarius*, an the number per trap ranged from 0 to ≥ 100, depending on the infestation level of the field and the altitude. No *E. postfasciatus* was detected even within the areas bordering the Dominican Republic.

Table 2.2. Detection of *Euscepes postfasciatus* (F)

Department	Communes/ Department	Sampling location/Commune	# of localities	Total traps/	Elevation range (m)	*Euscepes postfasciatus*
						Presence
Artibonite	**16**	6	11	16	10 -280	No
West	**20**	2	7	8	6-880	No
Central Plateau	**12**	7	21	33	145-590	No
Northeast	**13**	1	4	4	14-50	No
South	**18**	2	5	8	7-220	No
Total	**78**	**18**	**48**	**69**		

2.3.2. Weevil species emergence

Seventy-eight infested roots including those collected in mature sweetpotato field and those collected in regional markets were incubated and all emerged adult weevils collected during the six weeks incubation period were identified as *C. formicarius* (Fig. 2.3). Five roots from the Dominican Republic were collected in Cerca La Source and Belladere markets and were also incubated.The weevil emerged from these roots were also identified as *C. formicarius.*

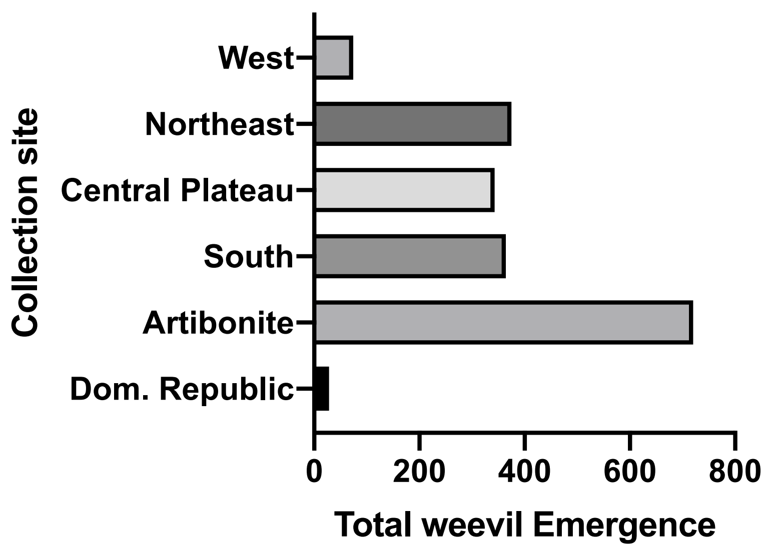
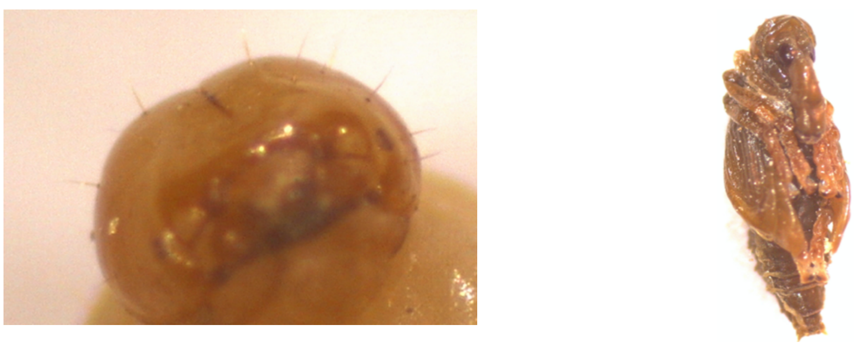


Fig. 2.3. Adult weevil Identification from the incubated sweetpotato roots

For the immatures, larvae and pupae stage observed, displayed the same characteristics discribed above (Materials and Methods) for *C. formicarius*. The 2nd hair was dorsally located to hair 1, the 3rd hair was dorso-mesally placed to the 4th, and the three lateral hairs were not long concluding that these observations were well corresponded to *C. formicarius* frontal head (Fig.2.3 A). Also, each of the 15 pupae observed was morphological different to the *E. postfasciatus* discribed by Sherman and Tamashiro (1954). Their legs and wings were folded and laid laterally on the thorax and fixed downward towards the abdomen. In addition, we could observed the tarsal claws were black even in alcohol. Comparing the samples observed (Fig. 2.3 B) with the characters presenting by Sherman and Tamashiro (1954), the findings revealed they were *C. formicarius* pupae.



A B

Fig. 2.4. Immature weevil identification. A: Frontal head of *C. formicarius* (S). B: Pupae identified as *C. formicarius* (S)

2.4. Discussion

The findings of this study show that all adults, pupae, and larvae collected in the field and from infested roots were *C. formicarius*. Even though ecological factors and environmental conditions were favorable for *E. postfasciatus* development, none were found. According to Jansson and McSorley (1991), proving the absence of a species in a region is not easy but the more thoroughly an area is sampled the more reasonable it is to accept the absence of the species. The sampling method used in this study covered the main sweetpotato growing regions and the

number of samples collected throughout represented a national sampling range. Moreover, the infested roots collected in regional markets represented of the sweet potato crop distribution within Haiti and parts of the Dominican Republic.

Scarce information is available about the population densities of *E. posfasciatus* in sweetpotato foliage. Kinjo (1995) reported an average population density of 10,000/ha stands for 1 insect/ m^2 despite only 1 to 2 traps per field. Even at this rate, we should have detected *E. posfasciatus* if it occured in these sweetpotato growing areas. Moreover, more than 90% of a weevil population lives below ground stated Jansson et al. (1990). However, no *E. postfasciatus* were detected from the infested sweetpotato roots incubated.

E. postfasciatus and *C. formicarius* are often sympatric (Raman and Alleyne, 1991) and use the same resources as food, oviposition sites, and larval habitats (Jansson and Raman 1991). However, their co-occurrence in an area in relation to competition for a resource can influence their population dynamics (Begon et al. 1999). Furthermore, in case of competition for the same limited resources can result in one of the species population decreasing, utilizing alternate resources, or migrating to another habitat (Kuriwada et al, 2012). Resources in Haiti for these two weevils are unlimited year-round since field sanitation is very poor. Moreover, sweetpotato is a low agricultural input crop and has a subsistence status in Haiti, thus, weed removal is done generally in the early stage of the production until harvest, creating habitats for the species. Furthermore, sweetpotato is exclusively propagated by vines among regions that would facilitate a quick spread of *E. postfasciatus* throughout all sweetpotato growing areas. Finally, agricultural exchange of products between Haïti and the Dominican Republic occur daily, representing potential entry of *E. postfasciatus* since the Division of Plant Protection and Quarantine cannot completely control the informal and illicit trade between the two countries

due to the non-functional nature of this division and the weakness of the Quarantine Service office (IICA, 2018).

Kuriwada (2012) reported the low competition abilities of the *E. postfasciatus* in the presence of *C. formicarius* stating that *E. postfasciatus* population density decreases when feeding in a sweetpotato root previously fed by *C. formicarius.* Since *C. formicarius* is prevalent in Haiti and reported to be the best competitor, this could constrain or obscure an obvious *E. postfasciatus* detection. Regardless, the findings of this study find no evidence for the presence of *E. postfasciatus* in Haiti. Thus, this study is consistent with the information suggesting the non-occurrence of *E. postfasciatus* in Haiti.

2.5. References

Alvarez, P. et al. 1996. Economic impact of managing sweet potato weevil (*Cylas formicarius*) with sex pheromones in the Dominican Republic. p. 83-94. In: T. Walker and C. Crissman (eds.), Case Studies of the Economic Impact of CIP Related Technology. *Internatioanl Potato Center* (CIP), Lima, Peru.

Athis, Guy. 2014. Assessment of the methods proposed by PNSA to control SPW (*Cylas formicarius*) at Verrettes. *State University of Haiti, Faculty of Agronomy and Veterinary Medicine*, Damien, Port-au-Prince, Haiti, 50.

Begon, M., Harper, J.L., and Townsend, C. R. 1999. Ecology. individuals, populations and communities. *Blackwell Scientific*, Oxford.

CIAT, 2018. International Centro for Tropical Agriculture. The first steps for biofortification in Haiti: Introducing and creating an enabling environment for biofortified yellow cassava and orange flesh sweet potato varieties in Haiti.http://ciat.cgiar.org/where-we-work/latin-america-and-the-caribbean-2/. Accessed on March 2019.

CNSA, 2013. Evaluation Prévisionnelle de la Performance des Récoltes de la Campagne Agricole de Printemps 2013-2014. Conseil Nationale de Sécurité Alimentaire (CNSA), Port-au-Prince, Haiti. http://www.cnsahaiti.org/Web/Etudes/Evaluation. Accessed on May 2018.

FEWSNET, (2018). Haiti, Staple Food Market Fundamentals. https://reliefweb.int/sites/reliefweb.int/files/resources/Hait. Accessed on 20 May 2018.

Great Lakes IPM, 2018. https://www.greatlakesipm.com/monitoring/ready-to-use-kits/row-amp-field-crops/glms32523k-sweet-potato-weevil. Accessed on May 2018.

Institut Interamericain de cooperation pour l'agriculture (IICA). 2018. Strategie IICA en Haiti 2014-2018. http://www.iica.int/en/countries/hait%C3%AD. Accessed on March 2018.

Jackson, M. D. and Bohac, J. 2006. Evaluation of Pheromones Traps for monitoring Sweet Potato Weevils. *Journal of Agriculture and Urban Entomology*, 23(3): 141-158.

Jean-Baptiste, P. L. O. 2018. Caractérisation agro-morphologique de seize (16) variétés de patate douce (*Ipomoea batatas* Lam.) sur la ferme de Damien. *State University of Haiti*, 83.

Jansson, R. K. and McSorley, R. 1990. Sampling plans for the sweetpotato weevil (Coleoptera: Curculionidae) on sweetpotato in Southern Florida. *Journal Economic of Entomolology*, 83: 1901-1906.

Joseph, Jean B. B. 1993. "Morphological characterization of Sweet Potato varieties found in Salagnac and assessment of their vulnerability to *Cylas formicarius*." State University of Haiti, *Faculty of Agronomy and Veterinary Medicine*, Damien, Port-au-Prince, Haiti, 58.

Kinjo, K. et al. 1995. Estimation of population Density, Survival and Dispersal Rates of the West Indian Weevil, *Euscepes postfasciatus* Fairmaire (Coleoptera: Curculionidae), with Mark and Re-capture Methods. *Applied Entomology and Zoology*, 30(2): 313-318.

Kuriwada, et al. 2012. Effects of intra- and inter-specific competition on fitness of sweetpotato weevil and West Indian sweetpotato weevil. *Journal of Applied Entomology*, 137:310-316.

McCaffrey et al. 2005. West Indian Sweetpotato Weevil. *Museum Victoria*. http://www.padil.gov.au/pests-and-diseases/pest/main/142363/44220. Accessed on June 2018.

Ministry of Agriculture, Natural Resources, and Rural Development (MARNDR). 2011. Politique de développement agricole 2010-2025. MARNDR, 1-28. Port –au-Prince, Haïti. http://agriculture.gouv.ht/view/01/IMG/pdf/Politique_de_developpement_agricole-Version_finale_mars_2011.pdf. Accessed on May 2019.

Ministry of Agriculture, Natural Resources, and Rural Development (MARNDR). 2012. Recensement General Agricole. Atlas Agricole d'Haiti. http://agriculture.gouv.ht/view/01/?Recensement-General/ Accessed on May 2019.

Nakamoto, Y. and Takushi, J. 2002. A newly developed LED (light emitting diode) trap for the West Indian sweetpotato weevil, *Euscepes postfasciatus* (Fairmaire) (Coleoptera: Curculionidae). *Japan Journal of Applied Entomology and Zoology*, 46:145-151. (in Japanese with English summary).

Nakamoto, N. Y., and Kuba, H. 2004. The effectiveness of a green light emitting diode (LED) trap at capturing the West Indian sweetpotato weevil, *Euscepes postfasciatus* (Fairmaire) (Coleoptera: Curculionidae) in a sweetpotato field. *Applied Entomology Zoology and Zoology,* 39(3): 491-495.

Okada, Y. et al. 2014. Sweetpotato Resistance to *Euscepes postfasciatus* (Coleoptera: Curculionidae): Larval Performance Adversely Effected by Adult's Preference to Tuber for Food and Oviposition. *Jourrnal of Economical Entomology*, 107(4): 1662-1673.

Oscar, M. and Raman, K. V. 1989. Strategies to Develop an Integated Pest management Program for Sweetpotato Weevil Complex in the Caribbean, 392-. In the 25th Annual Meeting, Guadelupe. *International Potato Center (CIP)*, Lima, Peru.

Raman, K. V. and Alleyne, E. H. 1991. Biology and Management of the West Indian Sweet Potato Weevil. *International Potato Center*, Lima Peru, 263-281.

Singh, B. and Cohen, M. J. 2014. Adaptation to Climate Changes: Case of Haiti. Research Report, *Montreal University, Oxfam of Americas*, 1-40. [French Version]. https://oxfamilibrary.openrepository.com. Accessed on May 2019.

Suah, J. R. R. 1981. Majors Pests of roots crops in the Caribbean: in short course on integrated Pest management of tropical crops. *Faculty of Agriculture, University of The West Indies*, Sainte Augustine, Trinidad.

Sherman, M. and Tamashiro, M. 1954. The sweetpotato weevil in Hawaii, their biology and control. Hawaii, *Agricultural Experiment Smtation, Technical Bulletin*, (23): 36.

World Bank, 2014. Hauteur moyenne des précipitations (mm par an). Fichiers électroniques et site Web de l'Organisation des Nations-Unies pour l'alimentation et l'agriculture. https://donnees.banquemondiale.org/indicateur/AG.LND.PRCP.MM?end=2014&locations=HT&most_recent_value_desc=true&start=2014&view=bar. Accessed on May 2019.

CHAPTER 3. GENETIC DISTINCTION OF *CYLAS FORMICARIUS* BIOTYPES OF HAITI AND LOUISIANA

3.1. Introduction

Of all known cultivated plants, sweetpotato is reported to be the world's highest yielding crop with a greater value and total food production per unit area exceeding even rice (ISAAA 2003). However, sweetpotato production is limited by *C. formicarius*, the most destructive pest of sweetpotato worldwide (Chalfant et al, 1990). Repeated outbreaks reported to occur in sweetpotato production regions of the US (Sorensen, 1987), China (Gitomer, 1996), and Japan (Ito et al., 1999) have led to the establishment of quarantine regulations within sweetpotato growing countries in order to decrease the potential risk of introduction of this pest via international trade (Nilakhe, 1991). Updating information about pest species in a country is very important because phytosanitary control breaches should be registered. Consistent data about *C. formicarius* populations is of major importance in order to control the spread of this pest. According to Hebert et al (2003), DNA sequences as taxon 'barcodes' is the most reliable and sustainable tool for the identification of a pest. For the past three decades, molecular markers based on DNA barcoding have been largely used in entomology and systematics because of their valuable contribution to the traditional phylogenetic methods for insect discrimination (Wagener et al. 2004). Also, DNA barcoding is used for agricultural purposes, either in determining insecticide resistance in sympatric species to designing host plant resistance for specific insect biotypes. In addition, barcodes allow researchers to track biotypes within the agro-ecoscape. For instance, recent results were reported on the development of genotyping-by-sequencing assays to help determine the resistance allele in *Rhyzopertha dominica* (Fabricius) (Kaur et al., 2013).

Findings on the expansion of *C. formicarius* reported similarities among biotypes in different sweetpotato growing countries and the potential pathway of its invasion. Data were reported on *C. formicarius* biotypes in different sweetpotato growing areas of the US including

Georgia and Hawaii (Kawamura et al. 2007) and Mississippi, North Carolina, Alabama, and Florida (Louis et al. 2006). However, no information is yet available about *C. formicarius* biotypes from Haiti and Louisiana since it is reported that the first occurrence of this pest was in New Orleans, Louisiana where European brought sweetpotato directly from Haiti (Cockerham, 1954). This study aimed to determine any potential distinction between *C. formicarius* biotypes from these two locations.

3.2. Materials and Methods

3.2.1. Insect collection

The *C. formicarius* samples from Haiti collected in chapter 2 (above) were used in this study. A total of 90 samples were collected within five departments in Haiti. Samples also include weevils trapped in Louisiana from five different sites (Table 3.1) and samples taken from the sweetpotato weevil colony maintained by the Soybean Entomology Laboratory under the direction of Dr. J. A. Davis in the Department of Entomology at Louisiana State University (LSU).

3.2.2. DNA extraction

DNeasy Tissue kit (QIAGEN Inc.) was used for DNA extraction following the protocol providing by the manufacturer. Between 0.0023 to 50 mg of insects were removed from the alcohol placed on filter paper and dried for approximately 10 min. Some weevils were blended into 1.5 autoclaved vials with 180 µl of 1x PBS or liquid nitrogen using 1.5 µl vials and pestle or mortar and pestle until yielding a maximum homogenization. 20 µl of proteinase K and 200 µl Buffer AL were added to the vials, vortexed throughout and incubated in water bath (Thermo Scientific®) at 56°C for 1 hour. 200 µl 100% ethanol were added to the vials and mixed using a vortex. Mixture was pipetted into DNeasy Mini spin column placed in a 2ml collection tube and centrifuged (Eppendorf® AG) at 8000 rpm for 1min. The DNeasy mini spin column were placed in a new 2 ml collection tube and 500 µl Buffer AW1 were added, and centrifuged at 8,000 rpm for 1 min. A

new 2ml collection tube was used and 500 µl Buffer AW2 were added and centrifuged at 14,000 rpm for 3 min. Finally, the DNeasy Mini spin column were placed in a autoclave 1.5 ml and 100 µl Buffer AE were pipetted directly onto the DNeasy membrane. The tube were incubated at room temperature in the water bath for 1 min and centrifuged at 8000 rpm for one min for elution. The DNA extracted were immediately placed in a -20°C freezer for further process.

3.2.3. DNA amplification

In order to DNA barcode samples, polymerase chain reaction (PCR) of the internal transcriptase subunit-1 (ITS-1) ITS-1 region of was amplified using ITS-1f (TTG ATT ACG TCC CTG CCC TTT) and ITS-1r (ACG AGC CGA GTG ATC CAC CG) (Taylor and Szalanski 1999, Kawamura et al. 2007). The PCR was performed in a total volume of 25 µl for each master mix containing 13 µl of Taq 2X Master mix w/standard Buffer (New England BioLabs), 0.5 µl forward primer, 0.5 µl reverse primer, 6.5 µl nuclease free water, and 5 µl of DNA template. The PCR was carried out on a Techne thermocycler (TECHNE® TC-42, Bibby Scientific) as follows: denaturation at 94°C for 3 min, follow by 30 cycles of denaturation at 94°C for 1 min, annealing at 60°C for 1 min, an extension of run at 72°C for 3 min, and a final extension at 72°C for 5 min. Visualization and separation of PCR products was done in a 1.5% of agarose gel, prepared in 1x TBE. A sample of 10 µl of PCR product was mixed in 4 µl of 6X glycerol/bromophenol blue loading dye and loaded onto the gel and run at 57 milliamperes for 30 min. The gel was then placed in 10 mg/ml of ethidium bromide solution for 30 min. Each gel contained a 1Kb plus DNA ladder (Invitrogen) and a positive and negative control. DNA visualization and pictures were taken with a High Performance UV Transilluminator (Mitsubishi) and photographed.

3.2.4. PCR product Cleanup

Purified PCR products were cleaned using a Monarch PCR and DNA Clean up kit (New England BioLabs Inc.) manufacturer protocols. Samples were sent for sequencing to CEIDR

Core Facilities and Services, School of Veterinary Medicine, LSU.

3.2.5 DNA sequencing

DNA samples were sent to the School of Veterinary Medicine in LSU for sequencing and were performed using Applied BioSystems BioDyenTerminator version 3.1. Reactions were run with a two AB Prism 3130, a four capillary based DNA sequencer.

3.2.6 Data analysis

The DNA sequences were analyzed using the Bioformatics software Clustal Omega® released by the European Bioformatics EMBL-EBI Institute Hinxton, Cambridge, United Kingdom. The output from the data, a multiple DNA alignment list and a phylogenetic tree, allowed us to determine monophyly or distance branches among the accession not only among Haiti and Louisiana but also with those of other countries.

3.3. Results

3.3.1 DNA Polymorphism Amplification

Ninety DNA samples were extracted representing five Department of Haiti including 32 samples from Central Plateau, 26 from Artibonite, 9 from Northeast, 9 from South, and 9 from West. Three samples were collected from Dominican Republic, however none of them showed bands. Of 18 DNA samples extracted from Louisiana, 11 showed bands (Table 3.1).

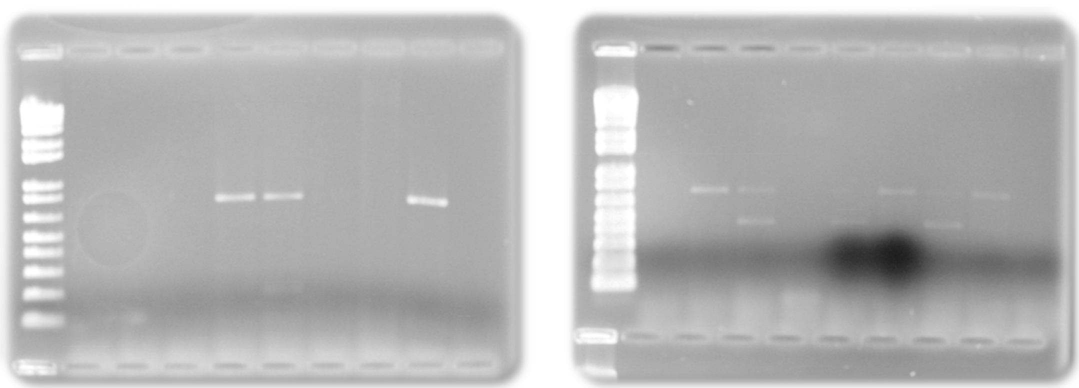
Table 3.1. Positive control of DNA samples

Samples/location	No of PCR products	Number of Samples dispalying positive DNA band	%
Artibonite	26	7	26.92
West	9	5	55.55
Central Plateau	32	13	40.62
Northeast	9	4	44.44

(Table cont'd)

Samples/location	No of PCR products	Number of Samples dispalying positive DNA band	%
South	9	5	55.55
Dom. Rep.	3	0	0
Louisiana	18	11	61.11
Total	**106**	**45**	**42.45**

Less than 50% of the total PCR products yielded DNA and, some were strongly stained and others fainted as it shown in Fig. 3.1. The samples from Louisiana yielded the highest percentage of DNA possibly due to the best storage (-20^{o} C) before DNA extraction. Compare to the other collection sites in Haiti, the Department of Artibonite present the lowest DNA results.



A B

Fig. 3.1. Electrophoresis PCR products for DNA. A: Strong DNA stained. B: Fainted DNA

3.3.2 DNA Sequence determination and relatedness

The sequence alignment (Fig.3.2) reveals high level of similarities between and within the countries. The length of DNA varied from 206 bp to 742 bp. Forty-nine DNA genomes form the phylogenetic tree (Fig.3.3) including thirty-two DNA genomes from Haiti, ten from Louisiana, and seven DNA accession from Georgia, and Hawaii of the USA; Vietnam, Philippines, Taiwan Tainan, and India Kerala from Asia, and St Kits and Nevis from the

Caribbean Basin. These DNA genomes are grouped in clusters to show similarities and distinction among *C. formicarius* biotypes. Globally, the phylogenetic tree is represented in two major clusters and each of them groups seventeen genotypes including fifteen paired that present no significant difference in genetic distances, and nineteen single genotypes. The first cluster is represented by seven sister genotypes including four related genotypes from Haiti, two paired genotypes from Louisiana and Haiti (estimating genetic distances: 0.00553-0.00853, and 0.00216-0.00098) and one separate branch from India Kerala. The second cluster is comprised of seven sister genotypes and ten single ones. From the seven paired genotypes, four of them are sister groups from Haiti and Louisiana the three other paired regroup Haiti with Taiwan Tainan, Vietnam, and Hawaii. The unpaired genotypes in this cluster are St-Kits, Georgia, Philippines, and others from Louisiana and Haiti.

Fig. 3.2. Clustal 0 (1.2.4.) multiple DNA sequence alignment of the biotype genomes based on ITS-1 region distance

```
GrdRackCenter           atcataGNCNNNNNNNNNGNNTNNGNTGCNNGNTANNTTAAG-GTGAAACCGCGAAAGGC   59
Burden2LA               -----gaTTAgCcaTGCATGTTTCAGTACAAGCCAAATTAAG-GTGAAACCGCGAAAGGC   54
CouponArtibonite        ----------NNNNNNNNNNNNNNNNNNNNNNNNNNGNNCNNTCNGCTCGNNNGNNCGAT   50
PepinCenter             ----------------------------gaTTgATGATTTAGTGAGGTCTTCGGACCGAT   32
AuvoisNE                ---------------------------gacgacgGtGGatCaCTCGGCTCGTNNacCGAT   33
India_Kerala            ------------------------------------------------------------   0
Damien1West             ----------------------ggGtGgagTAAGATCATCGG-TGGATCACTCGGCTCGT   37
TaiwanTainan            ------------------------------------------------------------   0
Vietnam                 ------------------------------------------------------------   0
StKits                  ------------------------------------------------------------   0
Georgia                 ------------------------------------------------------------   0
Philippines             ------------------------------------------------------------   0
MmeJackCenter           -------------------------------------------GNGGNNNNcggaccgat   17
MolXFCenter             --------------------------------TGNNNNNNTG-NGGTCtgTcGGACCGAT   27
FondwaWest              ----------------------NNNNNNNNNNNNNNNNNNNg-GTNCTNTCGGAcNNNNT   37
Laval1South             ----------------------ctaCCGATTGATGATTTAGT-GAGGTCTTCGGACCGAT   37
Burden3LA               ------------------------accGaTTGATGatTTAGT-GAGGTCTTCGGACCGAT   35
RoySavCenter            ---------------------ggagtgaNNtcatacggggGN-TcatcTcCGgACTCGNT   38
FerobienCenter          ---------------------------------------------GGNNtNNggAcNNGT   15
RR5                     -----------NNNNNNNNNNNNNNNNNNNNNNNNNNNNNGN-GGNNCTNNCGGAcCGAt   48
MatduFortWest           --------------------------------------------GNNTCGNNNGACCGAT   16
Losposos3Center         ----------------------ctaCCGATTGATGATTTAGT-GAGGTCTTCGGACCGAT   37
BossouArtib             ----------------------ctaCCGATTGATGATTTAGT-GAGGTCTTCGGACCGAT   37
Damien2West             -------------------------ccGATTGATGATTTAGT-GAGGTCTTCGGACCGAT   34
MakebaraNE              ---------------------------agtGagNtcaNtggg-gcTCtGTcagGACCGAt   32
AbricotsCenter          ------------------------gGGtgGagtaagATCATC-GGTGGATCACTCGGCTC   35
McheLekete              ----------------------------------------------------ggaccNNN   8
BVincentArtib           ---------------------------------------ggG-NGGGNNNNcggaccNGN   20
NanPaulArtibonite       ----------------------------------ttNgtgat-GNTCtgtcaGGACCGAT   25
DuperonSouth            --------------------------AtttgatggatTtaGT-GAGGTcTTCGGACCGAT   33
Losposos1Center         ---------------------------TTgatgNatTTAgtG-aGGTCTTCGGAcCGgAT   32
Dilaire1NE              --------------------------TNNNNNtGNNtTANTG-aGGTCtgTcGGACCGAT   33
1bWest                  ----------------------ctaccGaTTGaTGAtTTAGT-GAGGTCTTCGGACCGAT   37
Hawaii                  ------------------------------------------------------------   0
VyezoSouth              -------------------------------------------------NNNggACCGAT   11
PapayeCenter            ---------------------------------GNNNNNNNNNNNNTCGTNNGATCGANA   27
RR4                     ------------------------accGATTGATGAtTTAGTGAGGTCTTCGGACCGATA   36
LavalStDucSouth         -------------------------cgaTTGatNNatTTAGTGAGGTCTTCGGACCGATA   35
Burden4LA               -------------------------ccgaTTGATGAtTTAGTGAGGTCTTCGGACCGATA   35
Buden1LA                ---------------------------ttggaatGattTAgTGAGGTCTTCGGACCGATA   33
RR1                     ----------------------ctacCGATTGATGAtTTAGTGAGGTCTTCGGACCGATA   38
MPintadeCenter          --------------------------------GNNNNNNNNNgGNTCNNTCGGACCGATA   28
TrivilWest              --NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNtcggCTCGNNNNNNNNNNGATA   58
KobyeArtibonite         --------------------------------------ggggggTCatctcggACTCgTA   22
CrowleyLA               -------------------------atTgatNNNNNTNNNggaGgtCtCTCGGACNNNTa   35
LetangSouth             -------------------------ccgattgaTGatTTAGTGAGGTCTTCGGACCGATA   35
RR2                     --------------------------ttggagtGatttagTGAGGTCTgTCGGACCGATA   34
DeenLeeLA               --------------------ccGatttgaatGatttNagTGaGGtCcttNcgGAcCGATA   40
DesjarArtib             --------------------ccgaNtNgaatggatttNagtGAGGTCtNtCgGACCGATA   40


GrdRackCenter           TCATTAAATCAGTTATGGTTCCTTAGATCGTACCCACAATTACTTGGATAACTGTGGTAA   119
Burden2LA               TCATTAAATCAGTTATGGTTCCTTAGATCGTACCCACAATTACTTGGATAACTGTGGTAA   114
CouponArtibonite        ACGCCNATGGCGTTTCNGCGTCGTCNATGTGTCCGGGA---AGTTGACCANNCTT-GATC   106
PepinCenter             ACGCCGATGGCGTTTCGGCGTCNTcNNTGNGTCCGGgN---AGNTNNNTACCTTTCNNCG   89
AuvoisNE                ACgCcGANGGNNNTNCNNCGTCNNCNATGTGTCNNGGA---AGTTGACcNNNCT-TGATc   89
India_Kerala            ------------------------------------------------------------   0
Damien1West             ACNCCNATGGCGTTTCGGCGTCGTCGATGTGTCCGGGA---AGTTGACCAAACT-TGATC   93
TaiwanTainan            ------------------------------------------------------------   0
Vietnam                 ------------------------------------------------------------   0
StKits                  ------------------------------------------------------------   0
Georgia                 ------------------------------------------------------------   0
Philippines             ------------------------------------------------------------   0
MmeJackCenter           ACGCCGATGGCGTTTCGGCGTCGTCGATGTGTCCGGGA---AGTTGACCAAACT-TGATC   73
MolXFCenter             ACGCCGATGGCGTTTCGGCGTCGTCGATGTGTCCGGGA---AGTTGACCAAACT-TGATC   83
FondwaWest              ACGCCGATGGCGTTTCGGCGTCGTCGATGTGTCCGGGA---AGTTGACCAAACT-TGATC   93
Laval1South             ACGCCGATGGCGTTTCGGCGTCGTCGATGTGTCCGGGA---AGTTGACCAAACT-TGATC   93
Burden3LA               ACGCCGATGGCGTTTCGGCGTCGTCGATGTGTCCGGGA---AGTTGACCAAACT-TGATC   91

RoySavCenter            ACGCCGATGGCGTTTCGGCGTCGTCGATGTGTCCGGGA---AGTTGACCAAACT-TGATC   94
FerobienCenter          AcgCCGATGGCGTTTCGGCGTCGTCGATGTGTCCGGGA---AGTTGACCAAACT-TGATC   71
RR5                     aCGCCGATGGCGTTTCGGCGTCGTCGATGTGTCCGGGA---AGTTGACCAAACT-TGATC   104
MatduFortWest           ACGCCGATGGCGTTTCGGCGTCGTCGATGTGTCCGGGA---AGTTGACCAAACT-TGATC   72
Losposos3Center         ACGCCGATGGCGTTTCGGCGTCGTCGATGTGTCCGGGA---AGTTGACCAAACT-TGATC   93
BossouArtib             ACGCCGATGGCGTTTCGGCGTCGTCGATGTGTCCGGGA---AGTTGACCAAACT-TGATC   93
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(Figure cont'd)

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GrdRackCenter         TTCTAGAGCTAAT---ACATGCAAACAGAGGTCCGACCG-------GAG-----------   158
Burden2LA             TTCTAGAGCTAAT---ACATGCAAACAGAGGTCCGACCG-------GAG-----------   153
CouponArtibonite      ATTTANAGGAANTNNNANNCNTNNNNGGGTTNNNNNGGNNNNNNNNNNNNNNNNNNNNNN   166
PepinCenter           TNNAAAAAAAAAAAAAATTNNTT-TTNNGNNGNNNNTNNNNNANNNNNNNCANNGNNNNN   148
AuvoisNE              aTTTAGAGGAAGTAAAAGTCgTAACGNGGTTTCCGNAGGTGAANCTGCNGAANGATCATT   149
India_Kerala          ----------------------------------------------------------aa   2
Damien1West           ATTTAGAGGAAGTAAAAGTCGTAACAAGGTTTCCGTAGGTGAACCTGCGGAAGGATCATT   153
TaiwanTainan          ------------------------------------------------------------   0
Vietnam               ------------------------------------------------------------   0
StKits                ------------------------------------------------------------   0
Georgia               ------------------------------------------------------------   0
Philippines           ------------------------------------------------------------   0
MmeJackCenter         ATTTAGAGGAAGTAAAAGTCGTAACAAGGTTTCCGTAGGTGAACCTGCGGAAGGATCATT   133
MolXFCenter           ATTTAGAGGAAGTAAAAGTCGTAACAAGGTTTCCGTAGGTGAACCTGCGGAAGGATCATT   143
FondwaWest            ATTTAGAGGAAGTAAAAGTCGTAACAAGGTTTCCGTAGGTGAACCTGCGGAAGGATCATT   153
Laval1South           ATTTAGAGGAAGTAAAAGTCGTAACAAGGTTTCCGTAGGTGAACCTGCGGAAGGATCATT   153
Burden3LA             ATTTAGAGGAAGTAAAAGTCGTAACAAGGTTTCCGTAGGTGAACCTGCGGAAGGATCATT   151
RoySavCenter          ATTTAGAGGAAGTAAAAGTCGTAACAAGGTTTCCGTAGGTGAACCTGCGGAAGGATCATT   154
FerobienCenter        ATTTAGAGGAAGTAAAAGTCGTAACAAGGTTTCCGTAGGTGAACCTGCGGAAGGATCATT   131
RR5                   ATTTAGAGGAAGTAAAAGTCGTAACAAGGTTTCCGTAGGTGAACCTGCGGAAGGATCATT   164
MatduFortWest         ATTTAGAGGAAGTAAAAGTCGTAACAAGGTTTCCGTAGGTGAACCTGCGGAAGGATCATT   132
Losposos3Center       ATTTAGAGGAAGTAAAAGTCGTAACAAGGTTTCCGTAGGTGAACCTGCGGAAGGATCATT   153
BossouArtib           ATTTAGAGGAAGTAAAAGTCGTAACAAGGTTTCCGTAGGTGAACCTGCGGAAGGATCATT   153
Damien2West           ATTTAGAGGAAGTAAAAGTCGTAACAAGGTTTCCGTAGGTGAACCTGCGGAAGGATCATT   150
MakebaraNE            ATTTAGAGGAAGTAAAAGTCGTAACAAGGTTTCCGTAGGTGAACCTGCGGAAGGATCATT   148
AbricotsCenter        ATTTAGAGGAAGTAAAAGTCGTAACAAGGTTTCCGTAGGTGAACCTGCGGAAGGATCATT   151
McheLekete            ATTTAGAGGAAGTAAAAGTCGTAACAAGGTTTCCGTAGGTGAACCTGCGGAAGGATCATT   124
BVincentArtib         ATTTAGAGGAAGTAAAAGTCGTAACAAGGTTTCCGTAGGTGAACCTGCGGAAGGATCATT   136
NanPaulArtibonite     ATTTAGAGGAAGTAAAAGTCGTAACAAGGTTTCCGTAGGTGAACCTGCGGAAGGATCATT   141
DuperonSouth          ATTTAGAGGAAGTAAAAGTCGTAACAAGGTTTCCGTAGGTGAACCTGCGGAAGGATCATT   149
Losposos1Center       ATTTAGAGGAAGTAAAAGTCGTAACAAGGTTTCCGTAGGTGAACCTGCGGAAGGATCATT   148
Dilaire1NE            ATTTAGAGGAAGTAAAAGTCGTAACAAGGTTTCCGTAGGTGAACCTGCGGAAGGATCATT   150
1bWest                ATTTAGAGGAAGTAAAAGTCGTAACAAGGTTTCCGTAGGTGAACCTGCGGAAGGATCATT   153
Hawaii                ------------------------------------------------------------   0
VyezoSouth            ATTTAGAGGAAGTAAAAGTCGTAACAAGGTTTCCGTAGGTGAACCTGCGGAAGGATCATT   127
PapayeCenter          ATTTAGAGGAAGTAAAAGTCGTAACAAGGTTTCCGTAGGTGAACCTGCGGANGGATCNTT   143
RR4                   ATTTAGAGGAAGTAAAAGTCGTAACAAGGTTTCCGTAGGTGAACCTGCGGAAGGATCATT   151


Damien2West           ACGCCGATGGCGTTTCGGCGTCGTCGATGTGTCCGGGA---AGTTGACCAAACT-TGATC   90
MakebaraNE            aCGCCGATGGCGTTTCGGCGTCGTCGATGTGTCCGGGA---AGTTGACCAAACT-TGATC   88
AbricotsCenter        GTaNcGATGGcgTTTCGGCGTCGTCGATGTGTCCGGGA---AGTTGACCAAACT-TGATC   91
McheLekete            ACGCCGATGGCGTTTCGGCGTCGTCGATGTGTCCGGGA---AGTTGACCAAACTT-GATC   64
BVincentArtib         NcgCCGATGGCGTTTCGGCGTCGTCGATGTGTCCGGGA---AGTTGACCAAACTT-GATC   76
NanPaulArtibonite     ACGCCGATGGCGTTTCGGCGTCGTCGATGTGTCCGGGA---AGTTGACCAAACTT-GATC   81
DuperonSouth          ACGCCGATGGCGTTTCGGCGTCGTCGATGTGTCCGGGA---AGTTGACCAAACTT-GATC   89
Losposos1Center       ACGCCGATGGCGTTTCGGCGTCGTCGATGTGTCCGGGA---AGTTGACCAAACTT-GATC   88
Dilaire1NE            ACGCCGATGGCGTTTCGGCGTCGTCGATGTGTCCGGGA---AGTTGACCAAACTTGtATC   90
1bWest                ACGCCGATGGCGTTTCGGCGTCGTCGATGTGTCCGGGA---AGTTGACCAAACTT-GATC   93
Hawaii                ------------------------------------------------------------   0
VyezoSouth            ACGCCGATGGCGTTTCGGCGTCGTCGATGTGTCCGGGA---AGTTGACCAAACT-TGATC   67
PapayeCenter          CGCCGATGGCGNTNCNAGCNTCGTCNATGTGTCCGGGA---AGTTGACCAAACT-TGATC   83
RR4                   CGCC-GATGGCGTTTCGGCGTCGTCGATGTGTCCGGGA---AGTTGACCAAACT-TGATC   91
LavalStDucSouth       CGCC-GATGGCGTTTCGGCGTCGTCGATGTGTCCGGGA---AGTTGACCAAACT-TGATC   90
Burden4LA             CGCC-GATGGCGTTTCGGCGTCGTCGATGTGTCCGGGA---AGTTGACCAAACT-TGATC   90
Buden1LA              CGCC-GATGGCGTTTCGGCGTCGTCGATGTGTCCGGGA---AGTTGACCAAACT-TGATC   88
RR1                   CGCC-GATGGCGTTTCGGCGTCGTCGATGTGTCCGGGA---AGTTGACCAAACT-TGATC   93
MPintadeCenter        CGCC-GATGGCGTTTCGGCGTCGTCGATGTGTCCGGGA---AGTTGACCAAACT-TGATC   83
TrivilWest            CGCC-GATGGCGTTTCGGCGTCGTCGATGTGTCCGGGA---AGTTGACCAAACT-TGATC   113
KobyeArtibonite       CGCC-GATGGCGTTTCGGCGTCGTCGATGTGTCCGGGA---AGTTGACCAAACT-TGATC   77
CrowleyLA             CGCC-GATGGCGTTTCGGCGTCGTCGATGTGTCCGGGA---AGTTGACCAAACT-TGATC   90
LetangSouth           CGCC-GATGGCGTTTCGGCGTCGTCGATGTGTCCGGGA---AGTTGACCAAACT-TGATC   90
RR2                   CGCC-GATGGCGTTTCGGCGTCGTCGATGTGTCCGGGA---AGTTGACCAAACT-TGATC   89
DeenLeeLA             CGCC-GATGGCGTTTCGGCGTCGTCGATGTGTCCGGGA---AGTTGACCAAACT-TGATC   95
DesjarArtib           CGCC-GATGGCGTTTCGGCGTCGTCGATGTGTCCGGGA---AGTTGACCAAACT-TGATC   95
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(Figure cont'd)

```
LavalStDucSouth         ATTTAGAGGAAGTAAAAGTCGTAACAAGGTTTCCGTAGGTGAACCTGCGGAAGGATCATT   150
Burden4LA               ATTTAGAGGAAGTAAAAGTCGTAACAAGGTTTCCGTAGGTGAACCTGCGGAAGGATCATT   150
Buden1LA                ATTTAGAGGAAGTAAAAGTCGTAACAAGGTTTCCGTAGGTGAACCTGCGGAAGGATCATT   148
RR1                     ATTTAGAGGAAGTAAAAGTCGTAACAAGGTTTCCGTAGGTGAACCTGCGGAAGGATCATT   153
MPintadeCenter          ATTTAGAGGAAGTAAAAGTCGTAACAAGGTTTCCGTAGGTGAACCTGCGGAAGGATCATT   143
TrivilWest              ATTTAGAGGAAGTAAAAGTCGTAACAAGGTTTCCGTAGGTGAACCTGCGGAAGGATCATT   173
KobyeArtibonite         ATTTAGAGGAAGTAAAAGTCGTAACAAGGTTTCCGTAGGTGAACCTGCGGAAGGATCATT   137
CrowleyLA               ATTTAGAGGAAGTAAAAGTCGTAACAAGGTTTCCGTAGGTGAACCTGCGGAAGGATCATT   150
LetangSouth             ATTTAGAGGAAGTAAAAGTCGTAACAAGGTTTCCGTAGGTGAACCTGCGGAAGGATCATT   150
RR2                     ATTTAGAGGAAGTAAAAGTCGTAACAAGGTTTCCGTAGGTGAACCTGCGGAAGGATCATT   149
DeenLeeLA               ATTTAGAGGAAGTAAAAGTCGTAACAAGGTTTCCGTAGGTGAACCTGCGGAAGGATCATT   155
DesjarArtib             ATTTAGAGGAAGTAAAAGTCGTAACAAGGTTTCCGTAGGTGAACCTGCGGAAGGATCATT   155


GrdRackCenter           ----------------ACGGAAGGAGTGCTTTTATTAGATCAAAACCAATCGGTGGCGGG   202
Burden2LA               ----------------ACGGAAGGAGTGCTTTTATTAGATCAAAACCAATCGGTGGCGGG   197
CouponArtibonite        NNNNNGATNANNNNNNTTNNACAGTTTGTCNTTNNTTANTC-GAANNNNNTTTTGNCGCT   225
PepinCenter             CNCCNNATAATAAAAATTACACAGTTTGTCTTTATTTAGTC-GAACGAATTTTTGTCGCT   207
AuvoisNE                AaAGTGATAATAAAAATTACaCAGTTTGTCTTTATTTAGTCNAtAgcagAaTTTTTGTCN   209
India_Kerala            gtgataatataaaaaattacacagtttgttttatttcgtcgaacgtgaatttttgtcact   62
Damien1West             AAAGTGATAATAAAAATTACACAGTTTGTCTTTATTTAGTC-GAACGAATTTTTGTCGCT   212
TaiwanTainan            -aagtgataataaaaattacacagtttgtctttatttagtc-gaacgaatttttgtcgcc   58
Vietnam                 -aagtgataataaaaattacacagtttgtctttatttagtc-gaacgaatttttgtcgct   58
StKits                  --agtgataataaaaattacacagtttgtctttatttagtc-gaacgaatttttgtcgct   57
Georgia                 -aagtgataataaaaattacacagtttgtctttatttagtc-gaacgaatttttgtcgct   58
Philippines             -aagtgataataaaaattacacagtttgtctttatttagtc-gaacgaatttttgtcgcc   58
MmeJackCenter           AAAGTGATAATAAAAATTACACAGTTTGTCTTTATTTAGTC-GAACGAATTTTTGTCGCT   192
MolXFCenter             AAAGTGATAATAAAAATTACACAGTTTGTCTTTATTTAGTC-GAACGAATTTTTGTCGCT   202
FondwaWest              AAAGTGATAATAAAAATTACACAGTTTGTCTTTATTTAGTC-GAACGAATTTTTGTCGCT   212
Laval1South             AAAGTGATAATAAAAATTACACAGTTTGTCTTTATTTAGTC-GAACGAATTTTTGTCGCT   212
Burden3LA               AAAGTGATAATAAAAATTACACAGTTTGTCTTTATTTAGTC-GAACGAATTTTTGTCGCT   210
RoySavCenter            AAAGTGATAATAAAAATTACACAGTTTGTCTTTATTTAGTC-GAACGAATTTTTGTCGCT   213
FerobienCenter          AAAGTGATAATAAAAATTACACAGTTTGTCTTTATTTAGTC-GAACGAATTTTTGTCGCT   190
RR5                     AAAGTGATAATAAAAATTACACAGTTTGTCTTTATTTAGTC-GAACGAATTTTTGTCGCT   223
MatduFortWest           AAAGTGATAATAAAAATTACACAGTTTGTCTTTATTTAGTC-GAACGAATTTTTGTCGCT   191
Losposos3Center         AAAGTGATAATAAAAATTACACAGTTTGTCTTTATTTAGTC-GAACGAATTTTTGTCGCT   212
BossouArtib             AAAGTGATAATAAAAATTACACAGTTTGTCTTTATTTAGTC-GAACGAATTTTTGTCGCT   212
Damien2West             AAAGTGATAATAAAAATTACACAGTTTGTCTTTATTTAGTC-GAACGAATTTTTGTCGCT   209
MakebaraNE              AAAGTGATAATAAAAATTACACAGTTTGTCTTTATTTAGTC-GAACGAATTTTTGTCGCT   207
AbricotsCenter          AAAGTGATAATAAAAATTACACAGTTTGTCTTTATTTAGTC-GAACGAATTTTTGTCGCT   210
McheLekete              AAAGTGATAATAAAAATTACACAGTTTGTCTTTATTTAGTC-GAACGAATTTTTGTCGCT   183
BVincentArtib           AAAGTGATAATAAAAATTACACAGTTTGTCTTTATTTAGTC-GAACGAATTTTTGTCGCT   195
NanPaulArtibonite       AAAGTGATAATAAAAATTACACAGTTTGTCTTTATTTAGTC-GAACGAATTTTTGTCGCT   200
DuperonSouth            AAAGTGATAATAAAAATTACACAGTTTGTCTTTATTTAGTC-GAACGAATTTTTGTCGCT   208
Losposos1Center         AAAGTGATAATAAAAATTACACAGTTTGTCTTTATTTAGTC-GAACGAATTTTTGTCGCT   207
Dilaire1NE              AAAGTGATAATAAAAATTACACAGTTTGTCTTTATTTAGTC-GAACGAATTTTTGTCGCT   209
1bWest                  AAAGTGATAATAAAAATTACACAGTTTGTCTTTATTTAGTC-GAACGAATTTTTGTCGCT   212
Hawaii                  -aagtgataataaaaattacacagtttgtctttatttagtc-gaacgaatttttgtcgct   58
VyezoSouth              AAAGTGATAATAAAAATTACACAGTTTGTCTTTATTTAGTCcgaACGAATTTTTGTCgCT   187
PapayeCenter            ANNNTGatAATAAAAATTACACAGTTTGTCTTTATTTAGTC-GAACGAATTTTTGTCGCT   202
RR4                     AAAGTGATAATAAAAATTACACAGTTTGTCTTTATTTAGTC-GAACGAATTTTTGTCGCT   210
LavalStDucSouth         AAAGTGATAATAAAAATTACACAGTTTGTCTTTATTTAGTC-GAACGAATTTTTGTCGCT   209
Burden4LA               AAAGTGATAATAAAAATTACACAGTTTGTCTTTATTTAGTC-GAACGAATTTTTGTCGCT   209
Buden1LA                AAAGTGATAATAAAAATTACACAGTTTGTCTTTATTTAGTC-GAACGAATTTTTGTCGCT   207
RR1                     AAAGTGATAATAAAAATTACACAGTTTGTCTTTATTTAGTC-GAACGAATTTTTGTCGCT   212
MPintadeCenter          AAAGTGATAATAAAAATTACACAGTTTGTCTTTATTTAGTC-GAACGAATTTTTGTCGCT   202
TrivilWest              AAAGTGATAATAAAAATTACACAGTTTGTCTTTATTTAGTC-GAACGAATTTTTGTCGCT   232
KobyeArtibonite         AAAGTGATAATAAAAATTACACAGTTTGTCTTTATTTAGTC-GAACGAATTTTTGTCGCT   196
CrowleyLA               AAAGTGATAATAAAAATTACACAGTTTGTCTTTATTTAGTC-GAACGAATTTTTGTCGCT   209
LetangSouth             AAAGTGATAATAAAAATTACACAGTTTGTCTTTATTTAGTC-GAACGAATTTTTGTCGCT   209
RR2                     AAAGTGATAATAAAAATTACACAGTTTGTCTTTATTTAGTC-GAACGAATTTTTGTCGCT   208
DeenLeeLA               AAAGTGATAATAAAAATTACACAGTTTGTCTTTATTTAGTC-GAACGAATTTTTGTCGCT   214
DesjarArtib             AAAGTGATAATAAAAATTACACAGTTTGTCTTTATTTAGTC-GAACGAATTTTTGTCGCT   214
                                            * * ** * *  *

GrdRackCenter           TTCGCGCCCGACATCGTTCAACTTGGTGACTCTGAATAACTTTACGCTGATCGCA-----   257
Burden2LA               TTCGCGCCCGACATCGTTCAACTTGGTGACTCTGAATAACTTTACGCTGATCGCA-----   252
CouponArtibonite        GACGCNCCGTCNNANNNAT-A--TNNNGNCCNTNNTTNANNNNGNNNNNNNNNNNNTNNA   282
PepinCenter             GACGCTCGGTCGAAAATAT---CTCGCGTCNATCTTTNNNNNGNNCGNNNNNNNNTTNNA   264
AuvoisNE                CTGACgCTCGGNCNAAAAT-NNNTCGCGNCNATCTTTANNTCNGCCNCNANTNGNTTCNA   268
India_Kerala            gtcgctcggtcgaaaatat---ctcgcgtcgatctttaagtcggccgcgatcgaattcga   119
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(Figure cont'd)

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Damien1West           GACGCTCGGTCGAAAATAT---CTCGCGTCGATCTTTAAGTCGGCCGCGATTAAATTCGA   269
TaiwanTainan          gacgctcggtcgaaaatat---ctcgcgtcgatctttaagtcggccgcgattaaattcga   115
Vietnam               gacgctcggtcgaaaatat---ttcgcgtcgatctttaagtcggccgcgattaaattcga   115
StKits                gacgctcggtcgaaaatat---ctcgcgtcgatctttaagtcggccgcgattaaattcga   114
Georgia               gacgctcggtcgaaaatat---ctcgcgtcgatctttaagtcggccgcgattaaattcga   115
Philippines           gacgctcggtcgaaaatat-ctctcgcgtcgatctttaagtcggccgcgattaaattcga   117
MmeJackCenter         GACGCTCGGTCGAAAATAT---CTCGCGTCGATCTTTAAGTCGGCCGCGATTAAATTCGA   249
MolXFCenter           GACGCTCGGTCGAAAATAT---CTCGCGTCGATCTTTAAGTCGGCCGCGATTAAATTCGA   259
FondwaWest            GACGCTCGGTCGAAAATAT---CTCGCGTCGATCTTTAAGTCGGCCGCGATTAAATTCGA   269
Laval1South           GACGCTCGGTCGAAAATAT---CTCGCGTCGATCTTTAAGTCGGCCGCGATTAAATTCGA   269
Burden3LA             GACGCTCGGTCGAAAATAT---CTCGCGTCGATCTTTAAGTCGGCCGCGATTAAATTCGA   267
RoySavCenter          GACGCTCGGTCGAAAATAT---CTCGCGTCGATCTTTAAGTCGGCCGCGATTAAATTCGA   270
FerobienCenter        GACGCTCGGTCGAAAATAT---CTCGCGTCGATCTTTAAGTCGGCCGCGATTAAATTCGA   247
RR5                   GACGCTCGGTCGAAAATAT---CTCGCGTCGATCTTTAAGTCGGCCGCGATTAAATTCGA   280
MatduFortWest         GACGCTCGGTCGAAAATAT---CTCGCGTCGATCTTTAAGTCGGCCGCGATTAAATTCGA   248
Losposos3Center       GACGCTCGGTCGAAAATAT---CTCGCGTCGATCTTTAAGTCGGCCGCGATTAAATTCGA   269
BossouArtib           GACGCTCGGTCGAAAATAT---CTCGCGTCGATCTTTAAGTCGGCCGCGATTAAATTCGA   269
Damien2West           GACGCTCGGTCGAAAATAT---CTCGCGTCGATCTTTAAGTCGGCCGCGATTAAATTCGA   266
MakebaraNE            GACGCTCGGTCGAAAATAT---CTCGCGTCGATCTTTAAGTCGGCCGCGATTAAATTCGA   264
AbricotsCenter        GACGCTCGGTCGAAAATAT---CTCGCGTCGATCTTTAAGTCGGCCGCGATTAAATTCGA   267
MciheLekete           GACGCTCGGTCGAAAATAT---CTCGCGTCGATCTTTAAGTCGGCCGCGATTAAATTCGA   240
BVincentArtib         GACGCTCGGTCGAAAATAT---CTCGCGTCGATCTTTAAGTCGGCCGCGATTAAATTCGA   252
NanPaulArtibonite     GACGCTCGGTCGAAAATAT---CTCGCGTCGATCTTTAAGTCGGCCGCGATTAAATTCGA   257
DuperonSouth          GACGCTCGGTCGAAAATAT---CTCGCGTCGATCTTTAAGTCGGCCGCGATTAAATTCGA   265
Losposos1Center       GACGCTCGGTCGAAAATAT---CTCGCGTCGATCTTTAAGTCGGCCGCGATTAAATTCGA   264
Dilaire1NE            GACGCTCGGTCGAAAATAT---CTCGCGTCGATCTTTAAGTCGGCCGCGATTAAATTCGA   266
1bWest                GACGCTCGGTCGAAAATAT---CTCGCGTCGATCTTTAAGTCGGCCGCGATTAAATTCGA   269
Hawaii                gacgctcggtcgaaaatat---ctcgcgtcgatctttaagtcggccgcgattaaattcga   115
VyezoSouth            GACgCTCggtCgAAAATAT---CTCGCGTCNATCTTTAAGTCGGCCGCGATTAAATTCGA   244
PapayeCenter          GNNN--------------------------------------------------------   206
RR4                   GACGCTCGGTCGAAAATAT---CTCGCGTCGATCTTTAAGTCGGCCGCGATTAAATTCGA   267
LavalStDucSouth       GACGCTCGGTCGAAAATAT---CTCGCGTCGATCTTTAAGTCGGCCGCGATTAAATTCGA   266
Burden4LA             GACGCTCGGTCGAAAATAT---CTCGCGTCGATCTTTAAGTCGGCCGCGATTAAATTCGA   266
Buden1LA              GACGCTCGGTCGAAAATAT---CTCGCGTCGATCTTTAAGTCGgccgcgATTAaatTcgA   264
RR1                   GACGCTCGGTCGAAAATAT---CTCGCGTCGATCTTTAAGTCGGCCGCGATTAAATTCGA   269
MPintadeCenter        GACGCTCGGTCGAAAATAT---CTCGCGTCGATCTTTAAGTCGGCCGCGATTAAATTCGA   259
TrivilWest            GACGCTCGGTCGAAAATAT---CTCGCGTCGATCTTTAAGTCGGCCGCGATTAAATTCGA   289
KobyeArtibonite       GACGCTCGGTCGAAAATAT---CTCGCGTCGATCTTTAAGTCGGCCGCGATTAAATTCGA   253
CrowleyLA             GACGCTCGGTCGAAAATAT---CTCGCGTCGATCTTTAAGTCGGcCGCGATTAAATTCGA   266
LetangSouth           GACGCTCGGTCGAAAATAT---CTCGCGTCGATCTTTAAGTCGGCCGCGATTAAATTCGA   266
RR2                   GACGCTCGGTCGAAAATAT---CTCGCGTCGATCTTTAAGTCGGCCGCGATTAAATTCGA   265
DeenLeeLA             GACGCTCGGTCGAAAATAT---CTCGCGTCGATCTTTAAGTCGGCCGCGATTAAATTCGA   271
DesjarArtib           GACGCTCGGTCGAAAATAT---CTCGCGTCGATCTTTAAGTCGGCCGCGATTAAATTCGA   271


GrdRackCenter         -----CGGTCTCGCACCGGCGACGCATCTTTCAAATGTCTGCCTT--ATCAACTGTCGAT   310
Burden2LA             -----CGGTCTCGCACCGGCGACGCATCTTTCAAATGTCTGCCTT--ATCAACTGTCGAT   305
CouponArtibonite      NNNNNNNNANNNNNNNNNNNNNNNNNANNNNNNN---------------------------   315
PepinCenter           CGANTNNNNNNANNNCNCNACCA-------------------------------------   287
AuvoisNE              CGACTACNACGACCACTCNAC---CACCGNAAAAAAGGGGAGAGAGGANNGGANTANGA   325
India_Kerala          cgacgactactctccccgaccgacccccgtaaaaaaggggagagaggagaggagaggaga   179
Damien1West           CGACTACGACGACCACTCG---ACCACCGTAAAAAAGGGGAGAGA----GGAGTA---GG   319
TaiwanTainan                cgactacgacgaccactcg---accaccgtaaaaaagggagag  aggagtagg   164
Vietnam                 cgactacgacgactactcg---accaccgtaaaaaaggggagaga  ggagtaggagt   168
StKits                  cgactacgacgaccactcg---accaccgtaaaaaaggggagaga  ggagtaggagt   167
Georgia                 cgactacgacgaccactcg---accaccgtaaaaaaggggagaga  ggagtaggagt   168
Philippines             cgactacgacgactactcg---acccccgtaaaaaaggggagaga  ggagtagga--   168
MmeJackCenter           CGACTACGACGACCACTCG---ACCACCGTAAAAAAGGGGAGAGA  GGAGTAGGA--   300
MolXFCenter             CGACTACGACGACCACTCG---ACCACCGTAAAAAAGGGGAGAGA  GGAGTAGGA--   310
FondwaWest              CGACTACGACGACCACTCG---ACCACCGTAAAAAAGGGGAGAGA  GGAGTAGGA--   320
Laval1South             CGACTACGACGACCACTCG---ACCACCGTAAAAAAGGGGAGAGA  GGAGTAGGA--   320
Burden3LA               CGACTACGACGACCACTCG---ACCACCGTAAAAAAGGGGAGAGA  GGAGTAGGA--   318
RoySavCenter            CGACTACGACGACCACTCG---ACCACCGTAAAAAAGGGGAGAGA  GGAGTAGGA--   321
FerobienCenter          CGACTACGACGACCACTCG---ACCACCGTAAAAAAGGGGAGAGA  GGAGTAGGA--   298
RR5                     CGACTACGACGACCACTCG---ACCACCGTAAAAAAGGGGAGAGA  GGAGTAGGA--   331
MatduFortWest           CGACTACGACGACCACTCG---ACCACCGTAAAAAAGGGGAGAGA  GGAGTAGGA--   299
Losposos3Center         CGACTACGACGACCACTCG---ACCACCGTAAAAAAGGGGAGAGA  GGAGTAGGA--   320
BossouArtib             CGACTACGACGACCACTCG---ACCACCGTAAAAAAGGGGAGAGA  GGAGTAGGA--   320
Damien2West             CGACTACGACGACCACTCG---ACCACCGTAAAAAAGGGGAGAGA  GGAGTAGGA--   317
MakebaraNE              CGACTACGACGACCACTCG---ACCACCGTAAAAAAGGGGAGAGA  GGAGTAGGA--   315
AbricotsCenter          CGACTACGACGACCACTCG---ACCACCGTAAAAAAGGGGAGAGA  GGAGTAGGA--   318
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(Figure cont'd)

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McheLekete                CGACTACGACGACCACTCG---ACCACCGTAAAAAAGGGGAGAGA GGAGTAGGA--   291
BVincentArtib             CGACTACGACGACCACTCG---ACCACCGTAAAAAAGGGGAGAGA GGAGTAGGA--   303
NanPaulArtibonite         CGACTACGACGACCACTCG---ACCACCGTAAAAAAGGGGAGAGA GGAGTAGGA--   308
DuperonSouth              CGACTACGACGACCACTCG---ACCACCGTAAAAAAGGGGAGAGA GGAGTAGGA--   316
Lospos os1Center          CGACTACGACGACCACTCG---ACCACCGTAAAAAAGGGGAGAGA GGAGTAGGA--   315
Dilaire1NE                CGACTACGACGACCACTCG---ACCACCGTAAAAAAGGGGAGAGA GGAGTAGGA--   317
1bWest                    CGACTACGACGACCACTCG---ACCACCGTAAAAAAGGGGAGAGA GGAGTAGGA--   320
Hawaii                    cgactacgacgaccactcg---accaccgtaaaaaaggggagaga ggagtagga--   166
VyezoSouth                CGACTACGACGACCACTCG---ACCACCGTAAAAAANGGGAGAGA GGAGTANGANT   297
PapayeCenter           -------------------------------------------------------------   206
RR4                       CGACTACGACGACCACTCG---ACCACCGTAAAAAAGGGGAGAGA GGAGTAGGAGT   320
LavalStDucSouth           CGACTACGACGACCACTCG---ACCACCGTAAAAAAGGGGAGAGA GGAGTAGGAGT   319
Burden4LA                 CGACTACGACGACCACTCG---ACCACCGTAAAAAAGGGGAGAGA GGAGTAGGAGT   319
Buden1LA                  CGACTACgAcgACCACtcg---aCCaCCGtaaaaaagGggagaga ggAgtaggagt   317
RR1                       CGACTACGACGACCACTCG---ACCACCGTAAAAAAGGGGAGAGA GGAGTAGGAGT   322
MPintadeCenter            CGACTACGACGACCACTCG---ACCACCGTAAAAAAGGGGAGAGA GGAGTAGGAGT   312
TrivilWest                CGACTACGACGACCACTCG---ACCACCGTAAAAAAGGGGAGAGA GGAGTAGGAGT   342
KobyeArtibonite           CGACTACGACGACCACTCG---ACCACCGTAAAAAAGGGGAGAGA GGAGTAGGAGT   306
CrowleyLA                 CGACTACGACGACCaCTCG---ACCACCGTAAAA-AGGGGAgAGA gGAgtAGgagT   318
LetangSouth               CGACTACGACGACCACTCG---ACCACCGTAAAAAAGGGGAGAGA GGAGTAGGAGT   319
RR2                       CGACTACGACGACCACTCG---ACCACCGtAaAaaaggggagAGA GGAgtagGAgt   318
DeenLeeLA                 CGACTACGACGACCACTCG---ACCACCGTAAAAAAGGGGAGAGA GGAGTAGGAGT   324
DesjarArtib               CGACTACGACGACCACTCG---ACCACCGTAAAAAAGGGGAGAGA GGAGTAGGAGT   324

GrdRackCenter                        GGTAGGTTCTGCGC CTACCATGGTCGTAACGGGTAACGGG-GAAT   354
Burden2LA                            GGTAGGTTCTGCGC CTACCATGGTCGTAACGGGTAACGGG-GAAT   349
CouponArtibonite       -------------------------------------------------------------   315
PepinCenter            -------------------------------------------------------------   287
AuvoisNE                  NGNN NCNNACCGTGAGAAGTGTATTATACNCNNNCNTTAACATGCACCGTCTCGNC   381
India_Kerala           aggaggaggagcgccgacaaaattccgg-gtacc-tagacggaa-ctcgaagcgcgaaac   236
Damien1West            AGTAGGAGGAGCGCCGACAAAATTCCGG-GTACC-TAGACGGAA-CTCGAAGCGCGAAAC   376
TaiwanTainan           agtaggaggagcgccgacaaaattccgg-gtacc-tagacggaa-ctcgaagcgcgaaac   221
Vietnam                aggaggaggagcgccgacaaaattccgg-gtacc-tagacggaa-ctcgaagcgcgaaac   225
StKits                 ag---gaggagcgccgacaaaattccgg-gtacc-tagacggaa-ctcgaagcgcgaaac   221
Georgia                ag---gaggagcgccgacaaaattccgg-gtacc-tagacggaa-ctcgaagcgcgaaac   222
Philippines            -gtagaaggagcgccgacaaaattccgg-gtacc-tagacggaa-ctcgaagcgcgaaac   224
MmeJackCenter          -GTAGGAGGAGCGCCGACAAAATTCCGG-GTACC-TAGACGGAA-CTCGAAGCGCGAAAC   356
MolXFCenter            -GTAGGAGGAGCGCCGACAAAATTCCGG-GTACC-TAGACGGAA-CTCGAAGCGCGAAAC   366
FondwaWest             -GTAGGAGGAGCGCCGACAAAATTCCGG-GTACC-TAGACGGAA-CTCGAAGCGCGAAAC   376
Laval1South            -GTAGGAGGAGCGCCGACAAAATTCCGG-GTACC-TAGACGGAA-CTCGAAGCGCGAAAC   376
Burden3LA              -GTAGGAGGAGCGCCGACAAAATTCCGG-GTACC-TAGACGGAA-CTCGAAGCGCGAAAC   374
RoySavCenter           -GTAGGAGGAGCGCCGACAAAATTCCGG-GTACC-TAGACGGAA-CTCGAAGCGCGAAAC   377
FerobienCenter         -GTAGGAGGAGCGCCGACAAAATTCCGG-GTACC-TAGACGGAA-CTCGAAGCGCGAAAC   354
RR5                    -GTAGGAGGAGCGCCGACAAAATTCCGG-GTACC-TAGACGGAA-CTCGAAGCGCGAAAC   387
MatduFortWest          -GTAGGAGGAGCGCCGACAAAATTCCGG-GTACC-TAGACGGAA-CTCGAAGCGCGAAAC   355
Lospos os3Center       -GTAGGAGGAGCGCCGACAAAATTCCGG-GTACC-TAGACGGAA-CTCGAAGCGCGAAAC   376
BossouArtib            -GTAGGAGGAGCGCCGACAAAATTCCGG-GTACC-TAGACGGAA-CTCGAAGCGCGAAAC   376
Damien2West            -GTAGGAGGAGCGCCGACAAAATTCCGG-GTACC-TAGACGGAA-CTCGAAGCGCGAAAC   373
MakebaraNE             -GTAGGAGGAGCGCCGACAAAATTCCGG-GTACC-TAGACGGAA-CTCGAAGCGCGAAAC   371
AbricotsCenter         -GTAGGAGGAGCGCCGACAAAATTCCGG-GTACC-TAGACGGAA-CTCGAAGCGCGAAAC   374
McheLekete             -GTAGGAGGAGCGCCGACAAAATTCCGG-GTACC-TAGACGGAA-CTCGAAGCGCGAAAC   347
BVincentArtib          -GTAGGAGGAGCGCCGACAAAATTCCGG-GTACC-TAGACGGAA-CTCGAAGCGCGAAAC   359
NanPaulArtibonite      -GTAGGAGGAGCGCCGACAAAATTCCGG-GTACC-TAGACGGAA-CTCGAAGCGCGAAAC   364
DuperonSouth           -GTAGGAGGAGCGCCGACAAAATTCCGG-GTACC-TAGACGGAA-CTCGAAGCGCGAAAC   372
Lospos os1Center       -GTAGGAGGAGCGCCGACAAAATTCCGG-GTACC-TAGACGGAA-CTCGAAGCGCGAAAC   371
Dilaire1NE             -GTAGGAGGAGCGCCGACAAAATTCCGG-GTACC-TAGACGGAA-CTCGAAGCGCGAAAC   373
1bWest                 -GTAGGAGGAGCGCCGACAAAATTCCGG-GTACC-TAGACGGAA-CTCGAAGCGCGAAAC   376
Hawaii                 -gtaggaggagcgccgacaaaattccgg-gtacc-tagacggaa-ctcgaagcgcgaaac   222
VyezoSouth             AGGA--NGANNNNCNANNAAATTCCNGG-NNCCT-CN-NGNGAA-CNNNAANNTNNGNNN   351
PapayeCenter           -------------------------------------------------------------   206
RR4                    AGGA---GGAGCGCCgACaAAATTCCGG-GTACC-TAGACGGaA-CTCGaAGCgcgaAaC   374
LavalStDucSouth        AGGA---GGAGCGCCGACAAAATTCCGG-GTACC-TAGACGGAA-CTCGAAGCGCGAAAC   373
Burden4LA              AGGA---GGAGCGCCGACAAAATTCCGG-GTACC-TAGACGGAA-CTCGAAGCGCGAAAC   373
Buden1LA               agga--aggagcgccgacNNNNNNNNNN-NNNCC--------------------------   348
RR1                    AGGG--AGGAGCGCCGACAAAATTCCgG-GTACC-TAGACGGAA-CTCGAAGcgCGAAAC   377
MPintadeCenter         AGGA---GGANNNCCNACNAAATTCCNG-GTACC-TNNANGGAA-CTCGAATTNNNNANN   366
TrivilWest             AGGA---GGAGCGCCGACAAAATTCCGG-GTACC-TAGACGGAA-CTCGAAGCGCGAaac   396
KobyeArtibonite        AGGA---GGAGCGCCGACAAAATTCCGG-GTACC-TAGACGGAA-CTCGAAGNGNGAAAC   360
CrowleyLA              AGGA---GGAGCGCCgacaaaattCNgg-gtacc-TAGAcgGAa-ctcgaagcgcgAacc   372
LetangSouth            AGGA---GGAGCGCCGACAAAATTCCGG-GTACC-TAgACGGAA-CTCNAANCNCGAAAC   373
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(Figure cont'd)

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RR2                     agga---ggagcgccgacaaaattccgg-gtacc-tagacggaa-ctcgaagcGccaaac   372
DeenLeeLA               AGGA---GGAGCGCCGACAAAATTCCGG-GTACC-TAGACGGAA-CTCGAAGCGCGAAAC   378
DesjarArtib             AGGA---GGAGCGCCgACAAAATTCCGG-GTACC-tAgACgGAA-ctcNaN---------   369


GrdRackCenter           CAGGGTTCGATTCCGGAGAGGGAGCCTGAGAAAC---GGCTACCATATCCAAGGAGGGCA   411
Burden2LA               CAGGGTTCGATTCCGGAGAGGGAGCCTGAGAAAC---GGCTACCATATCCAAGGAGGGCA   406
CouponArtibonite        ------------------------------------------------------------   315
PepinCenter             ------------------------------------------------------------   287
AuvoisNE                NACGATTACNCGGTGTTCGTCGACNG----------------------------------   407
India_Kerala            ctagtttcg-cgctcgccgtcgacaaaataataggtgtcaaacgatattcgcccggagca   295
Damien1West             CTANTT-TCNCGCTCNCCGTCNACNAGGNG-------TNNANNNNNNGTNGCCNNN----   424
TaiwanTainan                 ctagtt-tcgcgctcgccgtcgacaaggtg  tcaaacgatattcgcccggagca   273
Vietnam                      ctagtt-tcgcgctcgccgtcgacaaggtg  tcaaacgatattcgcccggagca   277
StKits                       ctagtt-tcgcgctcgccgtcgacaaggtg  tcaaacgatattcgcccggagca   273
Georgia                      ctagtt-tcgcgctcgccgtcgacaaggtg  tcaaacgatattcgcccggagca   274
Philippines                  ctagtt-tcgcgctcgccgtcgacaaggtg  tcaaacgatattcgcccggagca   276
MmeJackCenter                CTAGTT-TCGCGCTCGCCGTCGACAAGGTG  TCAAACGATATTCGCCCGGAGCA   408
MolXFCenter                  CTAGTT-TCGCGCTCGCCGTCGACAAGGTG  TCAAACGATATTCGCCCGGAGCA   418
FondwaWest                   CTAGTT-TCGCGCTCGCCGTCGACAAGGTG  TCAAACGATATTCGCCCGGAGCA   428
Laval1South                  CTAGTT-TCGCGCTCGCCGTCGACAAGGTG  TCAAACGATATTCGCCCGGAGCA   428
Burden3LA                    CTAGTT-TCGCGCTCGCCGTCGACAAGGTG  TCAAACGATATTCGCCCGGAGCA   426
RoySavCenter                 CTAGTT-TCGCGCTCGCCGTCGACAAGGTG  TCAAACGATATTCGCCCGGAGCA   429
FerobienCenter               CTAGTT-TCGCGCTCGCCGTCGACAAGGTG  TCAAACGATATTCGCCCGGAGCA   406
RR5                          CTAGTT-TCGCGCTCGCCGTCgACAAGGTG  TCAAACGATATTCGCCCGGAGCA   439
MatduFortWest                CTAGTT-TCGCGCTCGCCGTCGACAAGGTG  TCAAACGATATTCGCCCGGAGCA   407
Losposos3Center              CTAGTT-TCGCGCTCGCCGTCGACAAGGTG  TCAAACGATATTCGCCCGGAGCA   428
BossouArtib                  CTAGTT-TCGCGCTCGCCGTCGACAAGGTG  TCAAACGATATTCGCCCGGAGCA   428
Damien2West                  CTAGTT-TCGCGCTCGCCGTCGACAAGGTG  TCAAACGATATTCGCCCGGAGCA   425
MakebaraNE                   CTAGTT-TCGCGCTCGCCGTCGACAAGGTG  TCAAACGATATTCGCCCGGAGCA   423
AbricotsCenter               CTAGTT-TCGCGCTCGCCGTCGACAAGGTG  TCAAACGATATTCGCCCGGAGCA   426
McheLekete                   CTAGTT-TCGCGCTCGCCGTCGACAAGGTG  TCAAACGATATTCGCCCGGAGCA   399
BVincentArtib                CTAGTT-TCGCGCTCGCCGTCGACAAGGTG  TCAAACGATATTCGCCCGGAGCA   411
NanPaulArtibonite            CTAGTT-TCGCGCTCGCCGTCGACAAGGTG  TCAAACGATATTCGCCCGGAGCA   416
DuperonSouth                 CTAGTT-TCGCGCTCGCCGTCGACAAGGTG  TCAAACGATATTCGCCCGGAGCA   424
Losposos1Center              CTAGTT-TCGCGCTCGCCGTCGACAAGGTG  TCAAACGATATTCGCCCGGAGCA   423
Dilaire1NE                   CTAGTT-TCGCGCTCGCCGTCGACAAGGTG  TCAAACGATATTCGCCCGGAGCA   425
1bWest                       CTAGTT-TCGCGCTCGCCGTCgACAAGGTG  TCAAACGATATTCGCCCGGAGCA   428
Hawaii                       ctagtt-tcgcgctcgccgtcgacaaggtg  tcaaacgatattcgcccggagca   274
VyezoSouth                   CCNNNTTCCNNCNNCNNCCCCNNCNNGNNN  TNNTNCNNNGGNNGNNNNGGNNN   404
PapayeCenter            ------------------------------------------------------------   206
RR4                          CTAgTT-TCgcgCTCgCcGTCgacaaGgtG  tcaAacgANANTNcgcCcNGAGC   426
LavalStDucSouth              CTAGTT-TCGCGCTCGCCGTCGACAAGGTG  TCaAACGATATTCGCCCGGagca   425
Burden4LA                    CTAGTT-TCGCGCTCGCCGTCGACAAGGtG  TCAAACGATATTCGCCCGGgAGC   425
Buden1LA                ------------------------------------------------------------   348
RR1                          CTAgTTTTCgcGCTCGccgtCgACAaGGtg  tcaAACGAtATtcgCCCGgagca   430
MPintadeCenter               CCNNNT-TCCNNCCCNNCCNCCACNNNGNN  NNNNANNNGNNNNNNNNGGGGNN   418
TrivilWest                   CTAGTT-TCgcGCTCGCCGTCgACAAGGTG  TCAAACGATATTCGCNNNNAGCA   448
KobyeArtibonite              CTANTT-TCNCGCTCCCCGNCNACAANGNG  NNNNACNANNNTNNNCNGGNNNN   412
CrowleyLA               NTNNNN-NNNNNNNNNNNNNNNGGNNNANNA-------N---------------------   402
LetangSouth                  CNANTT-TCNNGCTCNCCNNCNACNANGNG  TTNNNNNNNNNNNGCCNGGNNNN   425
RR2                          ctagtt-cgcgctcgccgtcNacNNNNNNN  NNNNAANATTNNNCNNNNNNNNC   424
DeenLeeLA                    CTAGTT-TCGCGCTCGCCGtCGACaAGGtG  TCAAACGAtATTCGCCCGGAgCa   430
DesjarArtib             ------------------------------------------------------------   369


GrdRackCenter           GCAGGCGCGCAAATTACCCACTC-----CCG---GCACGGGGAGGTAGTGACGAAAAAT-   462
Burden2LA               GCAGGCGCGCAAATTACCCACTC-----CCG---GCACGGGGAGGTAGTGACGAAAAAT-   457
CouponArtibonite        ------------------------------------------------------------   315
PepinCenter             ------------------------------------------------------------   287
AuvoisNE                ------------------------------------------------------------   407
India_Kerala                      ccgaagtat--acgta  cacccaccgcgaggaggtgtg-tatataaact   340
Damien1West             ------------------------------------------------------------   424
TaiwanTainan              c-cgaaaag-tacttacacccacccgcgacc  cgcgaggaggtgtgtatataaactt   327
Vietnam                   c-cgaaaag-tacttacacccacccgcgacc  cgcgaggaggtgtgtatataaactt   331
StKits                    c-cgaaaag-tacttacacccacccgcgacc  cgcgaggaggtgtgtatataaactt   327
Georgia                   c-cgaaaag-tacttacacccacccgcgacc  cgcgaggaggtgtgtatataaactt   328
Philippines               c-cgaaaag-tacttacacccacccgcgacc  cgcgaggaggtgtgtatataaactt   330
MmeJackCenter             C-CGAAAAG-TACTTACACCCACCCGCGACC  CGCGAGGAGGTGTGTATATAAACTT   462
MolXFCenter               C-CGAAAAG-TACTTACACCCACCCGCGACC  CGCGAGGAGGTGTGTATATAAACTT   472
FondwaWest                C-CGAAAAG-TACTTACACCCACCCGCGACC  CGCGAGGAGGTGTGTATATAAACTT   482
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(Figure cont'd)

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Laval1South          C-CGAAAAG-TACTTACACCCACCCGCGACC  CGCGAGGAGGTGTGTATATAAACTT   482
Burden3LA            C-CGAAAAG-TACTTACACCCACCCGCGACC  CGCGAGGAGGTGTGTATATAAACTT   480
RoySavCenter         C-CGAAAAG-TACTTACACCCACCCGCGACC  CGCGAGGAGGTGTGTATATAAACTT   483
FerobienCenter       C-CGAAAAG-TACTTACACCCACCCGCGACC  CGCGAGGAGGTGTGTATATAAACTT   460
RR5                  C-CGAAAAG-TACTTACACCCaCCCGCGACC  CGCGAGGAGGTGTGTATATAAACTT   493
MatduFortWest        C-CGAAAAG-TACTTACACCCACCCGCGACC  CGCGAGGAGGTGTGTATATAAACTT   461
Lospos os3Center     C-CGAAAAG-TACTTACACCCACCCGCGACC  CGCGAGGAGGTGTGTATATAAACTT   482
BossouArtib          C-CGAAAAG-TACTTACACCCACCCGCGACC  CGCGAGGAGGTGTGTATATAAACTT   482
Damien2West          C-CGAAAAG-TACTTACACCCACCCGCGACC  CGCGAGGAGGTGTGTATATAAACTT   479
MakebaraNE           C-CGAAAAG-TACTTACACCCACCCGCGACC  CGCGAGGAGGTGTGTATATAAACTT   477
AbricotsCenter       C-CGAAAAG-TACTTACACCCACCCGCGACC  CGCGAGGAGGTGTGTATATAAACTT   480
McheLekete           C-CGAAAAG-TACTTACACCCACCCGCGACC  CGCGAGGAGGTGTGTATATAAACTT   453
BVincentArtib        C-CGAAAAG-TACTTACACCCACCCGCGACC  CGCGAGGAGGTGTGTATATAAACTT   465
NanPaulArtibonite    C-CGAAAAG-TACTTACACCCACCCGCGACC  CGCGAGGAGGTGTGTATATAAACTT   470
DuperonSouth         C-CGAAAAG-TACTTACACCCACCCGCGACC  CGCGAGGAGGTGTGTATATAAACTT   478
Losposos1Center      C-CGAAAAG-TACTTACACCCACCCGCGACC  CGCGAGGAGGTGTGTATATAAACTT   477
Dilaire1NE           C-CGAAAAG-TACTTACACCCACCCGCGACC  CGCGAGGAGGTGTGTATATAAACTT   479
1bWest               C-CGAAAAG-TACTTACACCCACCCGCGACC  CGCGAGGAGGTGTGTATATAAACTT   482
Hawaii               c-cgaaaag-tacttacacccacccgcgacc  cgcgaggaggtgtgtatataaactt   328
VyezoSouth             NCNNAAAAN-NNN-NNNANCCNNCCNNN  NCCNNNNAAGNAGNGNGNNNNNAANT   456
PapayeCenter       ------------------------------------------------------------   206
RR4                NCCNNAAAN-NTNNNNNNNNCNNNCCNNNNNNNCNNNNNNGNAGGNNGNNNNNNNNANNN   485
LavalStDucSouth    CCG-AAAAG-TACTTACACCCACCCGCGA---CCCGC-GAGGAGGTGTGTATATAAaCTT   479
Burden4LA          ACCGAAAAG-TACTTACaCCCaCCCGCGAC--CCG-C-gAGGAGGtGtGTaTaTaAActT   480
Buden1LA           ------------------------------------------------------------   348
RR1                C-CgAAAag-TACTtacacCCcaCCCcgCgACCcgcg-aggaggtggtgtatataAacTT   487
MPintadeCenter       N-CNNAAAN-NNNNTAANNCCANCCGGNNCC  CNNNNNGNNGNGNGNNNNNAAANTT   472
TrivilWest           C-CGaaaag-tACTTACacCCACCCGCGACC  cGNNNGgAGGTGTGNATATAAAANN   502
KobyeArtibonite    N-NNAAAAG-NANNTA--------------------------------------------   426
CrowleyLA          ------------------------------------------------------------   402
LetangSouth          C-CNAAAAN-TACNTACNCCCACCCNCNNNC  CGNNAGGAGGTGTGTNNATAAACTT   479
RR2                C-NAAAAAN-NNNNNNNCCCCNCCCNCGNCC----CNNNNNGGAGGGGG-----------   467
DeenLeeLA            C-CgAAAAG-TACTTACaCCCaCCCgCGACC  CGCGAGGagGtGtGtaTatAAacTT   484
DesjarArtib        ------------------------------------------------------------   369


GrdRackCenter      --AACGATACGGGACTCATCCGAGGCCCCGTAATCGGAATGAGTACACTCTAAACCCTTT   520
Burden2LA          --AACGATACGGGACTCATCCGAGGCCCCGTAATCGGAATGAGTACACTCTAAACCCTTT   515
CouponArtibonite   ------------------------------------------------------------   315
PepinCenter        ------------------------------------------------------------   287
AuvoisNE           ------------------------------------------------------------   407
India_Kerala              ttaaaccggcgcgggtcgtccga  acgcgaacgcgcgcgcgcctaccgtctg   391
Damien1West        ------------------------------------------------------------   424
TaiwanTainan              t-aaaccggcgcgggtcgtccga  acgcgaacgcgcgcgcgcctaccgtctg   377
Vietnam                   t-aaaccggcgcgggtcgtccga  acgcgaacgcgcgcgcgcctaccgtctg   381
StKits                    t-aaaccggcgcgggtcgtccga  acgcgaacgcgcgcgcgcctaccgtctg   377
Georgia                   t-aaaccggcgcgggtcgtccga  acgcgaacgcgcgcgcgcctaccgtctg   378
Philippines               t-aaaccggcgcgggtcgtccga  acgcgaacgcgcgcgcgcctaccgtctg   380
MmeJackCenter             T-AAACCGGCGCGGGTCGTCCGA  ACGCGAACGCGCGCGCGCCTACCGTCTG   512
MolXFCenter               T-AAACCGGCGCGGGTCGTCCGA  ACGCGAACGCGCGCGCGCCTACCGTCTG   522
FondwaWest                T-AAACCGGCGCGGGTCGTCCGA  ACGCGAACGCGCGCGCGCCTACCGTCTG   532
Laval1South               T-AAACCGGCGCGGGTCGTCCGA  ACGCGAACGCGCGCGCGCCTACCGTCTG   532
Burden3LA                 T-AAACCGGCGCGGGTCGTCCGA  ACGCGAACGCGCGCGCGCCTACCGTCTG   530
RoySavCenter              T-AAACCGGCGCGGGTCGTCCGA  ACGCGAACGCGCGCGCGCCTACCGTCTG   533
FerobienCenter            T-AAACCGGCGCGGGTCGTCCGA  ACGCGAACGCGCGCGCGCCTACCGTCTG   510
RR5                       T-AAACCGGCGCGGGTCGTCCGA  ACGCGAACGCGCGCGCGCCTACCGTCTG   543
MatduFortWest             T-AAACCGGCGCGGGTCGTCCGA  ACGCGAACGCGCGCGCGCCTACCGTCTG   511
Losposos3Center           T-AAACCGGCGCGGGTCGTCCGA  ACGCGAACGCGCGCGCGCCTACCGTCTG   532
BossouArtib               T-AAACCGGCGCGGGTCGTCCGA  ACGCGAACGCGCGCGCGCCTACCGTCTG   532
Damien2West               T-AAACCGGCGCGGGTCGTCCGA  ACGCGAACGCGCGCGCGCCTACCGTCTG   529
MakebaraNE                T-AAACCGGCGCGGGTCGTCCGA  ACGCGAACGCGCGCGCGCCTACCGTCTG   527
AbricotsCenter            T-AAACCGGCGCGGGTCGTCCGA  ACGCGAACGCGCGCGCGCCTACCGTCTG   530
McheLekete                T-AAACCGGCGCGGGTCGTCCGA  ACGCGAACGCGCGCGCGCCTACCGTCTG   503
BVincentArtib             T-AAACCGGCGCGGGTCGTCCGA  ACGCGAACGCGCGCGCGCCTACCGTCTG   515
NanPaulArtibonite         T-AAACCGGCGCGGGTCGTCCGA  ACGCGAACGCGCGCGCGCCTACCGTCTG   520
DuperonSouth              T-AAACCGGCGCGGGTCGTCCGA  ACGCGAACGCGCGCGCGCCTACCGTCTG   528
Losposos1Center           T-AAACCGGCGCGGGTCGTCCGA  ACGCGAACGCGCGCGCGCCTACCGTCTG   527
Dilaire1NE                T-AAACCGGCGCGGGTCGTCCGA  ACGCGAACGCGCGCGCGCCTACCGTCTG   529
1bWest                    T-AAACCGGCGCGGGTCGTCCGA  ACGCGAACGCGCGCGCGCCTACCGTCTG   532
Hawaii                    t-aaaccggcgcgggtcgtccga  acgcgaacgcgcgcgcgcctaccgtctg   378
VyezoSouth                TTAAACCGGCGCGGGNCGNCCNA  ANNNNNANGCGCGCANNNCTANCNNNNG   507
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(Figure cont'd)

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PapayeCenter          ------------------------------------------------------------  206
RR4                   TNNANNNNN-NNN-----------------------------------------------  497
LavalStDucSouth              TAAAcCGGC-GCGGGTCGTCCgA  AANNNAANGCGCGCGCCTANNNTCTG  529
Burden4LA                    TaAACcggc-gcgGGTCGtCCga  AcgcgaAcgcgCgcgcgCctaccgtctg  530
Buden1LA              ------------------------------------------------------------  348
RR1                          taAAccGGc-gcgGgtcgtccgA  ANNNNNANNNNNNNNNNNNNNNNNNNN  537
MPintadeCenter               TNAANNNGNGCGGGNC-GNCCNA  AANNNNANGCGCGCNNNNCNANNNNNNG  522
TrivilWest                   TNANNNNNNNGNGGGTCNTCCNA  ACNCNAACGCGCGCGCGCCTACNGNCTG  553
KobyeArtibonite       ------------------------------------------------------------  426
CrowleyLA             ------------------------------------------------------------  402
LetangSouth                  TNNNCCNGCGCGGGNNNN-CCNA  AANNNAACGCGCGCGCGCCNANNNNNNN  529
RR2                   ------------------------------------------------------------  467
DeenLeeLA                    TaANccNGGcGCGGGTCGTCCGA  aCgCgaacgcgcgCgcgcCTACcGTCtG  535
DesjarArtib           ------------------------------------------------------------  369


GrdRackCenter         AACGAGGATCAATTGGAGGGCAAGTCTGGT---GCCAGCAGCCGCGGTAATTCCAGCTCC  577
Burden2LA             AACGAGGATCAATTGGAGGGCAAGTCTGGT---GCCAGCAGCCGCGGTAATTCCAGCTCC  572
CouponArtibonite      ------------------------------------------------------------  315
PepinCenter           ------------------------------------------------------------  287
AuvoisNE              ------------------------------------------------------------  407
India_Kerala                aatagagaacaacgggcgcgcggctaagc  gttccgtcggatagaccggc-cc  442
Damien1West           ------------------------------------------------------------  424
TaiwanTainan          aataataataaagaacaaagggcgcgcggcgaagtgttc--cgtcggttagaccggc-cc  434
Vietnam               aataataataaagaacaaagggcgcgcggcgaagtgttc--cgtcggatagaccggt-cc  438
StKits                aataat---aaagaacaaagggcgcgcggcgaagtgttc--cgtcggatagaccggc-cc  431
Georgia               aataataataaagaacaaagggcgcgcggcgaagtgttc--cgtcggatagaccggc-cc  435
Philippines           aataataaagaacaa---agggcgcgcggcgaagtgttccgtctcggatagaccggc-cc  436
MmeJackCenter         AATAATAAAGAACAA---AGGGCGCGCGGCGAAgtGTTCCGTCgG--AtaNACCGGc-CC  566
MolXFCenter           AATAATAAAGAACAA---AGGGCGCGCGGCGAAGTGttccgNCGGN-ANAgACcgGc-CC  577
FondwaWest            AATAATAAAGAACAA---AGGGCGCGCGGCGAAGTGTTC-CGTCGG-ATAGACCGGC-CC  586
Laval1South           AATAATAAAGAACAA---AGGGCGCGCGGCGAAGTGTTC-CGTCGG-ATAGACCGGC-CC  586
Burden3LA             AATAATAAAGAACAA---AGGGCGCGCGGCGAAGTGTTC-CGTCGG-ATAGACCGGC-CC  584
RoySavCenter          AATAATAAAGAACAA---AGGGCGCGCGGCGAAGTGTTC-CGTCGG-ATAGACCGGC-CC  587
FerobienCenter        AATAATAAAGAACAA---AGGGCGCGCGGCGAAGTGTTC-CGTCGG-ATAGACCGGC-CC  564
RR5                   AATAATAAAGAACAA---AGGGCGCGCGGCGAAGTGTTC-CGTCgG-ATAgACCGGC-CC  597
MatduFortWest         AATAATAAAGAACAA---AGGGCGCGCGGCGAAGTGTTC-CGTCGG-ATAGACCGGC-CC  565
Losposos3Center       AATAATAAAGAACAA---AGGGCGCGCGGCGAAGTGTTC-CGTCGG-ATAGACCGGC-CC  586
BossouArtib           AATAATAAAGAACAA---AGGGCGCGCGGCGAAGTGTTC-CGTCGG-ATAGACCGGC-CC  586
Damien2West           AATAATAAAGAACAA---AGGGCGCGCGGCGAAGTGTTC-CGTCGG-ATAGACCGGC-CC  583
MakebaraNE            AATAATAAAGAACAA---AGGGCGCGCGGCGAAGTGTTC-CGTCGG-ATAGACCGGC-CC  581
AbricotsCenter        AATAATAAAGAACAA---AGGGCGCGCGGCGAAGTGTTC-CGTCGG-ATAGACCGGC-CC  584
McheLekete            AATAATAAAGAACAA---AGGGCGCGCGGCGAAGTGTTC-CGTCGG-ATAGACCGGC-CC  557
BVincentArtib         AATAATAAAGAACAA---AGGGCGCGCGGCGAAGTGTTC-CGTCGG-ATAGACCGGC-CC  569
NanPaulArtibonite     AATAATAAAGAACAA---AGGGCGCGCGGCGAAGTGTTC-CGTCGG-ATAGACCGGC-CC  574
DuperonSouth          AATAATAAAGAACAA---AGGGCGCGCGGCGAAGTGTTC-CGTCGG-ATAGACCGGC-CC  582
Losposos1Center       AATAATAAAGAACAA---AGGGCGCGCGGCGAAGTGTTC-CGTCGG-ATAGACCGGC-CC  581
Dilaire1NE            AATAATAAAGAACAA---AGGGCGCGCGGCGAAGTGTTCC-GTCGG-ATAGACCGGC-CC  583
1bWest                AATAATAAAGAACAA---AGGGCGCGCGGCGAAGTGTTC-CGTCGG-ATAGACCGGC-CC  586
Hawaii                aataataaagaacaa---agggcgcgcggcgaagtgtttccgtcgg-atagaccggc-cc  433
VyezoSouth              AGTAA--NANANAANAAGG  GNNNNNGGGNANNTGNNCCNNNNNANNNACCGGN-NC  560
PapayeCenter          ------------------------------------------------------------  206
RR4                   ------------------------------------------------------------  497
LavalStDucSouth         ANNNNNNANNNNNNNNAAGG  GCGNNNNNGNAANTGTTCCGNNGGANNNNCCGGG-NN  584
Burden4LA             ataataAaNNNcaaagGGc----gcgcggcgaaNNNNNCNNNNNNNNNNNNNNNCNN----  582
Buden1LA              ------------------------------------------------------------  348
RR1                   NNNNNNNANNANNNNNNNGN----NNNNNNNNNNNNN-----------------------  569
MPintadeCenter          AGNNANAN-NNANANAAGG  GCNNNNGGGGANNTGTCCCGGNGNNNANACCGGA-NC  576
TrivilWest            AANAANAANANNA-----------------------------------------------  566
KobyeArtibonite       ------------------------------------------------------------  426
CrowleyLA             ------------------------------------------------------------  402
LetangSouth             GAGTNANANNNNACNAAGG  NNGCNNGGGGNNNTGNNCCNNNNNANACACCGGN-NC  584
RR2                   ------------------------------------------------------------  467
DeenLeeLA               AAtaataNNNNNANggcgc  gcggNNNANNNNNNGTCNGANNNNANNNNNCNNN-NA  590
DesjarArtib           ------------------------------------------------------------  369


GrdRackCenter         AATAGCGTATATTAAANTGNT-TTGCGGTTAN----------------------------  608
Burden2LA             AATAGCGTATATTAAAGTTGN-TTGCGGTTAA----------------------------  603
CouponArtibonite      ------------------------------------------------------------  315
PepinCenter           ------------------------------------------------------------  287
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(Figure cont'd)

```
AuvoisNE             ------------------------------------------------------------  407
India_Kerala         aaaatcgaactttatattttttttttac---------------------------------  471
Damien1West          ------------------------------------------------------------  424
TaiwanTainan           gaaatcgaactttttttt-t---tttacgtcgaaaggtatatacctcg  ccttccgta  486
Vietnam              gaaatcgaactttttttt-ttttt------------------------------------  460
StKits                 gaaatcgaactttttttt-tttttttacgtcgaaaggtatatacctcg  ccttccgta  486
Georgia                gaaatcgaactttttttt-tttttttacgtcgaaaggtatatacctcg  ccttccgta  490
Philippines          gaaatcgaactttttttttttt--------------------------------------  458
MmeJackCenter          NAANTCNAANTTTTTTTTTTTTTTTNNNNNNAANNNNNNNNNCNCNN  NNNNNNNNN  622
MolXFCenter            GAAaTCNAAcTTTTTTTTTTTTTTTNNNNNNANNGNNNNNNNNNCNN  NNNNNNNAA  633
FondwaWest             GAAATCGAACTTTTTTTTTTTTTTNCNNCAAANGGNNN-NNACCNCN  NNNNCNNNA  641
Laval1South            GaaaTCGAACTTTTTTTTTTTTTTTNNNNNNAANGGNNT-NNNCCNCN  CCTTCCGAA  641
Burden3LA              GAAATCGAACTTTTTTTTTTTTTTNCNNCN-AAAGGNNTANNCCNCN  CNTTCNNTA  639
RoySavCenter         GAAATCgAACTTTTTTTTTTTTTTTNNNNNNAANGGNNT---------------------  626
FerobienCenter         GAAATCGAACTTTTTTTTTTTTTTNCNNNNNAANNGNNNNNNNNNNN  NNNNNNNTTN  620
RR5                    gAAATCNAACTTTTTTTTTTTTTTTACNTCNAAAGGNNNATNNNNNN  NNNNNCNNN  653
MatduFortWest          GAAATCGAACTTTTTTTTTTTTTTTANNNCNNAAGGNATNTACCTCN  NNNNCCNNA  621
Lospsosos3Center       GAAATCGAACTTTTTTTTTTTTTTTANNNNNAAAGGNNNNNNNCTNN  NNNNNNG-A  641
BossouArtib            GAAATCGAACTTTTTTTTTTTTTTTANNNNNAAAGGNNNNNNNCTNN  NNNNNNG-A  641
Damien2West            GAAATCGAACTTTTTTTTTTTTTTTaCNtcaAAaGGtcTaTACCccc  cccTtCCga  639
MakebaraNE             GAAATCGAACTTTTTTTTTTTTTTTACgTCAAANGGtaTATcCctCc  CctTCCGTA  637
AbricotsCenter       GAAATCGAACTTTTTTTTTTTTTTTacgtcaAANggtatatN------------------  626
MCheLekete             GAAATCGAACTTTTTTTTTTTTTTTACNTCNAAAGGNATATNCCNCN  CCNTCCGTA  613
BVincentArtib          GAAATCGAACTTTTTTTTTTTTTTTACNNCNAANggNNNNNNccNcc  CCtTCCNNA  625
NanPaulArtibonite      GAAATCGAACTTTTTTTTTTTTTTTaCNTCaAAaGGtaTataCCtCc  CCNttCCGT  630
DuperonSouth         GAAATCGAACTTTTTTTTTTTTTTTAC---------------------------------  609
Lospsosos1Center       GAAATCGAACTTTTTTTTTTTTTTTACNTCNaAaGGtATaTaCCTCc  CCTTCCGTA  637
Dilaire1NE             GAAATCGAACTTTTTTTTTTTTTTTACNNCNAAAGGNNTNTACCNCG  CCTTCCGAA  639
1bWest                 GAAATCGAACTTTTTTTTTTTTTTTACNNCNAANGGNNTATNCCTCN  CNTTCCNNN  642
Hawaii               gaaatcgaactttttttttttttttac--------------------------------  460
VyezoSouth           NANTTTNANNNTTTTTTTTTTTTTTTNNNNNNNNNNNNNNNNNNGNNNNNNNTNNNCCGNA  620
PapayeCenter         ------------------------------------------------------------  206
RR4                  ------------------------------------------------------------  497
LavalStDucSouth      NNNNNNNNNNTTTTTTTTTTTTTTT-----------------------------------  609
Burden4LA                 ---NNNNNNNTTTTTTTTTTTTTNNNNNNNNNNNNNNNNNNNNNNN  NNNNNNNNA  632
Buden1LA             ------------------------------------------------------------  348
RR1                  ------------------------------------------------------------  569
MPintadeCenter       CNNNTNNAANNTTTTTTTTNTNNNTNNNANNNNNNNGNTTNNNNCNNNNN-NNCNCGNNA  635
TrivilWest           ------------------------------------------------------------  566
KobyeArtibonite      ------------------------------------------------------------  426
CrowleyLA            ------------------------------------------------------------  402
LetangSouth          NANNTNNANNNNTTTTTTTTTTNNNNNNNANNNNANNNNNNNNNNCNNNNN--NNNCNNNA  642
RR2                  ------------------------------------------------------------  467
DeenLeeLA            A-NNCNNANNNNNNNNNNNNNNNNNN----------------------------------  616
DesjarArtib          ------------------------------------------------------------  369


GrdRackCenter        ------------------------------------------------------------  608
Burden2LA            ------------------------------------------------------------  603
CouponArtibonite     ------------------------------------------------------------  315
PepinCenter          ------------------------------------------------------------  287
AuvoisNE             ------------------------------------------------------------  407
India_Kerala         ------------------------------------------------------------  471
Damien1West          ------------------------------------------------------------  424
TaiwanTainan           aa---aaatgtacgtg  tcgataacaacgc-cgacgactctttttcggagacgaaaa  538
Vietnam              ------------------------------------------------------------  460
StKits                 aa-aaaaatgtatgtg  tcgataacaacgc-cgacgactctttttcggagacgaaaa  540
Georgia                aa-aaaaatgtatgtg  tcgataacaacgc-cgacgactctttttcggagacgaaaa  544
Philippines          ------------------------------------------------------------  458
MmeJackCenter        NAAAAAANNNNNNNNN----NNNNNANN--------------------------------  646
MolXFCenter          AAAAANNN----------------------------------------------------  641
FondwaWest             AAAAAAANNNATGNNN  TNNANAACAANNC-CNNNNNNNNNTTTTNNGANNNNAAA-  695
Laval1South          AAAAAAANGTNNGNGN----NNNA--ANNNCNC-CNANNNNTCTTTTN------------  682
Burden3LA               AAAAAAANNNNNNNN  NCNANAANNNCNC-NNNNNACTCTTTTTTCNGANANNNAA  693
RoySavCenter         ------------------------------------------------------------  626
FerobienCenter       CNNNAAAAAA--------------------------------------------------  630
RR5                    NAAAAANGNNNNNNTC  NNNA--NCNNCNN-CNNNNNNNNNTTTNNNNNNNNAAAAN  706
MatduFortWest        AAAAAAAT----------------------------------------------------  629
Lospsosos3Center       AAAAAAAATGNATGNG  NNNNNAACAACNC-CGANNANTNNTTTTNNNNNNNNAAAA  696
BossouArtib            AAAAAAAATGNATGNG  NNNNNAACAACNC-CGANNANTNNTTTTNNNNNNNNAAAA  696
Damien2West            AAAAAAAANGTatGgG  tcaaaaAcaaCcC-CaANNACNNTTTTTTNNNANANAAAA  694
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(Figure cont'd)

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MakebaraNE             AAA-AAAAtGTAtGNg  TcaAaaacaaCcC-CaacaACttTTTTTCNG-ANANNAAA   690
AbricotsCenter       ------------------------------------------------------------   626
McheLekete             AA-AAAAATGNNNGTG  TCGANAACAACNC-CNACNACTCTTTTTCGN-ANANNNAA   666
BVincentArtib          AAAAAAANNNNNNNNN  NNNNAANNNNNNC-CNANNNCTCTTTTTCGG-ANNNNAAA   679
NanPaulArtibonite      AAAAAAAAtGTATGTG  tCGaTAAcaaCcC-CNNNNNACNNTTTTTCG-NNNANNAA   684
DuperonSouth         ------------------------------------------------------------   609
Losposos1Center        AA-AAAAANGtNTGTG  TCaATAACAACGC-CNAcaacTCTTTTTCGg-AaACaAAA   690
Dilaire1NE           AA-AAAAATG--------------------------------------------------   648
1bWest                 AA-AAAAANGTNNGTG  TCNNNAACAACNC-CNACNACNNTTTTTCGG-ANANAAAA   695
Hawaii               ------------------------------------------------------------   460
VyezoSouth           AAAAAAAAANNANNNNNNNNNNNNNNNNNNNNNCNNNNNNNNNNTTTTNNNNNNNAAAAAN   680
PapayeCenter         ------------------------------------------------------------   206
RR4                  ------------------------------------------------------------   497
LavalStDucSouth      ------------------------------------------------------------   609
Burden4LA            AAAN-N------------------------------------------------------   637
Buden1LA             ------------------------------------------------------------   348
RR1                  ------------------------------------------------------------   569
MPintadeCenter       AAAA-NNAAGGANNGGNNNA-NNNACCNNNNNNNANNNNNNNTTTNNNNNN-NNNNNAAA   692
TrivilWest           ------------------------------------------------------------   566
KobyeArtibonite      ------------------------------------------------------------   426
CrowleyLA            ------------------------------------------------------------   402
LetangSouth          NAAA-NAANNNNNNGNNNNN-NNNNCNNNNNNNNNNNNNNNTNNTTNNNNNN-NNNNNNAA   699
RR2                  ------------------------------------------------------------   467
DeenLeeLA            ------------------------------------------------------------   616
DesjarArtib          ------------------------------------------------------------   369


GrdRackCenter        ------------------------------------------------------------   608
Burden2LA            ------------------------------------------------------------   603
CouponArtibonite     ------------------------------------------------------------   315
PepinCenter          ------------------------------------------------------------   287
AuvoisNE             ------------------------------------------------------------   407
India_Kerala         ------------------------------------------------------------   471
Damien1West          ------------------------------------------------------------   424
TaiwanTainan         gc-gttaaaccgatcgaaaaaagat-----------------------------------   562
Vietnam              ------------------------------------------------------------   460
StKits               gc-gttaaaccgatcgaaaaaagat-----------------------------------   564
Georgia              gc-gttaaaccgatcgaaaaaagat-----------------------------------   568
Philippines          ------------------------------------------------------------   458
MmeJackCenter        ------------------------------------------------------------   646
MolXFCenter          ------------------------------------------------------------   641
FondwaWest           AGCNTNNNNCN-------------------------------------------------   706
Laval1South          ------------------------------------------------------------   682
Burden3LA            AANNNNN-----------------------------------------------------   700
RoySavCenter         ------------------------------------------------------------   626
FerobienCenter       ------------------------------------------------------------   630
RR5                  NNNNNNNNNNN-------------------------------------------------   717
MatduFortWest        ------------------------------------------------------------   629
Losposos3Center      NNNTTNAACCNNNNNAAAAAAANNTTNNCCNTNAA-------------------------   731
BossouArtib          NNNTTNAACCNNNNNAAAAAAANNTTNNCCNTNAA-------------------------   731
Damien2West          ANNNTTNAACCGATCNAAAAAAAANATTNCCCNAAA------------------------   729
MakebaraNE           AGNGTTNANCCGATCNAAAAAAAANNTNNCCCN---------------------------   722
AbricotsCenter       ------------------------------------------------------------   626
McheLekete           AGCGTTAANNNNATCGAAAAAA--------------------------------------   688
BVincentArtib        ANNNTTNANCNNATCNAAAA----------------------------------------   699
NanPaulArtibonite    AAGNGTTNNNNNATCGAAAAANNT------------------------------------   708
DuperonSouth         ------------------------------------------------------------   609
Losposos1Center      ANCGTTAaaccgATcNaAAAAAattNCCCTAaaN--------------------------   725
Dilaire1NE           ------------------------------------------------------------   648
1bWest               AGCGTTAANCNNATCNAAAAAAAANATTACCCTAAANGGGGG------------------   736
Hawaii               ------------------------------------------------------------   460
VyezoSouth           NNNNTTAANCCGNNNNNNAAANANNNNNNCNNNNNNNNGNNNNNNNNNNNNNNNNNNNNA   740
PapayeCenter         ------------------------------------------------------------   206
RR4                  ------------------------------------------------------------   497
LavalStDucSouth      ------------------------------------------------------------   609
Burden4LA            ------------------------------------------------------------   637
Buden1LA             ------------------------------------------------------------   348
RR1                  ------------------------------------------------------------   569
MPintadeCenter       ANCNTTAAACCNGNNNNAANA---------------------------------------   713
TrivilWest           ------------------------------------------------------------   566
KobyeArtibonite      ------------------------------------------------------------   426
```

(Figure cont'd)

```
CrowleyLA          ------------------------------------------------------------ 402
LetangSouth        NNNNNNNNNCNGNNNNNNANNNNAG----------------------------------- 724
RR2                ------------------------------------------------------------ 467
DeenLeeLA          ------------------------------------------------------------ 616
DesjarArtib        ------------------------------------------------------------ 369


GrdRackCenter          --    608
Burden2LA              --    603
CouponArtibonite       --    315
PepinCenter            --    287
AuvoisNE               --    407
India_Kerala           --    471
Damien1West            --    424
TaiwanTainan           --    562
Vietnam                --    460
StKits                 --    564
Georgia                --    568
Philippines            --    458
MmeJackCenter          --    646
MolXFCenter            --    641
FondwaWest             --    706
Laval1South            --    682
Burden3LA              --    700
RoySavCenter           --    626
FerobienCenter         --    630
RR5                    --    717
MatduFortWest          --    629
Losposos3Center        --    731
BossouArtib            --    731
Damien2West            --    729
MakebaraNE             --    722
AbricotsCenter         --    626
MacheLekete            --    688
BVincentArtib          --    699
NanPaulArtibonite      --    708
DuperonSouth           --    609
Losposos1Center        --    725
Dilaire1NE             --    648
1bWest                 --    736
Hawaii                 --    460
VyezoSouth             AA    742
PapayeCenter           --    206
RR4                    --    497
LavalStDucSouth        --    609
Burden4LA              --    637
Buden1LA               --    348
RR1                    --    569
MPintadeCenter         --    713
TrivilWest             --    566
KobyeArtibonite        --    426
CrowleyLA              --    402
LetangSouth            --    724
RR2                    --    467
DeenLeeLA              --    616
DesjarArtib            --    369
```

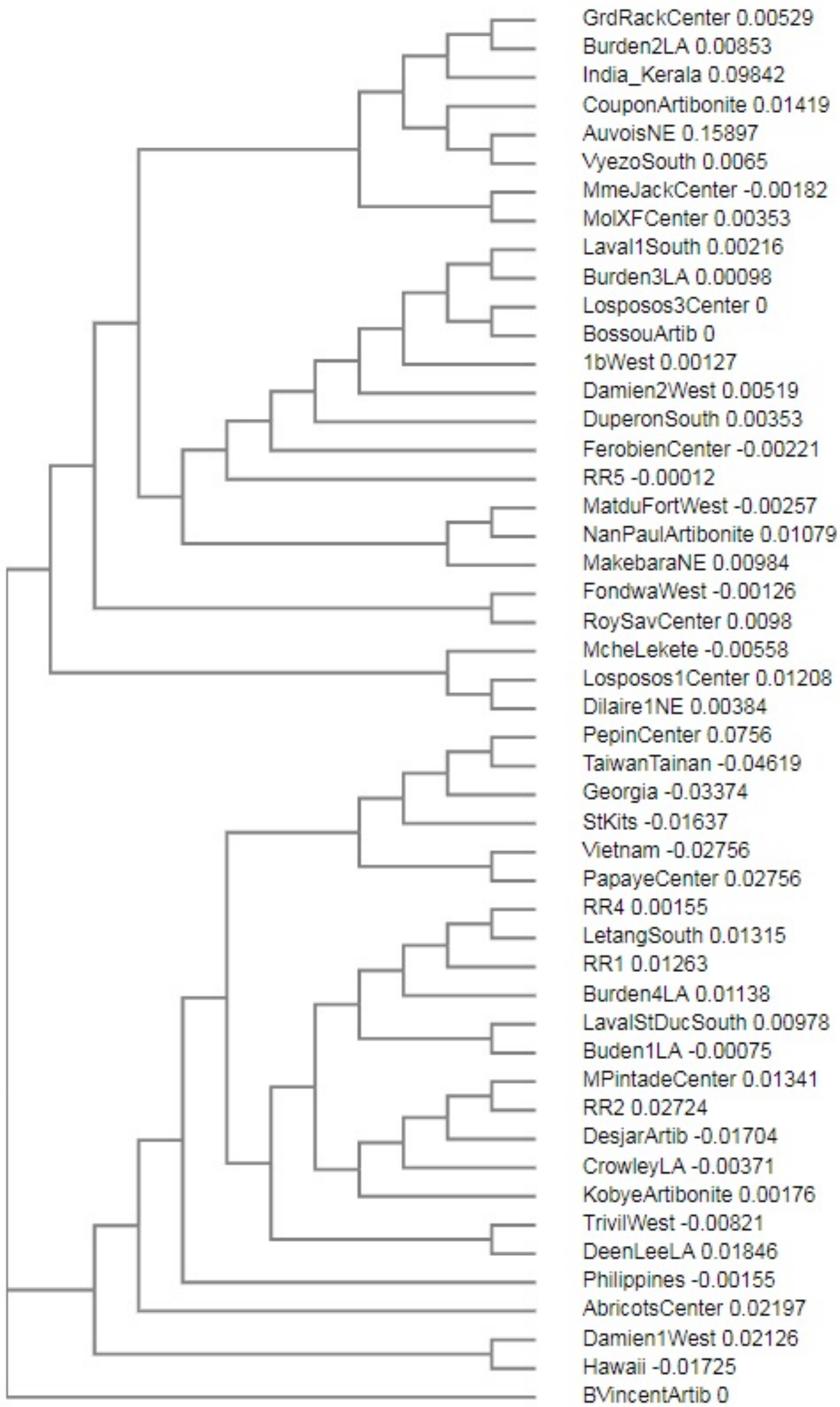

Fig. 3.3. Phylogenetic tree of the most related *C. formicarius* biotypes based on DNA ITS-1 region

3.4. Discussion

Traditionally, morphological characters were the primary or the only tool used for arthropods identification including insects. More recently, DNA-based identification was discovered as an additional method for insects and all biological subjects, and, is widely used in quarantine, and biological control for accurate pest identification (Gurr et al. 2016 ; Daane et al.2018). The main objective of this study was to display similarities or distinction among *C. formicarius* populations of Louisiana and Haiti based on the length of ITS-1 DNA region. These findings are consistent with the hypothesis that Louisiana was first infested by *C. formicarius* population from the Caribbean Basin (Cockerham, 1954) especially by the Haitian people who came from the New World in support against war. Also the results of this study display difference to those reported by Wolfe (1991) who stated that the sweetpotato became host in India when the Europeans first brought this plant from the West Indies. However, neither *C. formicarius* biotypes from Haiti nor St-Kits Nevis are related to those from India. Other controversial information with this study was reported by Kawamura et al. (2007) reported that *C. formicarius* DNA, based on ITS-1 region from Georgia (566bp), Hawaii (567bp), and St-Kitts Nevis (565bp) are similar and slightly different from those in India. The phylogenetic tree in this study displays no homogeneity among biotypes in these three locations. Despite that Haiti and St-Kits Nevis are from the Caribbean basin (West Indies) the biotypes of these two countries are different. The same case is underlined for the three USA States, the biotypes show no relatedness possibly because of the quarantine programs established for this insect.

The findings also display high rate of similarities *C. formicarius* biotypes within the Departments of Haiti that is consistent with the fact that this weevil is widely spread due to the inappropriate or absence of practices related to the propagation method of sweetpotato vines. . Since the findings of this study represent the biotypes that are generally present throughout the

sweetpotato growing regions in Haiti, this could be helpful in guiding a formal, systemic and sustainable management of *C. formicarius* in the country.

3.5. References

Chalfant, et al. 1990. Ecology and management of sweet potato insects. *Annual Review Entomology*, 35:157-180.

Cockerham, K. L. et al. 1954. The biology of the sweetpotato weevil. *Louisiana Technical Bulletin*, (483): 3.

Gitomer, S. 1996. Potato and Sweetpotato in China: Systems, Constraints, and Potential. *International Potato Center* (CIP), Lima, Peru, 191p.

Daane, K. M. et ale. 2018. Determining the geographic origin of invasive populations of the mealybug *Planococcus ficus* based on molecular genetic analysis. *PlosOne*, 13(3):1-12.

Gurr, M and You, M. 2016. Conservation Biological Control of Pests in the Molecular Era: New Opportunities to Address Old Constraints**.** *Frontiers in Plant Science*, 6: 1255.

Hebert, P. D. N. et al. 2003. Biological identifications through DNA barcodes. *The Royal Society,* 270:313–321.

ISAAA, 2008**.** Sweetpotato out-yields corn in Ethanol Production Study. http://www.isaaa.org/kc/cropbiotechupdate/article/default.asp?ID=3006 Accessed May 2019

Ito, S.1999. Occurrence and control of *Cylas formicarius* (Fabricius) in Kagoshima city [Japan] *Plant Protection Service* (Japan), (35):35-42.

Kawamura, K. et al. 2007. Genetic variation of sweet potato weevils, *Cylas formicarius* (Fabricius) (Coleoptera: Brentidae), in main infested areas in the world based upon the internal transcribed spacer-1 (ITS-1) region. *Applied Entomoly and Zoology,* 42 (1): 89-96.

Kaur, R. et al. 2013. Determining changes in the distribution and abundance of a *Rhyzopertha dominica* phosphine resistance allele in farm grain storages using a DNA marker. *Pest Management Science*, 69(6):685-8.

Louis, J. 2006. Occurrence and Intra-specific Variation of Sweetpotato Weevil (Brentidae: Coleoptera) in Relation to Its Potential Spread in SouthernUnited States of America and the Caribbean. Procedures l IS on Sweetpotato and Cassava. Ed. S.L. Tan. *Act Hort*, 703. ISFIS, 197-203pp.

Nilakhe, S. S. 1991. Quarantine programs for Cylas formicarius in the United States, pp. 247-262. In Jansson & Raman [eds.], Sweet potato Pest Management: a global perspective. *Westview, Boulder, CO*, USA.

Sorensen, K. A. 1987. Cultural, regulational and educational programmes on the sweet potato weevil in the United States. *Insect Sciences and Application*, 8:825-830.

Wagener, B. et al. 2004. A PCR-based approach to distinguish important Diadegma species (Hymenoptera: Ichneumonidae) associated with diamondback moth, Plutella xylostella (Lepidoptera: Plutellidae). *Bulletin of Entomological Research*, 94:465-471.

Wolfe, G. W. 1991. The origin and dispersal of the pest species of Cylas with a key to the pest species groups of the world. In Sweetpotato Pest Management, a Global Perspective (R. K. Jansson and K. V. Raman eds.). *Westview Press, Boulder*, 13–43.

CHAPTER 4. ASSESSMENT OF SWEETPOTATO LINES FOR HOST PLANT RESISTANCE TO *CYLAS FORMICARIUS* (S) IN LOUISIANA

4.1. Introduction

Sweetpotato is known as "the crop that feeds the world" since it is grown widely in the developing countries and is considered as a staple food. However, sweetpotato has widely gained attention in recent years due to its potential value. All parts of the sweetpotato are edible. The leaves are high in antioxidant phenolic compounds (Islam et al. 2002). The roots are rich in beta-carotene (Purcell and Walter, 1968b), vitamin C, and are widely used in processing (Utomo et al, 2005). Sweetpotato also has industrial value due in ethanol production and animal feed (Lareo et al.2013). For these reasons, USA sweetptotato production has increased significantly (USDA/NASS, 2019). This marked rise in sweetpotato production had been encouraged by promoting the nutritional value of this crop (Bond, 2017; Bowser et al. 2017) and development of new varieties containing both agricultural characters and pest resistance traits (LaBonte, 2008b).

Although many insects attack sweetpotato, *C. formicarius elegantulus* (Summers) is by far the most damaging (Jansson and Raman, 1991). This insect has a high reproductive capacity (Sherman and Tamashiro, 1954) and when only one pair mated escapes from control, an area can become rapidly infested (Abraham, 2007). Larvae represent the stage that is most threatening both in field and storage (Ray and Ravi, 2005). Due to their cryptic feeding habits (Chalfant et al, 1990), insecticides are the primary control. However, relying on a single control tactic can lead to resistance and thus, Cuthbert and Jones (1978) suggested that host plant resistance could be a very effective IPM component to manage *C. formicarius*.

Host plant resistance was also proposed as mechanism of pest control by Painter (1951) suggesting that any plant has a natural capacity to defend against pest attack by altering pest preference (antixenosis), limiting physiological functions and reducing pest fitness (antibiosis), or tolerating some insect injury. Sweetpotato storage roots are the preferred *C. formicarius*

oviposition tissue, assuring food availability for its offspring (Korada, 2010). *Cylas formicarius* induces response to sweetpotato storage roots by eliciting chemical compounds, including terpenoids, which makes the roots unmarketable (Uritani et al, 1975). Therefore, developing sweetpotato varieties resistant to insect injury is of high importance. Cockerham and Deen (1947) are the pioneers in sweetpotato phenotyping for weevil resistance and proposed variety breeding as a control method.

Phenotyping varieties is the first action in a breeding program (Barlow and Rolston, 1981). In developed sweetpotato production countries, infrastructure is available to conduct germplasm evaluations. In the United States, sweetpotato breeding programs were established more than 50 years ago (Jackson et al. 2012; Truong et al. 2018) in different sweetpotato production states including the Breeding Program at Louisiana State University Agricultural Center. A breeding program consists of a breeding nursery in which crosses are made to produce true seeds, which are then grown in a greenhouse to produce storage roots following by field tested to assess agronomic and pest characteristics prior to release (Clark and Smith, 2016). Although, many pest-resistant sweetpotato cultivars have been released, such as, Murasaki and Evangeline, many have not found grower acceptance due to poor yields. The objective of this study was to screen advanced breeding selections under laboratory no-choice conditions for resistance to *C. formicarius*.

4.2. Materials and Methods

4.2.1. *Cylas formicarius* colony

The *C. formicarius* colony at the Department of Entomology of Louisiana State University was established since 1989 and continuously mantained throughout years. The weevils are reared in ventilated plastic containers in which fresh sweetpotato roots are regularly

replaced to ensure food availability and oviposition sites. The colony is reared at 25°C with a relative humidity of 60-80% under dark conditions.

4.2.2. No-choice assay

A no-choice test was conducted in the spring of 2018 and 2019 on two set of sweetpotato lines, forthy-one and thirty, respectively, provided by Dr. D. LaBonte, Louisiana State University Agricultural Center Sweetpotato Breeder (Table 4.1). These lines were subjected to recurrent selection and are open pollinated. For the bioassay conducted in 2018, one root of each line was placed into a paper container, repeated ten times for each sweetpotato line. Each root was infested with six females randomly selected from the weevil colony. All adults were removed from the paper containers after five days. Storage roots were allowed to incubate for thirty days. Adult weevils were then counted, recorded, and removed three times per week for four weeks.

Table 4.1 List of sweetpotato lines assessed in 2018 and 2019

#	2018	2019
1	17-249w	16-173
2	17-241w	18-186w
3	17-250w	18-178w
4	17-251w	orleans
5	17-248w	16-209w
6	17-231w	14-116w
7	17-232w	18-184w
8	17-240w	18-194w
9	17-224w	B-63
10	16-250w	16-205w
11	w-Bx	18-189w
12	17-234w	18-173
13	17-233w	18-183w
14	17-247w	16-213w
15	17-225w	18-185
16	17-242w	18-174w
17	17-246w	16-212w
18	w-Murasaki	18-187w

(Table cont'd)

#	2018	2019
19	17-222w	08-7w
20	12-162w*	08-37w
21	17-226w	18-192w
22	17-117w	18-188w
23	17-230w	18-177w
24	17-221w	16-250w
25	17-220w	14-117w
26	17-237w	18-191w
27	17-236w	18-176w
28	17-119w	18-173w
29	17-118w	18-172
30	17-228w	18-193w
31	8-37w	
32	16-209w	
33	17-227w	
34	17-229w	
35	14-116w*	
36	17-245w	
37	17-243w	
38	17-223w	
39	17-235w	
40	17-239w	
41	17-244w	

4.2.3. Data analysis

Data were analyzed using SAS software, version 9.4 (SAS Institute 2013) to determine the mean number, in PROC MEANS, of weevil emegence over the four week period of incubation and R Software© version 3.6.0 was used to determine difference among the lines at $\alpha = 0.05$ in HSD test prior to ANOVA. The sweetpotato variety Murasaki was used to assess the level of resistance of the 2018 lines on the weevil behavior. Murasaki is generally used to test *Cylas formicarius* sweetpotato resistance in other experiments (LaBonte et al. 2008b ; Jackson, 2013, Chen, 2017).

4.3. Results

For the bioassay conducted in the spring 2018 the findings show four level of sweetpotato resistance to *C. formicarius (F* =3.05 *; df* =40 *; p*<.0001*)*, and most of the lines, thirty of them, present no difference in their resistance and ranged from 18 to 52.70 mean number of weevil emergence. However, Murasaki which is a commercial sweetpotato variety shown the lowest adult emergence followed by the line 17-221w with which no difference was recorded (Table 4.2). Compare to these two more resistant lines, Murasaki and 17-221w, the lines 17-223w, 17-241w, and 16-209w expressed also considerable resistance to C. formicarius with respectivevly a mean number of 15.00, 16.83, and 17.10 weevil emergence.

Table 4.2. Average number of *Cylas formicarius* emergence from the sweetpotato lines assessed in 2018 (F =3.05 ; df =40 ; p<.0001) PROC MEANS, ANOVA and HSD test for difference (p =0.05).

Line #	Sweetpotato Line Name	Mean	SE	HSD test
1	17-249w	47.60	±10.66	a-d
2	17-241w	16.83	±6.63	bcd
3	17-250w	36.22	±24.73	a-d
4	17-251w	40.89	±7.29	a-d
5	17-248w	52.20	±8.40	a-d
6	17-231w	29.40	±5.49	a-d
7	17-232w	60.30	±9.17	ab
8	17-240w	34.70	±5.60	a-d
9	17-224w	20.30	±9.04	a-d
10	16-250w	24.50	±9.94	a-d
11	w-Bx	43.50	±5.8	a-d
12	17-234w	64.60	±6.20	a
13	17-233w	55.60	±9.61	abc
14	17-247w	45.56	±5.64	a-d
15	17-225w	27.80	±13.10	a-d
16	17-242w	44.10	±4.58	a-d

(Table cont'd)

Line #	Sweetpotato Line Name	Mean	SE	HSD test
17	17-246w	35.00	±8.08	a-d
18	w-Murasaki	7.80	±3.09	d
19	17-222w	38.70	±7.25	a-d
20	12-162w*	25.10	±10.34	a-d
21	17-226w	41.60	±11.00	a-d
22	17-117w	43.59	±6.43	a-d
23	17-230w	47.00	±7.67	a-d
24	17-221w	7.90	±2.40	d
25	17-220w	33.10	±4.40	a-d
26	17-237w	31.80	±7.24	a-d
27	17-236w	40.45	±3.31	a-d
28	17-119w	51.67	±7.58	a-d
29	17-118w	50.20	±9.28	a-d
30	17-228w	52.70	±9.16	a-d
31	8-37w	31.30	±7.03	a-d
32	16-209w	17.10	±6.45	bcd
33	17-227w	37.86	±5.16	a-d
34	17-229w	35.70	±7.17	a-d
35	14-116w*	37.8	±6.61	a-d
36	17-245w	19.90	±3.52	a-d
37	17-243w	54.60	±8.86	abc
38	17-223w	15.00	±5.12	cd
39	17-235w	44.60	±6.15	a-d
40	17-239w	61.40	±11.30	ab
41	17-244w	55.80	±7.67	abc

During the Spring 2019, thirty sweetpotato lines also provided by the Sweetpotato Research Station, were tested to *C. formicarius* feeding and oviposition. The assessment was also conducted in the Soybean Entomology Laboratory by using a no-choice test under the same conditions described in the materials and methods above. However, for this experiment, the assessment was repeated eight times. The data analysis was performed using the same software previously for the bioassay in the spring 2018. Also, GraphPad Prism8© Software Inc. was perform to display more obviously the results the mean number and the standard error (Fig. 4.1).

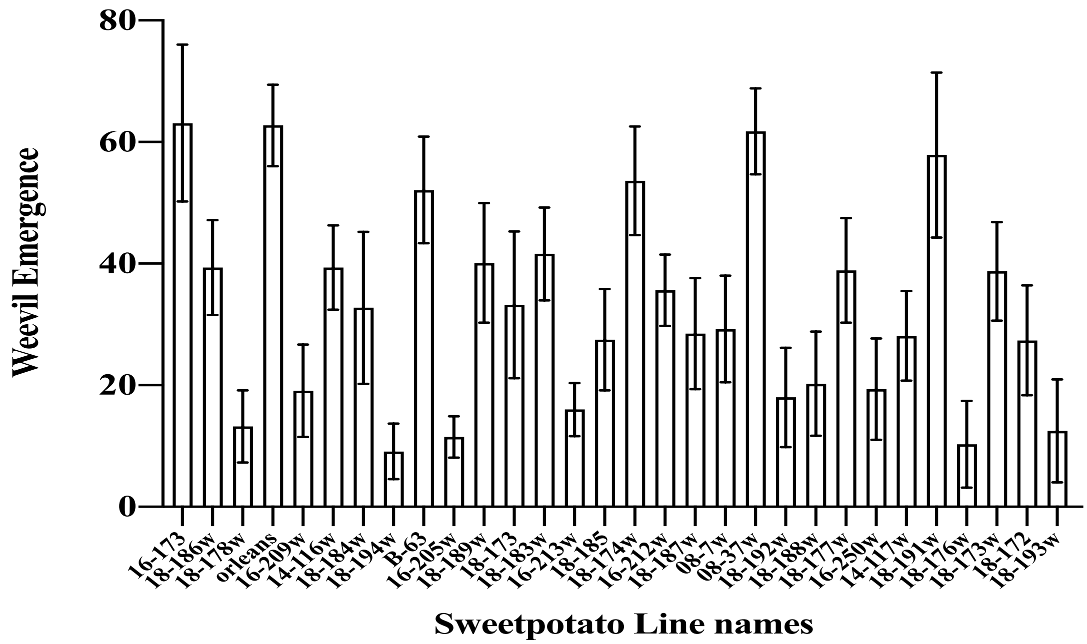

Fig. 4.1. Average number (±SE) of *Cylas formicarius* emergence from sweetpotato lines assessed in 2019 (GraphPad Prism 8, α =0.05).

Some lines previously assessed in 2018 were among these thirty lines for a second cycle of evaluation and the difference of their performance are presented in Table 4.3.

Table 4.3. Average number of *Cylas formicarius* emergence of the sweetpotato lines assessed in 2019 and performance of the four lines previously assessed in 2018 (F =3.05 ; df =40 ; p<.0001) PROC MEANS, ANOVA and HSD test for difference (p =0.05)

Line #	Sweetpotato Line Names	Mean	SE	HSD Test	Performance 2018	
1	16-173	72.14	±10.66	ab		
2	18-186w	45.00	±6.23	a-e		
3	18-178w	17.67	±7.06	de		
4	Orleans	62.75	±6.70	a-c		
5	16-209w	25.5	±8.68	c-e	17.1	±6.45
6	14-116w	45.00	±4.63	a-e	37.8	±6.61
7	18-184w	52.40	±13.40	a-e		

(Table cont'd)

Line #	Sweetpotato Line Names	Mean	SE	Tukey Test	Performance 2018	
8	18-189w	40.13	±9.83	a-e		
9	B-63	59-57	±5.34	a-c		
10	16-205w	11.50	±3.38	e		
11	18-189w	40.13	±9.83	a-e		
12	18-173w	53.20	±11.94	a-e		
13	18-183w	47.57	±5.54	a-e		
14	16-213w	18.29	±4.28	de		
15	18-185	31.43	±8.49	b-e		
16	18-174w	53.63	±8.96	a-d		
17	16-212w	35.63	±5.87	b-e		
18	18-187w	38.00	±9.14	a-e		
19	08-7w	46.80	±3.00	a-e		
20	08-37w	61.75	±7.06	a-c	31.3	±7.03
21	18-192w	28.80	±10.44	b-e		
22	18-188w	32.40	±10.37	b-e		
23	18-177w	44.43	±7.59	a-e		
24	16-250w	31.00	±10.23	b-e	24.5	±9.94
25	14-117w	37.50	±5.53	a-e		
26	18-191w	77.17	±6.72	a		
27	18-176w	24.00	±13.80	c-e		
28	18-173w	38.75	±8.10	a-e		
29	18-172	43.80	±6.99	a-e		
30	18-193w	20.00	±12.81	c-e		

The findings for 2019 show lines 16-205w, 18-194w, 18-178w, 16-213w, 18-193w, 18-176w, and 16-209w are more resistance to *C. formicarius* than susceptible checks. Based on the mean number, the four lines that were assessed previously in 2018 show lower level of resistance to *C. formicarius* compare to their performance in 2019 (Fig. 4.2.). However, significant difference was observed only in the line 08-37w upon the two years suggesting that introgression of resistance traits in susceptible sweetpotato varieties is a long precess and can last over five years to release a variety (LaBonte, 2015). In addition, expression of the insect resistance to the plant

chemical compounds and abiotic constrains can delay weevil-resistant variety time release (Collins and Mendoza, 1991) and, render breeding programs more difficult.

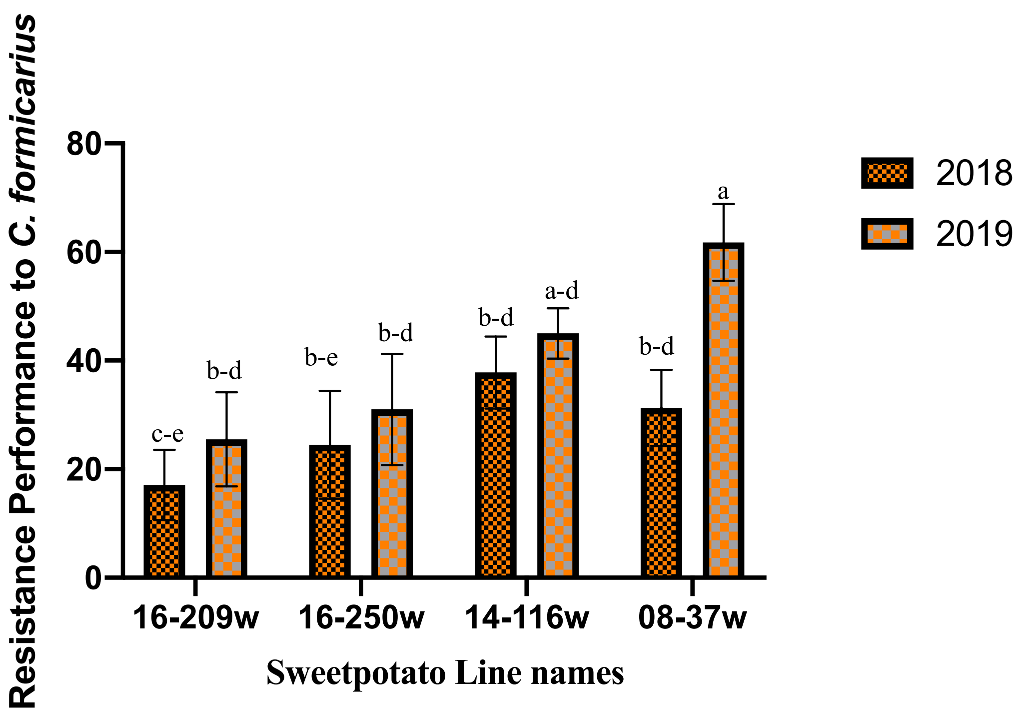

Fig. 4.2. Resistance Performance of the four sweetpotato lines on *C. formicarius* during the two cycle assessment (LSD test, p =*0.05*).

The non-linearity in the weevil resistance level of these four lines over the two cycles assessment does not automatically mean they will not be used for further work since they expressed appreciable preformance in 2019 on *C. formicarius*.

4.4. Discussion

The findings of this study show varietal resistance expressed by sweetpotato lines against *C. formicarius* under laboratory and no-choice tests. For the 2018 assessment, at least seven breeding lines expressed acceptable level of resistance to *C. formicarius* and were retained by the Sweetpotato Research Station for advanced work including the four lines assessed in the 2019 experiment. No sweetpotato cultivar, line, or variety has total immunity to *C. formicarius*

however, the level of responses are different (Mullen et al, 1980b; Rolston, 1979) and based on that, screening for germplasm can be performed for sweetpotato characterization. Wang and Krays (2002) reported that chemical compounds emitted by sweetpotato are different and not all of them attract female *C. formicarius* for oviposition. The resistant sweetpotato lines in this study possibly released repellent volatiles types against the weevil. Based on the type and concentration level of chemical compounds released by sweetpotato, in host-plant chemistry these volatiles have been experienced in modifying all stages of *C. formicarius* by constraining the weevil in the finding of their host plant for feeding or oviposition (Korada, 2010). Furthermore, the thickness of sweetpotatoto epidermis can also prevent weevils from puncturing the skin for feeding or oviposition (Chen, 2017). The findings of this study prove that host plant resistant (antibiosis, antixenosis, or tolerance) remains an important component in IPM programs.

4.5. References

Abraham, A. B. et al. 2007. Assessment of Risk of Introduction of *Cylas formicarius elegantulus* (Coleoptera: Brentidae) into Weevil-Free Areas in the Southern of the USA. *Journal of Economical Entomology*, 100(2): 315-321

Bond, J. 2017. U.S. Sweet Potato Production Swells.US Department of Agriculture (USDA). https://www.usda.gov/media/blog/2017/01/5/us-sweet-potato-production-swells. Accessed November 2017.

Bowser, T. J. et al. 2017.Promotion of orange flesh sweet potato by demonstration of acceptance and food product development. *African, Journal of Foof Science*, 11(12):383-388

Chalfant, et al. 1990. Ecology and management of sweetpotato insects. *Annual Review Entomology*, 35:157-180.

Chen, J. 2017. Evaluation of Control Tactics for Management of Sweetpotato Weevil (Coleoptera: Curculionidae). Ph. D. Dissertation. *Louisiana State University,* 1-124.

Clark, C. A and Smith, T. P. 2016. Sweet Potato Foundation Seed Production and the National Clean Plant Network Support. https://www.lsuagcenter.com/. Accessed on May 2019.

Cockerham, K. L., and Deen, O. T. 1947. Resistance of new sweetpotato seedlings and varieties to attack by the sweetpotato weevil. *Journal of Economic Entomology*, 40: 439-441.

Collins, W.W. and Mendoza, H.A. 1991. Breeding sweet potato for weevil resistance: Future outlook. p. 399-406. In: R.K. Jansson and K.V. Raman (eds.), Sweet Potato Pest Management: A Global Perspective. *Westview Press, Boulder*, CO, USA.

Cuthbert, F. P. Jr., and Jones, A. 1978. Insect resistance as an adjunct or alternative to insecticides for control of sweet potato soil insects. *Journal of Americas and Society, Hort. Science*, 103: 443-445.

Islam, M.S. et al. 2002. Identification and characterization of foliar polyphenolic composition in sweetpotato (*Ipomoea batatas* Lo) genotypes. *Journal of Agricultural Food and Chemistry*, 50: 3718–3722.

Jackson, D. M., Harisson, H. F. Jr, and Ryan-Bohac, J. R. 2012. Insect Resistance in Sweetpotato Plant Introduction Accessions. *Journal of Economic Entomology,* 105(2):651-658.

Jackson, D.M. and Harrison Jr, H.F. 2013. Insect resistance in traditional and heirloom sweetpotato varieties. *Journal of Economic Entomology*, 106(3):1456-1462.

Jansson, R.K. and Raman, K.V. 1991. Sweetpotato pest management: a global overview. Sweet Potato Pest Management – A Global Perspective (ed. by Jansson, R. K. and Raman, K. P.), 1–12 pp. *Westview Press*, *Boulder*, CO, USA.

Korada, R. R. et al. 2010. Differential volatile emission from sweetpotato plant: mechanism of resistance in sweetpotato for weevil *Cylas formicarius* (Fab.). *Current Science*, 99(9): 1597-1601.

LaBonte, D.R., Villordon, A.Q., Clark, C.A., Wilson, P.W. and Stoddard, C.S., 2008b. 'Murasaki-29'Sweetpotato. *HortScience*, 43(6):1895-1896.

LaBonte, D. R.2015. AgCenter plant breeders make Louisiana agriculture thrive. LouisianaState university Agricultural center (LSUAgCenter). https://www.lsuagcenter.com/portals/communications/leads/agcenter-plant-breeders-make-louisiana-agriculture-thrive. Accessed on June 2019.

Lareo, C. et al. 2013. Evaluation of sweet potato for fuel bioethanol production: hydrolysis and fermentation. *SpringerPlus*, 2(1):493

Miyatake, T., et al. 1997. Dispersal Potential of Male *Cylas formicarius* (Coleoptera: Brentidae) Over Land and Water. *Environmental Entomology*, 26 (2):271-276.

Mullen, M.A. et al. 1980b. Field selection of sweet potato lines and cultivars for resistance to the sweet potato weevil. *Journal of Economic Entomology*, 73:288-290.

Painter, R.H., 1951. Insect resistance in crop plants. *LWW*, 72(6): 481

Purcell, A.E. and Walter, W.M. 1968b. Carotenoids of centennial variety of sweetpotato, *Ipomoea batatas* (Lam.) during storage. *Journal of Agricultural Food and Chemistry* 16(5): 769–770.

Rolston, L.H. 1979. Field evaluation of breeding lines and cultivars of sweet potato for resistance to the sweet potato weevil. *Hort Science*, 14:634-635.

Truong, V. D et al. 2018. Sweetpotato Production, Processing, and Nutritional Quality. *John Wiley & Sons Ltd*, 2(35):812-838.

Uritani, I. et al. 1975. Induction of furanoterpenoids in sweetpotato roots by the larval components of the sweetpotato weevils. *Agricl. Biol. Chem.* 39 (9): 1857-1862.

USDA/NASS, 2019. US Department of Agrirculture. National Agricultural Statistics Services. https://www.nass.usda.gov/Statistics_by_Subject/result.php. Accessed on May 2019.

Utomo, J.S. et al. 2005. Physical and chemical characteristics of restricted sweetpotato sticks made from three sweetpotato cultivars. In: 2nd International Symposium on sweetpotato and cassava, Kuala Lumpur, Malaysia, 14–17 June, 2005, 221–222.

Ray, V. and Ravi, R.C. 2005. Post harvest spoilage of sweetpotato in tropics and control measures. *Critical Reviewa in Food Science and Nutrition*, 45(7-8):623-44.

Sherman, M. and Tamashiro, M. 1954. The sweetpotato weevil in Hawaii, their biology and control. *Hawaii Agricultural Experiment Station Technical Bulletin*, (23): 36.

Wang, Y. and Kays, J. 2002. Sweetpotato Volatile Chemistry in Relation to Sweetpotato Weevil (C. formicarius) Behavior. *Journal American Society and Hort Science,* 127(4):656-662.

CHAPTER 5. SUMMARY AND CONCLUSION

The West Indian sweetpotato weevil, *E. pstfasciatus*, is reported to be the most prevalent weevil in some Caribbean regions. From this statement on the non-exact regions of this species occurence lead to controversial information pertaining to the presence of this species in Haiti.The findings of this study show the importance for updating information pertaining to identification of plant pests especially when conflicting previous data are reported, and making appropriate decisions for management. Since *Euscepes postfasciatus* has the same feeding habit as *C. formicarius* and damage cannot be differentiated, it was of high importance to carry out this specific study to reveal that only *C. formicarius* is present in Haiti. However, further work can be conducted using other methods that could enhance the findings of this study about the occurrence of *E. postfasciatus* in Haiti.

Dispersal capacity of *C. formicarius* is very low and this insect can fly up to 2 km or maximum 4 km under laboratory conditions (Miyatake, 1997) meaning this pest is primary spread by human transportation. The DNA barcoding used in this study shows how fast this insect can spread. This study also displays the importance of DNA markers as rapid method to control potential pest invasion in an area and especially reveal the importance of quarantine regulations. The findings show that *C. formicarius* present in Louisiana are not similar to that in Georgia and Hawaii despite that these States are part of the USA.

Sweetpotato is one of the three crops in Louisiana that have high importance in the State's economy and this contribution is possible due to sweetpotato varieties improvement released by the continuous valuable work of the Breeding Program at Louisiana State University Agricultural Center. Since it is reported that the use of IPM approaches is best to control sweetpotato pests especially weevil, this study on assessment on sweetpotato lines revealed the importance of host plant resistance as an IPM tool to maintain sweetpotato germsplam and also show that

introgression of traits to susceptible sweetpotato can significatly decrease *C. formicarius* populations.

VITA

Marie Rachele Lexidort was born in Verrettes, Artibonite, Haiti. She received a Bachelor's degree at the State University of Haiti in Engineer-Agronomy in 2007. Soon after her Bachelor's degree, Mrs. Lexidort started working in Non-Governmental Organization (NGO) in food security projects in remote agricultural areas in assisting growers with advices for the improvement of their crop production including pest management. Mrs. Lexidort developed interest for further knowledge in agriculture while she was coordinating a food security and research project run by Laval University (Canada) in collaboration with the Ministry of Agriculture and The Faculty of Agronomy and Veterinary Medicine of Haiti. After being accepted in a scholarship program funded by the USAID and run by University of Florida Gainesville, Mrs. Lexidort left the project she coordinated and started her Master degree on Entomology under Dr. Jeffrey A. Davis at the Louisiana State University from August 2017 to August 2019. Mrs. Lexidort will receive her master's degree in August 2019 and will go back to Haiti with plans to work as an Entomologist.