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ECOLOGY OF THE ROSEAU CANE SCALE (NIPPONACLERDA BIWAKOENSIS, HEMIPTERA: ACLERDIDAE) IN COASTAL LOUISIANA

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**ECOLOGY OF THE ROSEAU CANE SCALE
(NIPPONACLERDA BIWAKOENSIS, HEMIPTERA:
ACLERDIDAE) IN COASTAL LOUISIANA**

A Thesis

Submitted to the Graduate Faculty of the
Louisiana State University and
Agriculture and Mechanical College
in partial fulfillment of the
requirements of the degree of
Master of Science

in

The Department of Entomology

by
Leslie Alejandra Aviles Lopez
B.S., Pan American Agricultural University El Zamorano,
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Abstract

Common reed, *Phragmites australis*, is the most dominant plant in the Mississippi River Delta (MRD), Louisiana. *Phragmites australis* stands reduce soil erosion from wave action, protect the oil infrastructure, and freshwater habitats. In the fall of 2016, widespread reed die-backs in the MRD were associated with outbreaks of an invasive scale *Nipponaclerda biwakoensis* (Hemiptera: Aclerdidae). Due to the recent detection of the scale, there was limited knowledge of its ecology in the adventive range, and its impacts on *P. australis* lineages. Therefore, the objectives of my thesis were to determine (1) the host specificity of the *N. biwakoensis* in important economic grasses closely related to *P. australis*, and (2) to evaluate the chemical defenses traits of *P. australis* lineages against *N. biwakoensis*.

The host specificity of *N. biwakoensis* was determined by conducting a no-choice experiment that demonstrated that nymphs established not only on *P. australis*, but also on *Spartina alterniflora*, and *Schoenoplectus californicus*. However, the survival to adult experiment revealed that *N. biwakoensis* only established on *P. australis*. Moreover, spill-over surveys at the MRD showed the presence of *N. biwakoensis* just on *P. australis*. Therefore, *N. biwakoensis* appears to be a specialist insect on *P. australis* and is unlikely to threaten other grasses with agronomic or environmental value in Louisiana.

The reed lineages Delta, European, Greeny, and Gulf are present in Louisiana. However, the Delta genotype is dominant in the MRD. In addition, native lineages are not present in Louisiana. Previous studies suggested that the European lineages are more resistant to herbivores. Research was needed to determine the defensive chemistry of the different reed lineages and their role in host-plant resistance. Therefore, we evaluated the survival of *N. biwakoensis*, quantified phenolics by the Folin-Ciocalteu method from leaf tissue; and silica by

the Molybdenum Blue calorimetric procedure. European lineages had less scale insect than Delta. In addition, the European lineages had 1.28- and 1.39-times more phenolics and silicon, respectively, than the Delta. Therefore, European lineages may have resistance traits against *N. biwakoensis*. This information will be critical when selecting common reed lineages during the implementation of restoration projects in the MRD.

Chapter 1. Background and Literature review

1.1. Common reed (*Phragmites australis*)

Common reed (*Phragmites australis*) (Cav.) Trin. ex. Steudel (Common reed, hereafter referred to as *Phragmites*) is the most prominent and distributed wetland grass worldwide (Den Hartog *et al.* 1989; Clevering and Lissner 1999). *Phragmites australis* belongs to the Arundineae tribe in the Poaceae family (Clevering and Lissner 1999). *Phragmites australis* is a perennial aquatic grass (Haslam 1972; Clevering and Lissner 1999; Brix 1999) that produces aerial shoots from vertical rhizomes. The vertical rhizomes are originated by large content of auxin-like growth regulator present in the horizontal rhizomes (Haslam 1972). The rhizomes have an oval-shape (Hurlimann 1951) and can live from 3 to 7 years (Bjork 1967; Hocking *et al.* 1983). The horizontal rhizomes can reach 20 to 100 cm depths (Bjork 1967; Haslam 1972; Hocking *et al.* 1983) and live longer than vertical rhizomes (Hocking *et al.* 1983). The aerial shoots grow and remain under the surface of the water until the end of the winter season. The stems are visible above water at the end of the spring and form a dense set of stems (Haslam 1972). The stems are hollow inside and divided by nodes that vary from 10 to 25 cm (Hocking *et al.* 1983). Stem diameter oscillates from 4 mm to 1m in the lower parts and becomes thinner in upper portions (Hocking *et al.* 1983). The height of a stem depends on the environmental conditions; stems with a height of 6-7 m can be found in the tropical and warm weather as well as low latitudes and altitudes (Haslam 1972). In addition, soil composition and water nutrients influence plant height (Haslam 1965). The leaves grow from the base of each node and cover the stems with a leaf sheath. Leaves are alternate, from 20 to 70 cm-long thin on the tip and narrow on the baseline of the node (Hocking *et al.* 1983). Common reed has a sizable panicle from 15-20 cm-long and from 10 to 25 cm-width. The panicle consists of a central branch with several spikelets. The

bloom varies from green to purple coloration (Hocking *et al.* 1983), depending on the genotype. The fruit is a simple dry and small caryopsis less than 2 mm-long (Hocking *et al.* 1983).

Phragmites australis is an aquatic plant that usually grows in swamps, especially, in areas with brackish water, sludge, organic and well fertilized substrate, and sometimes in seawater (Hocking *et al.* 1983); since it can tolerate salinity of up to 20 ppt (Achenbach *et al.* 2013; Pagter *et al.* 2009). In addition, it can be found in different conditions such as farm ponds, urban and ornamental ponds, drainage and irrigation ditches, highways edges, abandoned rice fields, river banks, in borders along lakes, and rivers (Martin *et al.* 1957; Wheat 1967; Clucas and Ladiges 1980; Rea 1983; Kiviat 1987).

Phragmites australis is affected by unsuitable environmental conditions such as high salinity, incompatible climate (Clapham *et al.* 1987), continuous flooding (Ostendorp 1991), and lower soil nutrient (Haslam 1972). However, *P. australis* can grow in soil with low nutrients as long as sufficient levels of phosphorus and nitrogen are available (Haslam 1970; Kershaw 1978). If the appropriate conditions are present, the density of stems increases varying from 70 to 100 shoots per m² and decreases in an unacceptable habitat that oscillates from 2 to 16 shoot per m² (Haslam 1972). The optimal pH for growth ranges from 5.5 to 7.5 (Gorham and Pearsall 1956; Matyulk 1960).

Phragmites australis has a high reproduction and high dispersal range; contributing perhaps to its cosmopolitan distribution (Den *et al.* Hartog 1989). In Europe, its economic value and extensive dispersal made *P. australis* a widely studied plant (Haslam 1972; Ostendorp 1989). Especially in the Danube Delta, the largest river in Europe, and Lake Felto, where these habitats are covered by *P. australis* (Brix 1999), and those stems were used for roofing and fencing (Brix 1999). In Romania, *P. australis* was processed for manufacturing cardboard,

printing paper, insulation, and thatching material (Brix 1999). In other parts of the world, such as Africa (Rodin 1985), South America (Heiser 1978), and Australia (Zola and Gott 1992), southern Canada and some regions of Mexico (Brix 1999), people rely on *P. australis* economically and socially (Brix 1999).

In the United States, fossil records shows that *P. australis* has been in America for a long period of time (Kearney and Pebbles 1960; Orson *et al* 1987). Indeed, according to Hansen 1978, data from the feces of the extinct *Nothrotheriops shastense* (Sinclair) demonstrated that *P. australis* has been in the U.S.A for at least 40,000 years (Hansen 1978). Kiviat and Hamilton (2001) suggested that this aquatic grass was brought to North America as an economic plant by the Dutch settlers. However, they also suggested that *P. australis* was brought in by the pre-Columbian people crossing the Bering Sea from Asia (Kiviat and Hamilton 2001). Although, the exact period of time when *P. australis* appears in the United States is unknown. Moreover, there is evidence that demonstrates *P. australis* was spreading rapidly through the country during the 1900s (Sipple 1972; Marks *et al.* 1994; Rice and Stevenson 1996). This accelerated expansion was due to changes in the wetland ecosystem, livestock reduction, lack of competitiveness from other plants, pollution on freshwater wetlands, reduction in salinity, fertilizers, sediments, and sewage (Mark *et al.* 1994; Pellegrin and Huber 1999; Chamber *et al.* 1999).

In the past, *P. australis* was essential to many tribes in the United States such as Apache, Blackfoot, Cahuilla, Chipewyan, Chippewa, Chumash, Cocopa, Gosiute, Havasupai, Hopi, Hualapai, Iroquois, Kawaiisu, Maricopa, Navajo, Papago, Pawnee, Pima, Seminole, Seri and Thompson. Especially in the south of the country where it was recorded as an economic resource for more than twenty native tribes. It was used to build arrow shaft, flute, pipestem, whistle, and knife; it was also used as smoking equipment (Kiviat and Hamilton 2001).

Currently, *P. australis* had become a crucial plant in the Mississippi River Delta (MRD) because of its ecological services (Knight *et al.* 2018). This aquatic grass has been helping MRD by sequestering carbon and maintaining water quality (Kiviat 2013). It provides habitat for wildlife species and conserved reservoirs areas for many bird species, which has been used for tourisms (Brix 1999). In addition, *P. australis* has been holding back sediments from the ocean wave due to its thick rhizomes (Rooth and Stevenson 2000); and preventing soil erosion from water movement and disturbance events such as hurricanes (Coops and Van der Velde 1995; Horppila *et al.* 2013). Lastly, *P. australis* protects the oil and natural gas infrastructures, and sustain navigation and fishing along the channels (Brix 1999).

These ecological services have been threatened at MRD since the fall of 2016. State biologists and stakeholders reported premature senescence, stunting, and death of stems of *P. australis*. Those symptoms were recognized as a die-back process (Armstrong and Armstrong 2001; Knight *et al.* 2018). Die-back is considered a vegetation loss process in which the flora dies, and the wetlands become less deep and more exposed to disturbance events resulting in wetland loss (Turner 1990; Webb and Mendelssohn 1996). This die-back process has been reported in *P. australis* in the past in Europe (Van der Putter 1997), and China (Li *et al.* 2013). In addition, United States reported the die-back on *Typha latifolia* (L.), *Schoenoplectus californicus* (C.A. Mey.) Steud, and other grasses in New Jersey, Maryland, and New York (Pancoast 1937; Dozier 1945; Dozier *et al.* 1948; McDonald 1955). Different causes have been attributed to the die-back process. In Europe, the main reason of the die-back was the eutrophication and the accumulation of phytotoxins, acetic acid and sulphide, created by byproducts of decomposing litter (Van der Putter 1997). In China, they attributed the die-back to the increases in salinity of water and the invasion of *Spartina alterniflora* (Loisel) (Li *et al.* 2013). In North America *P.*

australis die-back was related to the rise of water level during the winter of 1945-1946 (McDonald 1955). A previous study suggested that water elevation affected the dormant shoots and killed the growing shoots (McDonald 1955). In Louisiana, recently, the die-back process has been detected in *P. australis* growing at Pass-a-Loutre Wildlife Management Area at MRD (29°09' N, 89°13' W) (Knight *et al.* 2018). A scale insect was found between the stem and leaf sheaths of *P. australis*. This species was identified as *Nipponaclerda biwakoensis* (Kuwana) (Hemiptera: Acleridae), and it was associated with die-back of common reed in Louisiana (Knight *et al.* 2018).

1.2. Roseau cane scale

Roseau cane scale, *Nipponaclerda biwakoensis* (Kuwana), is a new invasive species from China and Japan (Kawai 1980). The population of the scale in Louisiana was identified using morphological and genetic characters (Knight *et al.* 2018). First, it was identified by morphological characters based on taxonomic keys of species in the genera *Aclerda* and *Nipponaclerda* (McConnell 1954). Second, it was identified by sequencing the mitochondrial COI barcoding region (Knight *et al.* 2018), resulting in 99.8% of similarity in the sequences data of *N. biwakoensis* (Park *et al.* 2010; Knight *et al.* 2018).

1.2.1. Taxonomy

Nipponaclerda biwakoensis belongs to the superfamily Coccoidea inside the suborder Sternorrhyncha, in the Hemiptera order (Teague 1925; Miller 2005). The Coccoidea superfamily is composed of scale insects; it is divided into two informal groups based on the number of abdominal spiracles: The Archaeococcoids with spiracles present and Neococcoids with spiracles absent (Kondo *et al.* 2008). *Nipponaclerda biwakoensis* belongs in the group Neococcoids since these scales insect lack abdominal spiracles (Kondo *et al.* 2008) in their adult female stages

(Teague 1925). The Neococcoid group contains 17 families, in which *N. biwakoensis* pertain to the Aclerdidae family (Kondo *et al.* 2008). The Aclerdidae are described as flat grass scales, which are a phytophagous insect that sucks the plant's sap by an ensemble of styles (Miller 2005).

The family Aclerdidae was first described by Milton M. Teague. (Ben-Dov and Hodgson 1997). This family is a distinct group because some morphological features are different than the rest of the scale insects of the Coccoidea superfamily. This family lacks a unique ring in the anal opening. In addition, they have a single plate in the anal opening with a cleft portion, but it is not entirely separated (Teague 1925). Also, they have a slender and tubular alimentary canal (Teague 1925). It was identified through morphological characteristics such as marginal tuberculate setae with similar shape and size around the body, and three anal setae (Figure 1.1 A, B).

Nipponaclerda biwakoensis has large thoracic spiracles on immatures stages, numerous ventral and dorsal macrotubular ducts, and a short and wide anal cleft (McConnell 1954)

1.2.2. Biology

Nipponaclerda biwakoensis is categorized as a hemimetabolous insect. The main reason is that they have incomplete metamorphosis, and they do not have distinguishing morphological changes in their life stages. In addition, the adult female is considered paedomorphic because they remain in a nymphal form even when they are mature (Kondo *et al.* 2008).

Crawlers are the first stage of the life cycle of *N. biwakoensis* (Figure 1.1A; Figure 1.2 A). They are the only stage that disperse and colonize new areas (Figure 1.1C) (Magsig-Castillo *et al.* 2010). The dispersal is affected by different factors such as temperature, humidity, dustiness, host species, and population density (Greathead 1972; Willard 1973; Beardsley and

Gonzalez 1975). Previous studies stated that crawlers could disperse short distance by air currents (Greathead 1972; Magsig-Castillo *et al.* 2010). However, several studies suggested that crawlers can disperse for a few meters, and even up to hundreds of kilometers (Greathead 1972). One example are the Diaspididae crawlers such as *Aulacaspis tegalensis* (Zehntner) crawlers, and *Aonidiella aurantii* (Maskell) crawlers (Greathead 1997). Two morphological characteristics, such as a flat body and long filamentous setae, allows the crawler to stay airborne in air currents (Gullan and Kosztarab 1997). Recent studies conclude that leg morphology has adapted for dispersal. The crawler's legs are formed by a single tarsal claw and four digitules with a suction cup-like structure at the tip (Figure 1.1 D). The suction cup-like structure could allow the crawler to attach to vector for dispersing long distances (Magsig-Castillo *et al.* 2010). The crawler loses its legs when they settle down and find a suitable host (Magsig-Castillo *et al.* 2010). They produce waxy secretions from the epidermal wax gland (Waku and Foldi 1984) to the body surface (Gullan and Kosztarab 1997). The wax filaments, partially, cover only the scale body margin once they have settled (Figure 1.2 B) (Kawai and Tamaki 1967). The wax secretions are responsible for protecting scales from deteriorating climatic effects, water loss, predators, and pathogen (Tamaki and Yushima 1969). In addition, the wax secretion prevent the scale to contaminate with their honeydews (Gullan and Kosztarab 1997). The waxy secretions are a combination of lipids, resins, and other secretions (Gullan and Kosztarab 1997).

Nymphs of *N. biwakoensis* are oval in shape, and they possess milky white color (Figure 1.2C) (Kuwana 1932). Before becoming an adult female, the nymphs go through variation of color (Camacho and Chong 2015) from white to reddish color in *N. biwakoensis*; and differences in size (Marotta 1997).

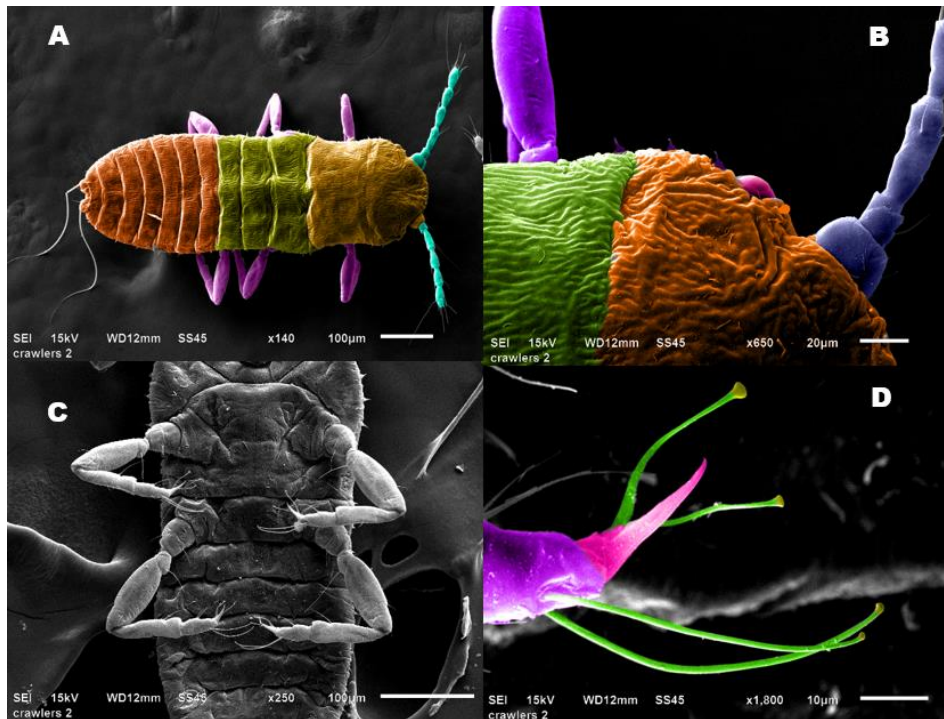


Figure 1.1. SEM colored pictures of *Nipponaclerda biwakoensis* crawlers. (A): flat crawler; (b): filamentous setae; (c): functional legs; (d) four digitules with suction cup-like structure at the tip.

Adult females are flattened and oval in most cases. However, females differ in shape because the scales bend and twist to fit in tight spaces between the leaf sheaths and the stems; since they live in large aggregations (Teague 1925). Adult females' length varies from 5 to 15 mm-long (Kuwana 1932; McConnell 1953) compared with nymphs which measure from 2 to 4 mm-long (McConnell 1953). The adult female's scales turn into reddish-orange color when they are gravid or reach maturity (Figure 1.2D). Females are ovoviviparous meaning that the eggs hatch inside of the body (Figure 1.2E) (Teague 1925). One adult female can produce 400 to 600 hundred crawlers (Kaneko 1995). In Louisiana, one female can produce up 300 crawlers (Aviles, L., unpublished data.). Gravid females are recognized by the presence of eggs which are visible through the integument. When the crawlers emerge from the adult female, the life cycle starts all over again (Figure 1.2F).

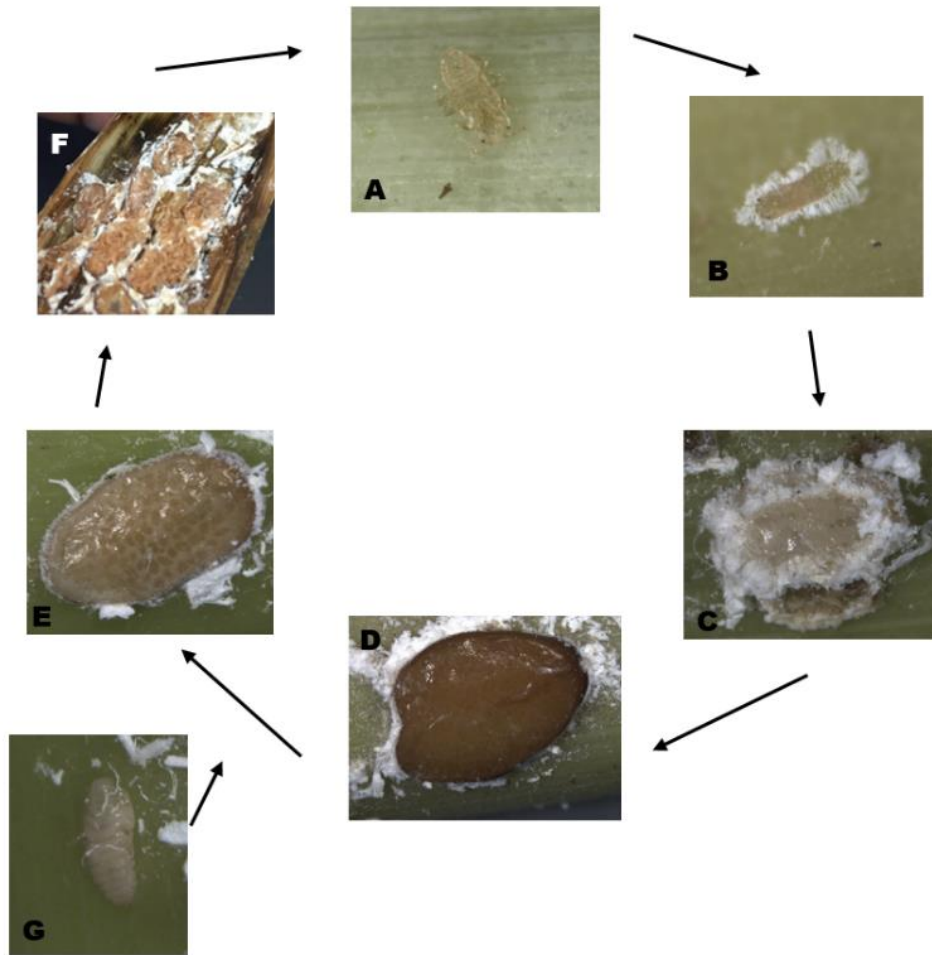


Figure 1. 2. Life cycle of *Nipponaclerda biwakoensis*. (A): crawler; (B): crawler settled with wax filaments clearly visible (C): milky white color nymph (D): adult female; (E): gravid female with visible eggs; (F): crawler hatching from the adult female; (G): male

Males are much smaller than females; their size is 1.1 mm-long and 0.44 mm-width (Figure 1.2 G) (McConnell 1954). They are usually difficult to find because of their size (Kaneko 2004, McConnell 1953). Males are apterous (Kuwana 1932; McConnell 1953). Males have a distinctive metamorphosis; including the presence of a pre-pupal and pupal stages before becoming an adult male (Miller & Kosztarab 1979). Adult males are delicate, ephemeral, and without functional mouthparts (Gullan and Kosztarab 1997). An adult male in the Coccoidea superfamily locates females using pheromones (Moreno *et al.* 1972, Millar *et al.* 2012; Gullan

and Kosztarab 1997). Males mate with females located near to them (branch, stem) because they are weak and fragile (Camacho and Chong 2015).

1.3. Host range of the roseau cane scale in the native range

Nipponaclerda biwakoensis is native from China, Japan and Korea (Kuwana 1907).

The scale insect can be on different host including *P. australis* (Kuwana 1907), *Agropyron cristatum* (L.) (Poaceae), and *Juncus acutus* (L) (Juncaceae) (Wang 1994).

1.4. Distribution and dispersal

Nipponaclerda biwakoensis is the first species of the genus recognized as a pest (Schneider 2019). This scale insect can be found in several countries in Asia such as Japan, China, and Korea, and occurs in wetlands, lakes, and rivers where *P. australis* is present (Kaneko 2004). In 2016, *N. biwakoensis* was detected in United States, and the timing and means of dispersal to North America are unknown (Schneider 2019). However, there were some specimens of *N. biwakoensis* reported in quarantine in California, United States. These specimens came from Japan to California, and it was found three times in quarantine; it was also found once in Hawaii, between 1959 and 1961 (Schneider 2019). Despite these early detections, there is not enough evidence that demonstrates that these samples were the first introductions of *N. biwakoensis* in the United States (Schneider 2019).

In Louisiana, the parishes with reports of *N. biwakoensis* were Cameron, Vermillion, Jefferson David, Calcasieu, Iberia, Terrebonne, Lafayette, Saint Martin, Lafourche, San Mary, Saint James, Saint Charles, Plaquemines, Saint Bernard (Knight *et al.* 2018). Recently, there is a record of *N. biwakoensis* in eastern Texas (Schneider 2019). While monitoring for *N. biwakoensis* in Louisiana, LSU entomologists there discovered another scale insect associated

with *P. australis*. This scale insect was identified as *Aclerda holci* Teague (Hemiptera: Aclerdidae), and it is native to the USA (Knight *et al.* 2018). Teague described *A. holci* from the specimens that were shipped from New Orleans with the help of Mr. H. K. Plank (Teague 1925). Later on, it was included on a taxonomic key to identify species of the Aclerdidae family (Teague 1925).

Aclerda holci is elongate almost with spherical form; its posterior portion is a relatively pointed angle shape. Morphological differences between *N. biwakoensis* and *A. holci* include the smaller size and presence of marginal and anal setae in *A. holci* (McConnell 1954). The only visual difference between both scale insects is that *A. holci* can be recognized by their partial sclerotized abdomen with black color at the pointed angle shape, and just a few scales insect can be found per stems (Knight *et al.* 2018). *Aclerda holci* was also recorded infesting two economic important grasses, Johnsongrass, *Sorghum halepense* (L.) Pers. (Ferris 1955), and sugar cane, *Saccharum officinarum* (L.) (Meagher and Gallo 2008) in the United States. In addition, the United States National Entomology Collection (USNM) has reports of *A. holci* preserved on two marshgrasses such as of *A. holci* collected on *Arundo donax*, *Cynodon dactylon* (L.) Pers., and *Sporobolus wrightii* Munro. Ex Scribn (Knight *et al.* 2018).

1.5. Plant damage by *N. biwakoensis*

Nipponaclerda biwakoensis could be associated with the die-back of *P. australis* due to the massive densities found per stem (Knight *et al.* 2020) up to thousands scale in the worse cases. In addition, scale insects produce honeydew that could allow the presence of pathogens that could affect the host plant; it could also disrupt the photosynthesis process (Malumphy 1997). More research needs to be done to determine the exact reason for the die-back, and whether *N. biwakoensis* is the primary cause.

1.6. Management of *P. australis* in China

Phragmites australis is a source for paperboard production, wastewater treatment, phytoremediation, and as fuel pellets in China. Consequently, Chinese agriculturists developed a different technique to deal with pests of *P. australis* called shaotang; “shao” means burning, and “tang” wetland grass; in other words this means burning the cane or burning *P. australis*.

Burning of the cane is done only where the scale insect was already established because it will help to destroy eggs on the stems (Brix et al. 2014). Other, effective technique was the removal of the stems and flooding the wetland. Previous studies showed that flooding increased *N. biwakoensis* mortality (Qi et al. 2003). According to Brix et al (2014) submersion of stem in the early winter for 40 days resulted in 57 % scale mortality compared to 40% in the control plots (Brix et al. 2014).

1.7. Natural enemies of *N. biwakoensis*

There are five parasitoids on *N. biwakoensis* reported in China including *Astymachus japonicus* Howard and, *Boucekiella depressa* Hoffer in the family Encyrtidae; and *Aprostecetus* sp., in the Eulophidae. Two additional parasitoids were not identified to species but belong to the Encyrtidae (Kaneko 2004; Kaneko 2005). Natural enemies were sufficient to control *N. biwakoensis* in China, with reports of 45% of parasitism rates, and 85% of scale reduction because of bird predation in the winter season (Xiong et al. 2010). In Louisiana, field surveys in 2017 and 2018 revealed the presence of parasitized *N. biwakoensis* females. These species were identified as *Neastymachus japonicus* (Tachikawa), *B. depressa* (Hoffer), and *Astymachus* sp. (Knight et al. 2018). The impact of the parasitoids in Louisiana is under investigation

1.8. Host plant resistance on *P. australis*

1.8.1. Lineages of Common reed

Several lineages of *P. australis* have been described (Clayton 1967; Haslam 1972). Their high genetic variation has been associated with phenotypic plasticity; it means that the phenotypic characteristic of the plant can change in different environments conditions. Consequently, *P. australis* is considered a polymorphic species since it is affected by genetic and environmental factors (Clevering and Lissner 1999). Since 2002, the lineages of *P. australis* have received a lot of attention in the United States. Two non-coding chloroplasts DNA (cpDNA) markers were used to sequence the DNA of the lineages present in the United States (Saltonstall 2002). The first sequenced lineages was the Native, these lineages are endemic to North America, and consist of up 14 haplotypes (Table 1.1) (Saltonstall 2002, 2003; Saltonstall et al. 2004). The native genotype was found in almost all states of West, Midwest, and Northeast of the United States. However, it is not present in the Southeast, and especially at the Mississippi Delta River in Louisiana. The second sequenced genotype was the Gulf. It was identified by Pellegrin and Hauber (Pellegrin and Hauber 1999). It contains a single haplotype (Table 1.1), and it can be found at the Gulf Coast in North America and Mexico, as well as in California, and South America (Saltonstall 2002; Meyerson *et al.* 2010). It is possible that the Gulf lineages expanded its range from Mexico (. However, there is not enough evidence to support this theory, and more studies need to be done. Moreover, there is no record of introducing a Gulf lineages to North America (Saltonstall 2002). The third genotype was the European. It contains a single haplotype (Table 1.1), and it is closely related to European and Asian strains. The European genotype has extended all over the world in the last 100 years, and it is considered an introduced genotype in North America (Saltonstall 2002; Howard *et al.* 2008) including several populations

at the Mississippi River Delta. The fourth genotype is the Delta, and it consists of a single haplotype (Table 1.1). It is originated from the North African and Mediterranean region, and it is the dominant genotype in the Mississippi River Delta (Hauber et al. 2011; Lambertini et al. 2012). Lastly, the combination of three haplotypes like M, AD, and AI was named as a Greeny which can be found in very isolated populations at the Mississippi River Delta (Knight et al. 2018).

Table 1.1. List of the lineages of *Phragmites australis* in the United States, and their presence in the Mississippi River Delta (MRD), Louisiana.

Lineages	Haplotype	Source	Presence at MRD
Native	A, B, C, D, E, F, G, H, S, Z, and AA	Saltonstall 2002	NO
Gulf	I	Saltonstall 2002	NO
European	M	Saltonstall 2002	YES
Delta	M1	Hauber <i>et al.</i> 2011	YES
Greeny	M, AD, and AI	Lambertini <i>et al.</i> 2012	YES

1.8.2. Possible plant resistance among common reed lineages

The European lineages of *P. australis* is considered an invasive species (Saltonstall 2002). European lineages became dominant in marshes and brackish water in North America (Meyerson *et al.* 2000 a, b; Kiviat 2013). The European lineages provides negative and positive effects in the new environment where it is established (Cronin *et al.* 2016). Negative effects include the displacement of native lineages and the alteration in communities of herbivores (Cronin *et al.* 2016). In addition, European lineages has positive effects such as habitat for birds, fishes, insects, and other communities of organisms, a large number of above and belowground biomass (Meyerson *et al.* 1999, 2000a, b, Kiviat 2013), sea level and wave action protection

(Hershner and Havens 2008), evapotranspiration from the land surface and plant reduction, carbon sequestration, and heavy metal elimination (Kiviat 2013, Cronin *et al.* 2016).

Natural selection should increase plant defense traits and reduce herbivore palatability in plants that are located at lower latitudes relative to plants located in higher latitudes (Coley and Aide 1991). This is in agreement with a previous study that revealed that the European lineages of *P. australis* suffers less damage by herbivores such as leafminers, stemborers, and aphids at lower latitudes (Bhattarai *et al.* 2017). In the same study, the authors also found 46% lower aphid damage in European lineages compared with native lineages at the *P. australis* garden at Louisiana State University, Baton Rouge, Louisiana (LSU; 30°35' N, 91°14' W) compared with the 18% of aphid damages in the University of Rhode Island, Kingston, Rhode Island (URI; 41°49' N, 71°54' W (Bhattarai *et al.* 2017). In addition, the same study revealed that European lineages contains 1.2-times more phenotypic plasticity than natives (Bhattarai *et al.* 2017). Indeed, other studies confirm that the European lineages has greater tolerance to salinity (Vasquez *et al.* 2005; Achenbach and Brix 2014; Eller *et al.* 2017).

Field observations suggested that stands of European lineages were healthier in appearance than those of Delta lineages in the MRD (Knight *et al.* 2020). In addition, the number of *N. biwakoensis* per meter of stem on the European lineages of *P. australis* is lower than that of the Delta lineages (Knight *et al.*, 2020). Moreover, the percentages of stems with green leaf tissue were greater in European lineages compare with Delta lineages (Knight *et al.* 2020). These observations suggest that there is a probability that the European lineages could be more resistant to *N. biwakoensis* than the Delta lineages.

Several studies have tried to understand how plants produce chemical defenses against insects (Duffey and Stout 1996). Chemical defenses change the physiology of the insect such as

molting hormones, plant analogs of juvenile insect hormones, pheromone production, and primary and secondary metabolomics (Harmatha and Dinan 2003). In addition, some plant act as toxins that poison the insects; meanwhile, other plants fabricate complex substances to prevent insects' digestion (Rosenthal 1986). Two crucial plant chemicals in that deter insect in Poaceae grasses are phenolic and silicon compound (Morgan 1984; Reynolds *et al.* 2016).

Silicon content in grasses tissue enhances chemical defenses traits against herbivores (McNaughton and Tarrants 1983; Fawe *et al.* 1998). A previous study has demonstrated that silicon can reduce the pupal production of *Spodoptera exempta* on European grass species: *Agrostis capillaries* (L.), *Brachypodium pinnatum* (L.), *Festuca ovina* (L.), *Lolium perenne* (L.), and *Poa annua* (L.) (Massey *et al.* 2008). Similarly, *Schistocerca gregaria* (Forsskål) removed fewer leaves from *Poa annua* (L.) with silicon content (Garbuzov *et al.* 2011). All the plants mentioned above belong to the Poaceae family. In addition, according to Schaller *et al.* 2013, *P. australis* can also uptake large amounts of silicon from the soil (Schaller *et al.* 2013). Moreover, a previous study has demonstrated that silicon has positive effects in the biomass of Poaceae plants (Eneji *et al.* 2008; Schaller *et al.* 2013).

Several studies have demonstrated that high phenolic contents in plants reduce the occurrences of insects. For example, phenolic content found in tomato leaves reduce the incidence of *Heliothis zea* (Boddie) (Duffey and Isman 1981). Also, increased phenolic content on tomato and cotton increased the mortality of *Heliothis virescens* (L.) and *Helicoverpa zea* (Boddie) (Hoover *et al.* 1998). Similarly, *Phaseolus vulgaris* (L.) had increased phenolic, such as tannins and α -amylase; these two types of phenolic are considered chemical plant defenses tools against phytophagous insects (Lattanzio *et al.* 2006). According to Reddy and Krishnan 2013, *Saccharum officinarum* (L.) and *Bambusa vulgaris* (Schrad), both contain 89.69

µg/g of phenolic (Reddy and Krishnan 2013). Moreover, previous studies have revealed large quantities of the phenolic compound on grasses such as *Triticum* (L.) species (Kharazain and Rahiminejad 2009), *Amaranthus hybridus* (L.), *Brachiaria brizantha* (A. Rich.), and *Panicum Maximum* (Jacq.) (Chitindingu et al. 2006); *Miscanthus sinensis* (Ander.), *Dichanthium caucasicum* (Trin.) S.K.Jain & Deshp, and *P. australis* (Balcerek et al. 2009; Croy et al. 2020, Bhattarai et al. 2017, Cronin et al. 2015).

1.9. Project Rationale

Due to the lack of knowledge of the biology and ecology of *N. biwakoensis*, there was a need to study its host range and determine its threat to other grass species. In addition, The background of plant defenses traits of the European lineages, the observation of different scale abundances between different lineages of *P. australis*, and the previous research about plant defenses demonstrated that some plant chemicals may inhibit insect pressure. Consequently, it was critical to characterize the phenolic, silicon, and lipids of *P. australis* lineages present in Louisiana. These data could help in the formulation of plant genotypes suitable for restoration projects at the Mississippi River Delta.

Therefore, the objectives of this study were to:

1. Evaluate the host specificity of *N. biwakoensis* on economic and environmental important grasses in Louisiana.
2. Characterize silicon and phenolics among *P. australis* the lineages present in Louisiana.

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Chapter 2. Survival and population growth of roseau cane scale (*Nipponaclerda biwakoensis*) on *Phragmites australis* and related grass species

2.1. Introduction

Common reed, *Phragmites australis* (Cav.) Trin. ex Steud, is an emergent wetland grass that has spread around the world (Den Hartog *et al.* 1989; Clevering and Lissner 1999).

Common reed is a robust plant that propagates from rhizomes (Haslam 1972; Clevering and Lissner 1999; Brix 1999). The rhizomes, the modified subterranean plant stems (Granéli *et al.* 1992), confer to *P. australis* the ability to rapidly reproduce and effectively disperse (Den Hartog *et al.* 1989). *Phragmites australis* usually grows in swamp, sludge, organic and high nutrient substrates, and from saltwater, brackish to fresh water conditions (Rodewald-Rudescu 1974; Hocking *et al.* 1983). In the United States, research on *P. australis* started when invasive lineages from Europe replaced diverse plant communities and breed with natives lineages (Meyerson *et al.* 2010; Saltonstall *et al.* 2010)

Phragmites australis has become an important economic plant on which the livelihood of many people around the world depends (Kobbing *et al.* 2013). In Europe, *P. australis* is used for manufacturing cardboard, printing paper, insulation and thatching material, and wastewater management (Brix 1999). In southern United States, more than twenty native tribes consider *P. australis* as essential resource for making arrows shaft, rods, mat, nets, whistle, knife, and smoking equipment (Brix, 1999i; Kiviat and Hamilton 2001). Nowadays, *P. australis* plays an important role by offering environmental services (Knight *et al.* 2018). *Phragmites australis* helps with carbon sequestration, maintains water quality and conserves habitat resources for wildlife species (Kiviat 2013). More importantly, *P. australis* is the dominant vegetation at the MRD and the Gulf Coast (Kiviat and Hamilton 2001). Therefore, it is considered as a vital

element for holding back sediments (Rooth and Stevenson 2000); preventing soil erosion from wave action and storms (Coops and Van der Velde 1995; Horppila *et al.* 2013), protecting oil infrastructure and preserving navigation channels (Brix 1999).

In the fall of 2016, *P. australis* die-back were reported in hundreds of hectares of the Pass-a-Loutre Wildlife Management Area at the MDR (29°09' N, 89°13' W) (Knight *et al.* 2018). The die-off can be characterized by premature senescence, stunting, and dead stems (Armstrong and Armstrong 2001). Although, the die-back was detected recently, a multi-year analysis from satellite imagery of the MRD suggests that the die-back might have been occurring since 2014 (Ramsey and Rangoowala 2017). During a die-back of dominant plant species, wetlands undergo a vegetation loss process resulting in shoreline retraction, increase in substrate depth, and increased exposure to disturbance events resulting in even more wetland loss (Turner 1990; Webb and Mendelssohn 1996). Die-back of *P. australis* has been reported in the past in Europe and attributed in part to eutrophication and the accumulation of phytotoxins (acetic acid and sulphide) (Van der Putter 1997). In China, the die-back observed in 2008 was attributed to increase of salinity and the invasion of *Spartina alterniflora* (Loisel) (Li *et al.* 2013). In Louisiana, large densities of a scale insect were found between the stem and leaves sheaths of *P. australis* at the die-back sites (Knight *et al.* 2018). This species was identified as *Nipponaclerda biwakoensis* (Kuwana) (Hemiptera: Aclerdidae), also known as roseau cane scale (Knight *et al.* 2018).

Nipponaclerda biwakoensis is an invasive species native from China and Japan (Kawai 1980). The presence of scale in Louisiana is considered the first record of an established population in North America (Knight *et al.* 2018). The current distribution of *N. biwakoensis* includes 14 counties in Louisiana and one county in Texas (Knight *et al.* 2018, Schneider 2019).

As in other species of the Coccoidea, crawlers have functional legs, thus, they are responsible for the dispersal and colonialization of new plants (Magsig-Castillo *et al.* 2010). Once the crawler settles and starts feeding on the sap, the legs become non-functional (Magsig-Castillo *et al.* 2010), and remains in a specific plant location until the adult stage (Kaneko 2004, Kaneko 2005, Kondo *et al.* 2008). Nymphs produce waxy secretions from the epidermis (Waku and Foldi 1984; Gullan and Kosztarab 1997), and cover the scale along the margins (Kawai and Tamaki 1967). The second instar of *N. biwakoensis* is oval in shape, and is milky white in color (Kuwana 1932). The shape of females varies based on space available between the leaf sheath and the stem, and the crowding conditions created by other females in the same location (Teague 1925). Female size varies from 5 to 15 mm-long compared to nymphal stages lengths which vary from 2 to 4 mm-long (McConnell 1954). Mature females can be recognized by a reddish-orange color and/or when the eggs can be observed through the integument. Crawlers emerged from dead females. Males are apterous, 1.1 mm long (Kuwana 1932; McConnell 1954), have functional legs, and lack functional mouthparts (Gullan and Kosztarab 1997).

Most species in the family Aclerdidae have been collected from grasses (Poaceae) and occasionally on sedges (McConnell 1954, Ferris 1955). *Nipponaclerda biwakoensis* has been found on *P. australis* in China (Wang, 1994), Japan, Taiwan, and Korea (Kuwana 1907). In addition, there are reports of *N. biwakoensis* on wheatgrass, *Agropyron cristatum* (L.), and rush (*Juncus* sp.) (Wang 1994). Except for studies of parasitoids in its native range (Kaneko 2004, 2005), there is no published information on the biology and ecology of *N. biwakoensis*. Host records revealed that *Nipponaclerda* spp. and *Aclerda* spp. have been collected from *Sorghum halepense* (L.) (Ferris 1955), *Saccharum officinarum* (L.) (Meagler and Gallo, 2008), *Sasa*

borealis (Hack.) (Suh 2020), *Arundo donax* (L.), *Cydodon dactylon* (L.) and *Sporobolus wrightii* (L.) (Knight *et al.* 2018; ScaleNet 2020).

The outbreaks of *N. biwakoensis* and the close relation of Acleridae with grasses warranted the study of the host specificity of the populations present in Louisiana. Particularly, since invasive species cause production losses in agriculture, and the U.S. Department of Agriculture has spent more \$580 million per year to mitigate invasive species impact through the country since 2001 (Andersen *et al.* 2004). Moreover, stakeholders and biologists were concerned due to the ecological consequences of potentially invasive species. According to Baurick 2018, biologists described this problem as a cancer that is infesting the *P. australis* (Baurick 2018). Those consequences could be the reduction of plant community (Didham *et al.* 2005); or the increasing in the area taken by others invasive species like *Hydrilla verticillata* (L.f.) resulting in changes in the vegetation biodiversity (Haram *et al.* 2020). Therefore, the overall goal of this study was to study the host specificity of *N. biwakoensis*. The specific objectives were: (i) to determine the nymphal development of scale on different crops and grasses; (ii) to evaluate the survival the scale on crops and grasses affected by the scale; and (iii) to study the host specificity under outbreak conditions at the MRD. To achieve these objectives, no-choice greenhouse assays and field surveys were conducted.

2.2. Material and methods

2.2.1. Collection of *N. biwakoensis*

Nipponaclerda biwakoensis was collected from *P. australis* stands at Tangipahoa, Louisiana (30 ° 30' N and 90°40' W); this site was named site “A” and at the Pass-a-Loutre Wildlife Management Area 29 °09' N and 89 ° 13' at the MRD, Louisiana. Infested stems were

collected in summer of 2018 and 2019. Upon arrival to the Department of Entomology at Louisiana State University, infested stems were cut in 10-15 cm long segments. Each segment contained from three to five gravid females. Afterwards, segments were placed inside a plastic container ($40 \times 27 \times 11$ cm) with wet sand. The bottom of the stem was inserted vertically in the sand to provide moisture, and to guarantee healthy stems and scales insect for experiments. All segments were maintained at laboratory conditions (20-25° C and 55-70% RH) for no more than three days before inoculation.

2.2.2. Procurement of test plants

Plant species were selected based on the Centrifugal Phylogenic Method (Wapshere 1989); and suggestions from experts from the United States Department of Agriculture (USDA), and the Louisiana Department of Wildlife and Fisheries (LDWF). Most of the plant species selected were in the Poaceae family and several known hosts of *N. biwakoensis* (ScaleNet 2020). In addition, special consideration was given to economically (crops) and environmentally (marsh grasses) important species (Table 1). Plants material was collected from different locations and sources. Marsh grasses were collected from wild populations with the help of the USDA and LDWF. Seeds of *Sorghum bicolor* (L.), *Oryza sativa* (L.), *Zea mays* (L.), and propagules of *Saccharum officinarum* (L.) were collected from the Sugar Research Station of Louisiana State University. Plant propagules were placed in nursery pot ($17 \times 19 \times 14$ cm) containing Miracle-Gro Premium Potting Mix (The Scotts Miracle-Gro, Marysville, OH). All marsh grasses were placed inside of a kiddie pool ($91 \times 91 \times 18$ cm) filled with water until the middle (46 cm). Seeds were sowed in the germination soil to a depth of no more than 4 cm directly to nursery pot ($17 \times 19 \times 14$ cm) containing Miracle-Gro Premium Potting Mix (The Scotts Miracle-Gro, Marysville, OH). All plants were fertilized with one tablespoon of slow-release fertilizer OsmocotePlus[®]

(19N-6P-12K, The Scotts Miracle-Gro, Marysville, OH). All crops were watered daily and the kiddie pools of the marsh grasses were filled with water twice per week. Plant pests were controlled with natural insecticide (Potassium Salts of Fatty Acids 49.52%, Safer ® Insecticidal Soap, Lititz, PA). Plant colonies and experiments were conducted at the Department of Entomology or at the Louisiana State University Agricultural Center (APMCG) greenhouses. Experiments were conducted in summer and fall of 2018 and 2019.

2.2.3. No-choice experiment with crops and marsh grasses

The objective of this experiment was to compare the development of immatures *N. biwakoensis* in different crop and marsh grasses. The experiment was conducted in summer and fall 2018 under greenhouse conditions which fluctuated between 25–30° C, 40–60% RH. Due to intrinsic differences in growth forms and phenology of each plant species, the size of test plants was not standardized (Ex. Diaz *et al.* 2014), but test plants had at least three to five stems per pot. Therefore, the experiment was conducted in three groups (July, August and November) based on the availability of plant material and each group included *P. australis* as a positive control (Table 1). Five pots each with 3-5 stems per species were placed inside of mesh cage (60 × 60 × 140 cm, BioQuip products Inc., Compton, CA). When possible, 10 potted plants (2 cages) per species were tested. For cages containing marsh grasses, a plastic tray was added to maintain flooded conditions. The inoculation of *N. biwakoensis* in each potted plant was made by attaching three scale-infested segments to the test plant. Stems were attached using twist tie gently pressed against the test stem. This technique allowed the crawlers to emerge and explore the stems in their surroundings. Sixty days after the inoculation, test plants were harvested which consisted of the removal of the stem at the base of the plant. Stems were labelled, placed in plastics bags, and transported in coolers to the Department of Entomology. In the laboratory, the

stem height, and the number of live scale per stem were recorded. Stem height was measured from the nearest centimeter of stem base to the tallest leaf. Leaf sheaths were removed and the number of scales were counted. The number of scales per meter of stem was used to compare densities among plant species.

Table 2.1. Plant species evaluated in the no choice host specificity experiment of *Nipponaclerda biwakoensis*

Scientific name	Common name	Importance
Category 1- Genetic types of the target weed species found in North America		
POALES		
Poaceae		
<i>Phragmites australis</i> (Cav.) Trin. ex Steud.	Common reed	Invasive weed
Category 2- North America species in other genera in the same family as the target weed		
POALES		
Poaceae		
<i>Arundo donax</i> L.	Giant reed	Marsh grasses
<i>Panicum hemitomon</i> Schult	Maidencane	Marsh grasses
<i>Paspalum vaginatum</i> Sw	Seashore paspalum	Grass
<i>Oryza sativa</i> L.	Rice	Cultivated
<i>Saccharum officinarum</i> L.	Sugarcane	Cultivated
<i>Sorghum bicolor</i> L.	sorghum	Cultivated
<i>Spartina alterniflora</i> Loisel.	Smooth cordgrass	Marsh grasses
<i>Urochloa mutica</i> (Forssk.) T.Q. Nguyen	Para grass	Marsh grasses
<i>Zea mays</i> L.	Corn	Cultivated
<i>Zizaniopsis miliacea</i> (Michx.) Döll & Asch.	Giant cutgrass	Marsh grasses
<i>Zizania hemitomon</i> L.	wild rice	Marsh grasses
<i>Arundinaria gigantea</i> Muhl.	Hog cane	Marsh grasses
Cyperales		
Cyperaceae		
<i>Cladium mariscus</i> L.	Jamaica swamp sawgrass	Marsh grasses
<i>Schoenoplectus californicus</i> (C.A. Mey.) Palla	California bulrush	Marsh grasses

2.2.4. Survival of *N. biwakoensis* on four grasses species

The objective of this experiment was to evaluate the survival of *N. biwakoensis* on those plant species where nymphs successfully develop in the no-choice experiment. The experiment was conducted in summer a 2018 under greenhouse conditions which fluctuated between 25–30° C, 40–60% RH. *Spartina alterniflora*, *Schoenoplectus californicus*, *Sorghum bicolor*, and *P. australis* as a control were tested. *Sorghum bicolor* was included in this experiment because a preliminary study conducted in 2017 demonstrated that three *N. biwakoensis* nymph established on *S. bicolor* (Appendix A, Table A.1). *Spartina alterniflora*, *S. californicus*, and *Phragmites australis* cages contained a plastic trays maintaining flooding conditions. *Sorghum bicolor* pots were watered daily. Four pots from single species were placed inside of one cage (60 × 60 × 140 cm), with three cages per species, therefore, twelve pots per species. Each pot contained from 2 to 10 stems due the intrinsic difference in growth form and phenology. The inoculation of *N. biwakoensis* on the four grasses was made when the plants reached had stems of more than 30 cm long and 2-3 open leaves. The scale inoculation was made by placing three scale-infested segments of *P. australis* next to the target plant. Each infested segment contained between two to three *N. biwakoensis* gravid females for standardization. Twenty one days after the inoculation, all plants were evaluated for the presence or absence of *N. biwakoensis* on the stems. To measure scale densities, stems were harvested after 30 and 60 days of the scale inoculation. The purpose of the first harvest was to assess nymph development, and was made 30 days after the inoculation on July 2019. One stem per pot was haphazardly selected, cut at ground level, labeled, and transported to the Department of Entomology at Louisiana State University. The purpose of second or final harvest was to assess in adult development, and was made 60 days after the inoculation on August 2019. The remaining stems in each pot were harvested and

followed the steps describe in the first harvest. Data on plant variables from each harvest included the stem height, and the number of live scales per stem. Stem height were measured from the nearest centimeter of stem base until the tallest leaf. Total numbers of scales, including immature and adult scale, were measured by peeling the leaf sheath of each stem, and counting the numbers of scales. The number of *N. biwakoensis* was reported as densities per meter of stem.

2.2.5. Measuring spill over risk under outbreaks in the Mississippi River Delta

The objective of this experiment was to determine field host specificity of *N. biwakoensis* under outbreak conditions experienced at the Mississippi River Delta. Eight sites were selected according to the presence of *N. biwakoensis*, *P. australis* and others possible host plants. The sites were located at the Mississippi River Delta, Plaquemines Parish, Louisiana (Figure 2.1). The Pass-A-Loutre is 46,540 ha in size (Knight *et al.* 2020) and is one of the four channels ‘Bird’s Foot’ of the Mississippi River Delta. This area encompasses a complex of minor passes, man-made canals, mudflats, ponds, and marshes (White *et al.* 2004). Environmental conditions include salinity levels of less than 1 parts per thousand, the annual temperatures of 17.2 -25°C, water depths varies from 7 cm up to more than 100 cm, and it is composed by Belize and Larose soils (Knight *et al.* 2020). Each site was located along a channel edge and was accessible by airboats.

Sites were selected based on the presence of potential non-targets grasses that were in direct contact or surrounded by an outbreak of *N. biwakoensis*. All sites were separated by at least 60 m and contained low plant species diversity since the dominant grass was *P. australis*. The first site was called LS1, it was located in a mudflat at 29°12'24" N and 89°20'5" W with 2 m of elevation. LS1 contained four marsh grasses such as *Zizaniopsis miliacea* (Michx.) Döll &

Asch, *Schoenoplectus californicus* (C.A. Mey.) Palla, and *Typha latifolia* (L.). The second site was called LS2, it was located in a swamp at 29°13'07" N and 89°22'44" W with 10 cm deep. LS2 only had *Typha latifolia* (L.). The third site was called LS3, it was located in marsh at 29°10'82" N and 89°20'63" W, with 30 cm deep. LS3 had only *Zizania aquatica* (L.). The fourth site was called LS4, it was located in a mudflat at 92°11'96" N and 89°20'55" W. LS4 had only *Panicum hemitomon* (Schult). The fifth site was called K1, it was located in a swamp at 29°11'79" N and 89°20'35" W with 24 cm deep. K1 was composed just for one grass, *Urochloa mutica* (Forssk.) T.Q. Nguyen. The sixth site was called K2, it was located at 29°11'63" N and 89°20'05" W with 24 cm deep. K2 was the site with greater plant diversity, it was comprised by *Zizaniopsis miliacea* (Michx.) Döll & Asch, *Zizania aquatica* (L.), *Urochloa mutica* (Forssk.) T.Q. Nguyen and *Typha latifolia* (L.). The seventh was called K3, it was settled at 29°10'66" N, 89°18'49" W with a 34 cm deep. K3 contained juts *Schoenoplectus californicus* (C.A. Mey.) Palla. Lastly, the ultimate site was called K4, it was situated at 29°12'38" N and 89°19'00" W. K4 had only *Spartina alterniflora* Loisel.

At each site, thirty stems per species were inspected in the field and clipped just below the water level. During field inspection, only the presence or absence of the scale was reported. Only 10 stems out of 30 stems that where infested with *N. biwakoensis* were haphazardly selected and stored in a plastic trash bag. Stems were transported in a cooler the Department of Entomology. Two field surveys were conducted at Pass-a-Loutre Wildlife Management Area (one in July 24 and the other in October 18 of 2018). Data on plant variables from each harvest included the stem height, and the number of live scales and parasitized scale per stem. Stem height was measured from the nearest centimeter of stem base until the tallest leaf. Total numbers of scales, including immature and adult scale, and the parasitized scales were measured

by peeling the leaf sheet of each plant, and counting each scale found in the whole stem individually. The number of *N. biwakoensis* and the parasitized scale was reported as densities per meter of stem.

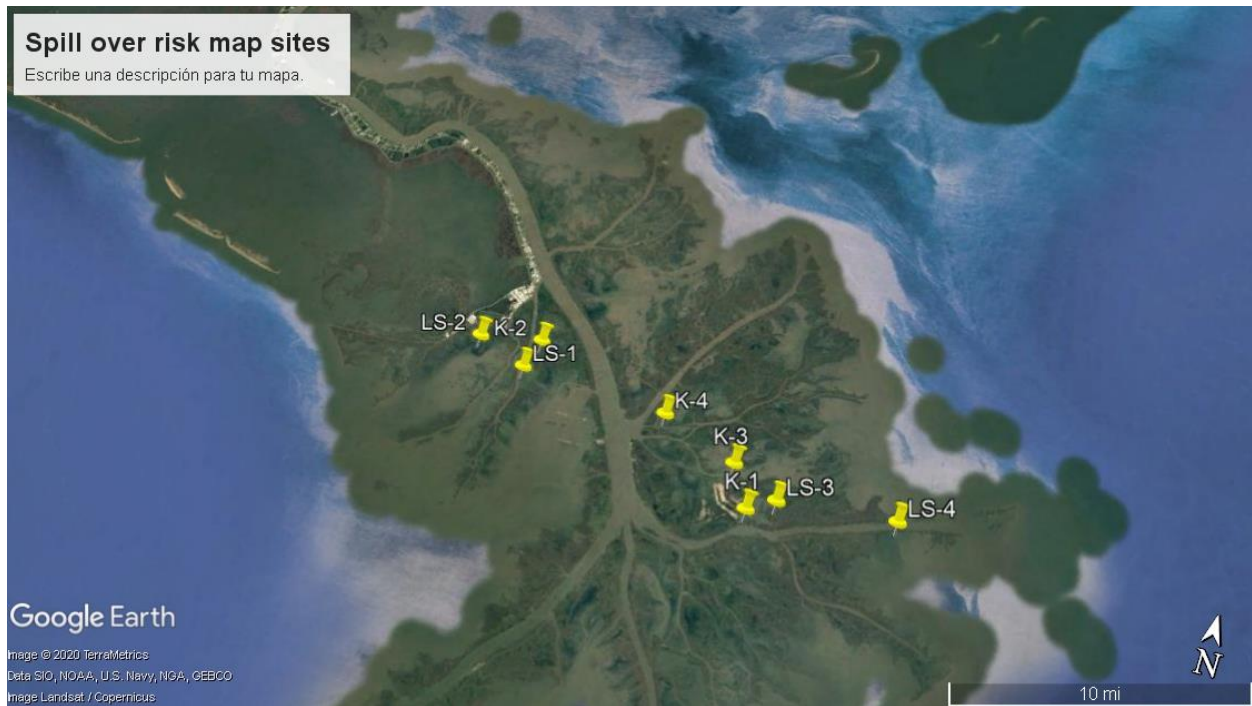


Figure 2.1. Map of the locations of the spill-over survey sites at the Mississippi River Delta, Plaquemines, Louisiana, USA. LS and K means the initials names of the people in charge of the field surveys.

2.2.6. Data analysis

Means of live *N. biwakoensis* in all grasses of the no-choice experiment and the Survival of scale were analyzed per species. Therefore, we analyzed by generalized linear mixed models (PROC GLIMIX, SAS instituted 2009). In the case that grasses did not present any scale insect, those data will be not include in the statistical analysis. Effects were considered significant when p-values were less than 0.05. Means *N. biwakoensis* in the grasses of the no-choice experiment and the Survival of scale of were pairwise and evaluated with Tukey's Post-hoc test. No statistical

analyzed were required for the spill over risk experiment because *N. biwakoensis* scale were found only on the control, *P. australis*.

2.3. Results

2.3.1. No-choice experiment with crops and marsh grasses

No Scales were found on *Z. mays*, *S. bicolor*, *C. mariscus*, *A. donax*, *A. gigantea*, *P. hemitomom*, *P. vaginatum*, *S. officinarum*, *S. cynosuroides*, *U. mutica*, *Z. miliacea*, and *Z. hemitomom*; instead dry and death scale were found in those grasses (Figure 2.2). Nymphs of *N. biwakoensis* were found on *S. californicus*, *S. alterniflora* and *P. australis*. The mean number of nymphs per plant species was significantly different ($F_{3,25} = 3.03$; $P = 0.0482$) (Figure 2.3). *Phragmites australis* is the species with more nymphs per meter, followed by *S. alterniflora*, and lastly, *S. californicus* (Figure 2.3). Due to the time frame of the experiment, no *N. biwakoensis* adults were found in each species.

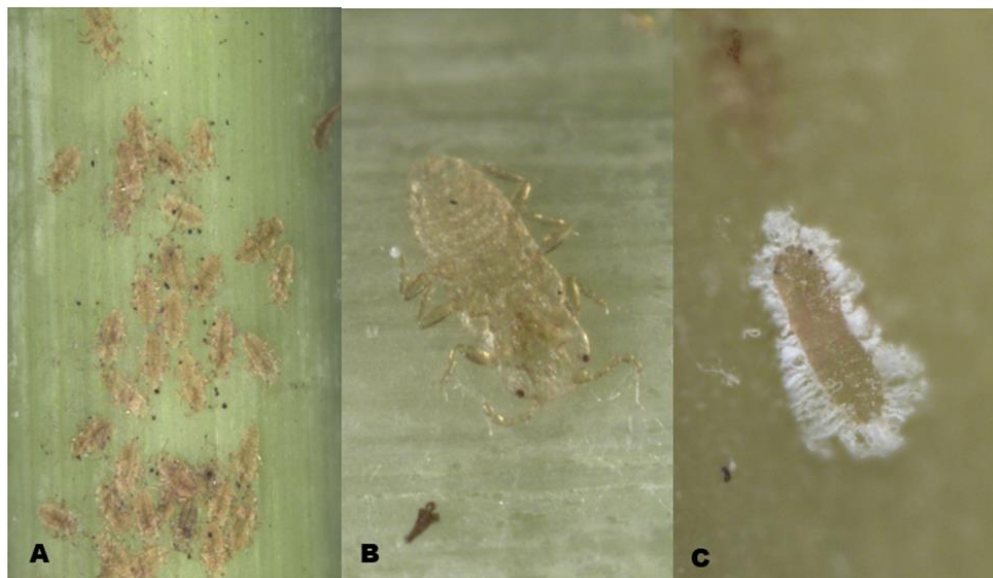


Figure 2.2. . Dry and dead crawlers on *Arundo donax* stem (A); a single crawler on *Arundo donax* stem (B), and crawler with visible wax filaments around its body on *Schoenoplectus californicus* (C)

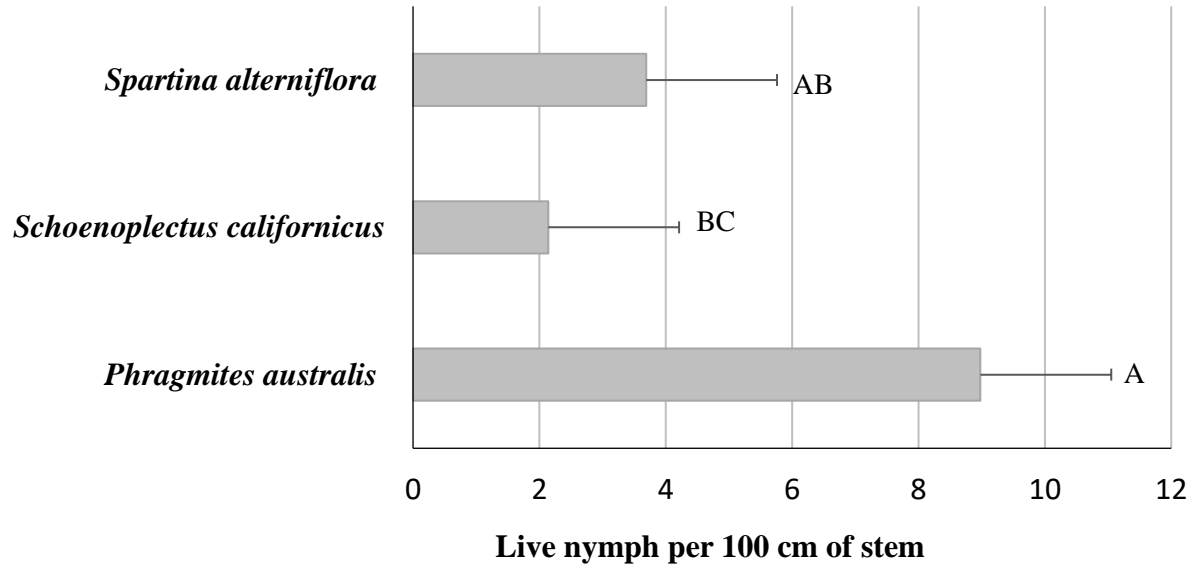


Figure 2.3. Mean number per 100 cm of stem (+ SE) of *Nipponaclerda biwakoensis* after thirty days of inoculation on *Spartina alterniflora*, *Schoenoplectus californicus*, and *Phragmites australis* under no-choice condition ($F_{3, 25} = 3.03$; $P = 0.0482$). Plant species with different letter with in a species are significantly different ($P < 0.05$).

2.3.2. Survival of *N. biwakoensis* on four grasses species

Thirty days after inoculation, the density of nymphs per plant species varied. The number of nymphs on *S. californicus* (26.08 ± 34.92 , mean \pm SE) was 3.7-times lower than on *P. australis* (96.83 ± 95 , mean \pm SE) (Figure 2.4; $F_{2, 33} = 8.36$; $P = 0.0012$). The mean for *S. bicolor* was 2.08 ± 4.38 scale. There were no nymphs on *S. alterniflora*. Sixty days after inoculation, scales were found only on *P. australis* with a mean of 67.56 ± 50.52 scales per meter of stem.

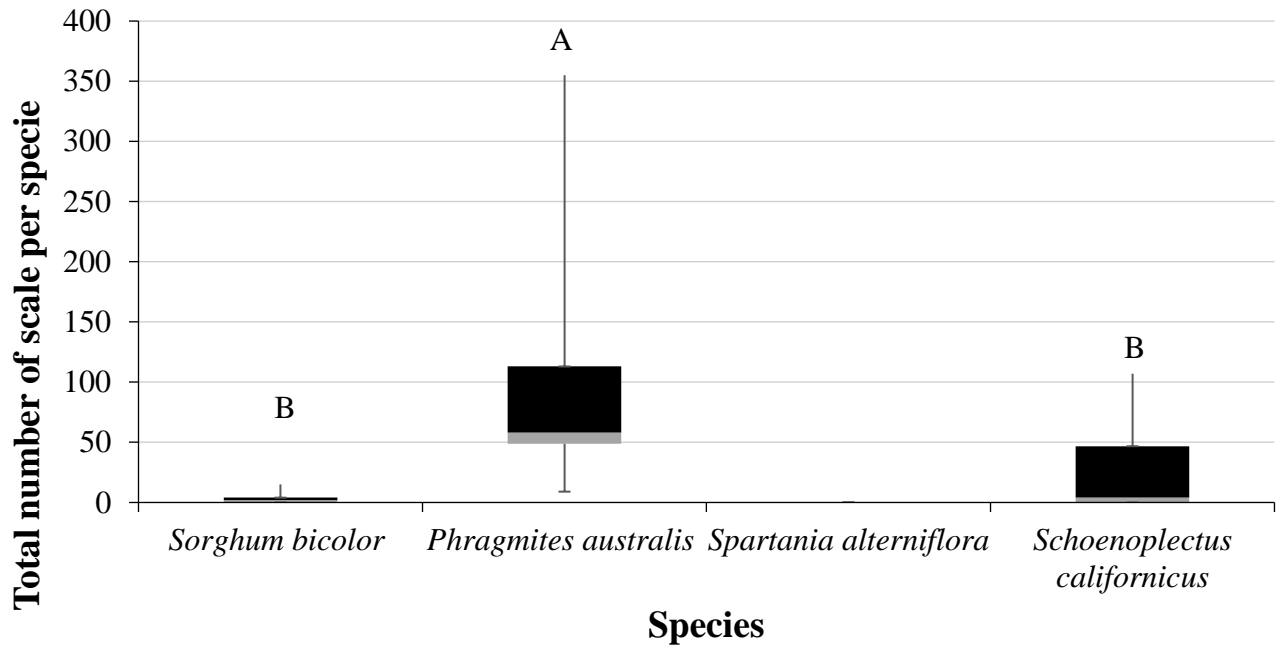


Figure 2.4. Box plots graph were used to compare the data from the Survival of *N. biwakoensis* on four grasses 30 days after the inoculation. Box plots display batches of data. Five values are used to create the graph the extremes, the upper and lower quartiles, and the median. However, the graph demonstrated two box one from the lower quartile to median (gray color), and the other from the median to upper quartile (blank color). Only *Nipponaclerda biwakoensis* nymph were found on *Sorghum bicolor*, *Phragmites australis*, *Schoenoplectus californicus*, and no nymphs on *Spartina alterniflora*

2.3.3. Measuring spill over risk under outbreaks in the Mississippi River Delta

Field surveys during outbreak conditions revealed that *N. biwakoensis* was found only on *P. australis* (Table 2.2). The densities of live and parasitized *N. biwakoensis* scales found on *P. australis* ranged from 17 to 115 scales, and from 1 to 6 scales per 100 cm of stem, respectively (Table 2.2).

Table 2.2 Mean \pm standard error of live and parasitized *Nipponaclerda biwakoensis* collected from several plant species at the Pass-A-Loutre Wildlife Management Area at the Mississippi Delta River during summer and fall 2018

Site	Grass specie	Mean <i>N. biwakoensis</i> and parasitoids per 100 cm of stem			
		Live scales		Parasitized scales	
		July	October	July	October
LS-1	<i>Zizaniopsis miliacea</i>	0	0	0	0
	<i>Schoenoplectus californicus</i>	0	0	0	0
	<i>Typha latifolia</i>	0	0	0	0
	<i>Phragmites australis</i>	17 \pm 17	40 \pm 31	5 \pm 7.8	2 \pm 2.2
LS-2	<i>Typha latifolia</i>	0	0	0	0
	<i>Phragmites australis</i>	10 \pm 9	104 \pm 57	4 \pm 5.8	3 \pm 3.2
LS-3	<i>Zizania aquatica</i>	0	0	0	0
	<i>Phragmites australis</i>	21 \pm 18	18 \pm 11.9	0 \pm 0.7	0 \pm 0.0
LS-4	<i>Panicum hemitomon</i>	0	0	0	0
	<i>Phragmites australis</i>	61 \pm 116	69 \pm 36.1	6 \pm 8.3	0 \pm 0.3
K1	<i>Urochloa mutica</i>	0	0	0	0
	<i>Phragmites australis</i>	65 \pm 56	48 \pm 31	4 \pm 5.9	1 \pm 1.3
K2	<i>Typha latifolia</i>	0	0	0	0
	<i>Zizaniopsis miliacea</i>	0	0	0	0
	<i>Zizania aquatica</i>	0	0	0	0
	<i>Urochloa mutica</i>	0	0	0	0
	<i>Phragmites australis</i>	76 \pm 76	51 \pm 24	3 \pm 8.4	0 \pm 0.4
K3	<i>Schoenoplectus californicus</i>	0	0	0	0
	<i>Phragmites australis</i>	72 \pm 59	89 \pm 55	3 \pm 5.5	4 \pm 7.4
K4	<i>Spartina alterniflora</i>	0	0	0	0
	<i>Phragmites australis</i>	42 \pm 31	115 \pm 71	1 \pm 2.5	2 \pm 2.6

2.4. Discussion

The determination of the host range of *N. biwakoensis* was critical to address the risks associated with arrival of this invasive species. Results of the no-choice experiment demonstrated that *N. biwakoensis* nymphs were highly specific to *P. australis*. Under greenhouse conditions, most of the crawlers settled and developed waxy filaments on *P. australis*. However, very few crawlers were able to settle on *S. californicus* and *S. alterniflora*. In addition, a

preliminary experiment showed that three *N. biwakoensis* crawlers were able to establish on *S. bicolor* stems. The implications of the establishment and limited development on *S. californicus*, *S. alterniflora*, and *S. bicolor* remains unknown. One could speculate that the limited period of feeding on these non-target plants, might not be enough to cause a significant damage to the plant. Results of the Survival of *N. biwakoensis* experiment revealed that nymphs can develop partially on *S. californicus*, *S. bicolor*, and *P. australis* but not on *S. alterniflora*. However, the large number of scales on *P. australis* demonstrated its ideal quality as a host for *N. biwakoensis*. Finally, the sampling conducted after 60 days revealed that *S. californicus*, *S. alterniflora*, *S. bicolor* could not sustain the development of *N. biwakoensis*. The density of the scales decreased later in the life cycle, probably due to the impact of parasitoids present in the cages. The spillover survey revealed that under outbreak conditions, *N. biwakoensis* was found only on *P. australis*.

Most of the phytophagous insects that are considered invasive species feed on one plant species or a few genera, or from a singular family or subfamily (Bernays 1988). The validation of the host specificity to a single species exhibited by *N. biwakoensis* may be related to different factors. According to Futuyma 1983, phytophagous insects have evolved a behavioral level in which they become specialists in response to the abundance of a particular species (Futuyma 1983), which is the case of *N. biwakoensis*, since *P. australis* is the dominant vegetation at the MRD. In addition, some herbivores with narrow host range switched their behavior to increase mate-finding (Colwell 1986), since *N. biwakoensis* movement is limited among a stem, I suspect that the females scales become host specific to be found by males who only can walk.

The potential protective mechanism involved in narrow host range could determine the specificity of the *N. biwakoensis* with other hosts and between *P. australis* genotypes. The poor

performance of the sap-sucking hemipterans between hosts have been attributed to the differences in nutritional composition such as the amino acids in phloem (Sandström and Petterson 1994), plant nitrogen (Van klinken 1999), plant carbon (Awamack and Leather 2002), and variation in carbohydrates storages in Poales tissue cell wall like in rice (Vega-Sánchez *et al.* 2013). In addition, differences in defensive compounds among Poales grasses could deter *N. biwakoensis* from becoming generalists. Peroxidases a chemical compounds are limited to C4 grasses such as *S. bicolor* (Scully *et al.* 2016), and *Paspalum vaginatum* (Sw.) (Campbell 2005). In contrast, *N. biwakoensis* could evolved resistance traits against the peroxidases present in *P. australis* since this plant is a C3 plant, further investigation of *P. australis* defenses compounds are necessary to complete understand the host specificity of scale insect. Other factors that explained the host specificity of the scale insect could be the viscosity among grasses tissues (Diaz *et al.* 2010), seasonal variations in the hosts features (Brodbeck *et al.* 2004; Bi *et al.* 2007), and lack of feeding stimulants (Ciepiela and Sempruch 1999).

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Chapter 3. Comparison of resistances-related traits in lineages of *Phragmites australis* that differ in resistances to

3.1 Introduction

Roseau cane scale, *Nipponaclerda biwakoensis* (Kuwana), is an invasive species native to China, Japan and Taiwan (Kawai 1980). *Nipponaclerda biwakoensis* was reported in the fall 2016 in Louisiana and has been associated with the *Phragmites australis* die-back process in the Mississippi River Delta (MRD) in Louisiana (Knight *et al.* 2018). This scale insect can be found between the leaf sheaths and stem of *P. australis*. *Nipponaclerda biwakoensis* can be identified by its distinctive nymph-form throughout all its life stages except for the first instar, the crawlers (Kondo *et al.* 2008). The crawlers are the only stage with functional legs and are in charge of its dispersal (Magsig-Castillo *et al.* 2010). The scale possess long styles that allow it to feed from thick and hard stems (Gullan and Kosztarab 1997). The presence of this scale in Louisiana is considered the first record of an established population in North America (Knight *et al.* 2018).

Phragmites australis is a cosmopolitan wetland grass (Den Hartog *et al.* 1989; Clevering and Lissner 1999) and is considered of environmental value in some regions (Haslam 1972; Ostendorp 1989). This wetland grass has been used for roofing, fencing, manufacturing cardboard, printing paper, insulation and thatching material (Heiser 1979; Rodin 1985; Zola and Gott 1992; Brix 1999). In the United States, the exact time of arrival of *P. australis* is unknown. However, *P. australis* has spread all over the United States since more than 100 years ago (Rice and Stevenson 1996; Chambers *et al.* 1999). At the MRD, *P. australis* plays an important role by offering ecosystems services (Knight *et al.* 2018) including carbon sequestration, maintaining water quality, and conserving habitat for wildlife species (Kiviat 2013). *Phragmites australis* is also a vital element for holding sediments and preventing soil erosion (Coops and Van der Velde

1995; Rooth and Stevenson 2000; Horppila *et al.* 2013), guarding oil infrastructure, and protecting navigation channels (Brix 1999).

There is a large diversity of *P. australis* populations with several lineages that have been described and sequenced in many countries. In the United States, since 2002, several lineages have been found by using two non-coding chloroplast DNA (cpDNA) sequences (Saltonstall 2002a). Five different lineages were found in the United States. The first sequenced lineage was called Native, this lineage is endemic to North America, and consist of 13 haplotypes such as A, B, C, D, E, F, G, H, S, Z, and AA (Saltonstall 2002a, 2002b; Peterson *et al.* 2004). The second lineage was called Gulf. It was identified by Pellegrin and Hauber (Pellegrin and Hauber 1999), and it contains a single haplotype called 'T' (Saltonstall 2002). The third lineage was called the European. European lineage as same as Gulf, only contains a single haplotype called 'M' (Howard *et al.* 2008). The fourth genotype was called Delta, and it contains a single haplotype called as 'M1' (Hauber *et al.* 2011; Lambertini *et al.* 2012). Finally, the combination of three lineages, such as M, AD, and AI was known as a Greeny (Lambertini *et al.* 2012). Except for the native lineages, all lineages are present in Louisiana (Knight *et al.* 2018). Although the Native lineages is not present in the south, this lineages has been found in almost all states of West, Midwest and Northeast of the United States (Saltonstall 2002a). The Gulf lineages dominates the Gulf Coast of the United States (Saltonstall 2002a) and the Delta lineages is the more abundant at the MRD (Knight *et al.* 2018). The European lineages has extended all over the country in the last 100 years, and it is considered an introduced lineages in North America (Howard *et al.* 2008).

At the lower MRD, scientists observed that stands of European lineage had healthier stems compared with Delta lineage during a die-back in May 2017 until July 2019; this

observations was associated with lower densities of *N. biwakoensis* on European lineages compared to Delta lineages stems (Knight *et al.* 2020). Moreover, another study also revealed that the European lineages suffers less damage by herbivores (Bhattarai *et al.* 2017). Therefore, one could hypothesize that the European lineages has greater quantities of defenses or resistances related traits against *N. biwakoensis* compared with Delta lineages. Scientists have been studying the coevolution in which plants have developed a set of defenses traits against herbivores damage (Ehrlich and Raven 1964; Karban and Baldwin 1997; Karban and Agrawal 2002). Plants have evolved chemical defenses (second metabolites) and physical barriers (trichomes and waxy cuticles), against herbivores (Carmona *et al.* 2011; Mitchell *et al.* 2016).

Silicon is associated with resistances against insects because plants deposit silicon crystals in the epidermal cells resulting in more leaf thickness and obstruction of insect feeding (Raven 1983, Bélanger *et al.* 2003, Morales *et al.* 2004, Kvedaras and Keeping 2007). Several studies have demonstrated the efficacy of silicon content against herbivores. One study discovered that silicon could reduce the pupal production of *Spodoptera exempta* (Walker) on European grasses (Massey *et al.* 2008). In addition, *Schistocerca gregaria* (Forsskål) removed leaves from *Poa annua* (L.) with high silicon content when they selected their food (Garbuzov *et al.* 2011). According to Schaller *et al.* 2013, *P. australis* can uptake large amounts of silicon from the soil (Schaller *et al.* 2013; Croy *et al.* 2020). However, no previous studies had evaluated the role of silicon in *Phragmites* resistances lineages.

Phenolic content are secondary metabolites that constitute one of the largest groups of substances in plants (Lattanzio *et al.* 2006). Phenolic content have been associated with herbivore deterrence. One study found a lower density of *Mahanarva fimbriolata* (Stal) and increases in total phenolics on *Saccharum officinarum* (L.) (Valverde 2012). Similarly. Lower

number of nymphal stages of *Nilaparvata lugens* (Stal.) were found in genotypes of *Oryza sativa* (L.) with higher Total phenolic content (Udayasree *et al.* 2020). Moreover, previous studies have revealed large quantities of phenolic content on grasses such as *Triticum* (L.) species (Kharazain and Rahiminejad 2009), *Amaranthus hybridus* (L.), and *Brachiaria brizantha* (A. Rich.) Stapf, *Panicum Maximum* (Jacq.) (Chitindingu *et al.* 2006), *Miscanthus sinensis* (Ander.), *Dichanthium caucasicum* (Trin.) S.K.Jain & Deshp, *Saccharum officinarum*, *Bambusa vulgaris* (Schrad), (Reddy and Krishnan 2013), and *P. australis* (Balcerek *et al.* 2009; Cronin *et al.* 2015, Bhattarai *et al.* 2017; Croy *et al.* 2020). Therefore, silicon and phenolics could be associated with pest resistance among *P. australis* lineages.

Due to the genetic variation in *P. australis* populations present in United States (Clevering and Lissner 1999), there is a great opportunity to study whether this variation is associated with pest resistance. Therefore, the goal of this study was to understand the variation in resistance and expression of two resistances related traits among four lineages of *P. australis* and their role in the host plant resistance against *N. biwakoensis*. The specific objectives were: (i) to evaluate the population growth of the scale insect on different lineages of *P. australis* (ii), and to quantify the total phenolic content and silicon content of *P. australis* lineages. We hypothesized that phenolic and silicon content of European lineages might confer resistance traits against *N. biwakoensis*.

3.2. Material and Methods

3.2.1. Collection of *N. biwakoensis*

Nipponaclerda biwakoensis was collected from either ‘Site A’ located at Akers, Tangipahoa Parish, Louisiana (30°30' N and 90°40' W) or at the Pass-a-Loutre Wildlife Management Area (29°09' N and 89°13' W) at the MRD, Louisiana. Scale-infested stems of *P.*

australis were collected in summer and fall of 2018 and 2019. After the arrival to the Department of Entomology at Louisiana State University, stems were cut in 10 -15 cm segments containing at least three to five gravid females. Scales were held on the stem to keep them alive because gravid females die when they are removed from the stem. Subsequently, infested segments were positioned inside a white plastic container (40 × 27 × 11 cm) with wet sand. The lower part of the infested segments were submerged in sand to provide moisture, and to guarantee healthy stems and scales insect for experiments. All segments were maintained at laboratory conditions (20-25° C, 55-70% RH) for no more than three days before inoculation.

3.2.2. *Phragmites* collection and maintenance

A *Phragmites* common garden was established in 2009 at Louisiana State University (LSU), Baton Rouge, East Baton Rouge Parish, Louisiana (30°35' N, 91°14' W) (Bhattarai *et al.* 2017). All stems were cut down every winter, this procedure is meant to minimize maternal environmental effects on plant performance, and to enhance healthy and young stems every year. Rhizomes were collected from Gulf, Pacific, and Atlantic coasts of North America. *Phragmites australis* rhizomes were grown in sand in 20-L nursery pots and maintained outdoors. *Phragmites australis* were watered three times per day with an automatic irrigation system. Pots were fertilized, early in spring and at the middle of the year, with one cup of a mix of one tablespoon of Miracle-Gro® (24N-8P-16K, The Scotts Miracle-Gro, Marysville, OH) and 132 ml of Liquinox GROW® (10N-10P-5K, Liquinox CO, Orange, CA) per every 11.36 L of water. Pots were fertilized with one 120 ml of slow-release fertilizer OsmocotePlus® (19N-6P-12K, The Scotts Miracle-Gro, Marysville, OH) twice per year. In the flowering season, *P. australis* flowers were removed to prevent seed production. As of 2020, the common garden contains more than 90 populations, each population comprising in one site and one lineage (Aviles personal

observation). The number populations used in each experiment varied because of shoot failures, plant mortality, poor propagation, and slow growth of some lineages like Native lineage. Populations were randomly distributed within the common garden. Temperature and relative humidity in summer at the common garden in Baton Rouge vary from 23-28.7° C and 60-80%, respectively (PRISM, Climate group 2020).

3.2.3. Comparison of *N. biwakoensis* growth rate on different *P. australis* lineages

The objective of this experiment was to compare the population growth of *N. biwakoensis* on different *P. australis* lineages. Sixty-nine and fifty-one populations were tested in the summer of 2018 and 2019, respectively. In the summer 2018, 12 Delta, 22 Gulf, 26 European, and 13 Native populations were evaluated (Appendix A, Table A.2). Plants were taller than one meter, which allowed for the standardization of the phenology of the lineages. Before the inoculation, plants were inspected to determine if *N. biwakoensis* was already present. The inoculation of *N. biwakoensis* was made by attaching next to target plant five infested segments per pot. Each infested segment contained between two to three *N. biwakoensis* gravid females. Using this technique allowed the crawlers to emerge and colonize the potted stems. In summer 2019, 2 Delta, 19 Gulf, 24 European and 6 Natives were evaluated (Appendix A, Table A.2). The population numbers varied between 2018 and 2019 due to sprout failures, mortality, propagation, and slow growth rate among genotypes resulting from the previous cut downs. The inoculation and harvest processes were the same as described for the 2018 experiment

Sixty days after the inoculation, six randomly selected stems per pot were cut and transported to the Department of Entomology. Data on plant variables from each harvest included the stem height, and the number of live scales per stem. Stem height was measured from the nearest centimeter of stem base to the tallest leaf. Total number of scales per stem,

including immature and adult scale, were counted by peeling the leaf sheet of each plant, and counting each scale found in the whole stem individually. The number of *N. biwakoensis* was reported as densities per meter of stem.

3.2.4. Quantification of total phenolic content of *P. australis* lineages

Sample collection

The objective of this experiment was to measure the amount of total phenolic present in individuals of the *P. australis* populations. Eight Delta, 22 Gulf, 25 European and 12 Native, a total of sixty-seven populations were analyzed in fall 2018 (Appendix A, Table A.3). Samples were collected from the populations of the common garden by cutting random leaves and stems (0.5g) from pot. Each sample was placed into a paper bag ($17 \times 8.1 \times 5.8$ cm), and moved into a plant drying oven set at 50°C for one week.

Phenolic content extraction

Each dried sample of *P. australis* was cut into small pieces with sterile scissors. Afterwards, 0.5g of each dry sample was weighted on an XS 105 Dualrange (Mettler-Toledo, Columbus, OH) analytical balance, and placed inside a 20ml scintillation vials that contained 5 ml 50% methanol and 5 ml 50% distilled water. Samples were let sit for 24 hours.

Reagent preparations

Folin-Ciocalteu reagent was prepared by diluting 500 ml of the 2N reagent in 500 ml of distilled water. The 20% Na_2CO_3 solution was prepared in a 250 ml Erlenmeyer flask by adding 20 grams of Na_2CO_3 to 100 ml of distilled water. Then, the solution was heated for 20 min, cooled down and sealed with Parafilm. The gallic acid was mixed by dissolving 8.5 mg of gallic acid in a solution of 50% of methanol and 50% of distilled water.

Phenolic content analysis

The total phenolic content was evaluated from methanolic plant extracts described above by using the Folin-Ciocalteu method (Waterman and Mole 1994). 250 microliters of each sample extracts was placed in a test tube and filled with distilled water to adjust the total volume to 2.75 ml. Later on, each tube was filled with 500 microliters (µl) of the Folin-Ciocalteu reagent. After approximately 30 seconds, 500 µl of 20% sodium carbonate was added to each sample. Each sample was vortexed and allowed to sit for ninety minutes at room temperature. Then, each sample was transferred to cuvettes and the absorbance was read at 720 nm in a UV 6300 PC Spectrophotometer (Avantor™, Radnor, PA). The standard curve was developed with a concentration of 0, 5, 10, 25, 50, 100, and 200 mg/ml of gallic acid. Estimations of phenolic content were calculated from the standard curve obtained with Gallic acid, and the calibration equation was $y = 7.5471 + 212.2970 \times \text{Abs}$. (Figure 3.1). Total phenolics were expressed as gallic acid equivalents (nmoles /g plant extract). Phenolic content of each sample was determined by replacing the absorbance into this formula:

$$\frac{abs - b}{m}$$

Where:

Abs = absorbent data from the spectrophotometer

b = slope of the equations

m = the weight of the plant extract in g

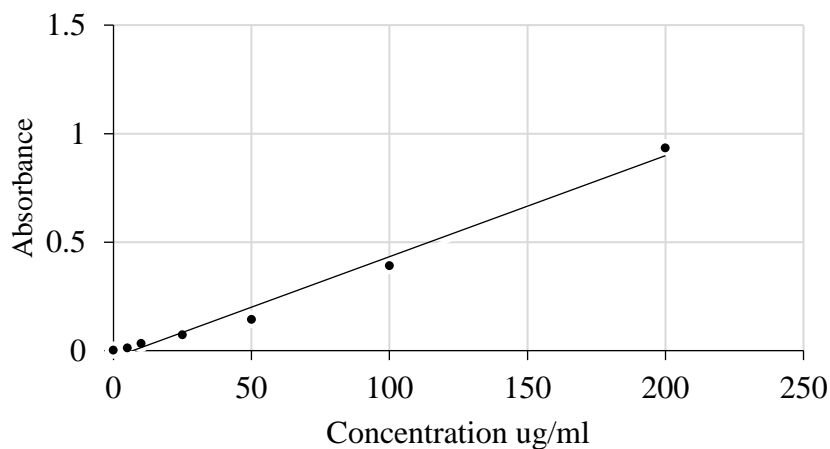


Figure 3.1. Standard curve of Gallic Acid used in the quantification of total phenolic content

3.2.5. Quantification of silicon content of *P. australis* lineages

The objective of this experiment was to quantify the amount of silicon present in the *P. australis* populations. Eight Delta, 22 Gulf, 24 European and 5 Native, a total of fifty-nine populations were analyzed by the Oven-induced Digestion procedure (Kraska and Breitenbeck 2010) followed by Molybdenum Blue calorimetric procedure (Hallmark *et al.* 1982) (Appendix A, Table A.4). Samples were collected from the populations of the common garden by cutting leaves and stems of *P. australis* population pot. Each sample was put into a lunch paper bag (17 × 8.1 × 5.8 cm), and placed into a plant drying oven for one week. Later on, dry plant tissue was grounded in a plant mill model Wiley mill No. 3 (Arthur H. Thomas CO, Philadelphia, PA). 5 mg ground plant tissue were weight XS 105 Dualrange (Mettler-Toledo, Columbus, OH) analytical balance, and placed it inside of 50 ml polyethylene centrifuge tubes for further analysis.

Oven-Induced digestion reagents preparation

Ammonium fluoride (NH_4F) was prepared by mixing 0.23 g of NH_4F and 100 ml of distilled water volumetric flask. Then, distilled water was added to make up a volume of 1000 L. In another volumetric flask, 500g of hydrogen peroxide (H_2O_2), was prepared in 400 ml of distilled water.

Oven-Induced digestion

Five drops of octyl alcohol and 2 ml of H_2O_2 was added to the 50 ml polyethylene centrifuge tubes of each sample before placing in the oven at 95°C for 30 min. Then, 4 ml of 50% sodium hydroxide (NaOH) was added to each sample, and each centrifuge tubes were loosely capped, and place back in the oven. Samples were taken out of the oven and gently mixed every 15 min for 4 hours. After 4 hours, 1 ml of NH_4F was added to the samples, and mixed. Later on, each sample was filled with distilled water for diluting the digested solution to 50 ml.

Molybdenum Blue calorimetric reagents preparation

Ammonium molybdate [$(\text{NH}_4)_6\text{Mo}_7\text{O}_{21}$] were prepared by dissolving 321.32 g of $(\text{NH}_4)_6\text{Mo}_7\text{O}_{21}$ and 800 ml of distilled water, and blending the solution in a magnetic stirrer vortex until the mixture was dissolved. Then, distilled water was added to make up a 1000 L volume. 200 ml of concentrate acetic acid was mixed with 600 ml of distilled water, and the solution was blended before adding 400 ml of distilled water to make up 1000 L. volume. 200 ml of concentrate tartaric acid was prepared with 600 ml of distilled water, and the solution were mixed before adding 400 ml of distilled water to make up 1000 L. volume. Lastly, ANSA reagent were prepared by mixing 0.5 g of ANSA, 1.0 g of sodium sulfite and 30 g sodium

bisulfite in 100 ml of distilled water before adding 150 ml of distilled water to make p 250 L. volume.

Molybdenum Blue calorimetric procedure

Two ml of plant digested solution was obtained and placed into 30 ml centrifuge tube. 10 ml of 20% acetic acid and 2 ml of 0.26 M $[(\text{NH}_4)_6\text{Mo}_7\text{O}_{24}]$ were added to the samples, and samples were allowed to sit for 5 min. Then, 2 ml of 20% tartaric acid were added and allowed to sit for 2 min before adding 2 ml of ANSA (reducing agent composed by 0.5 mg of 1-amino-2-naphthol-4-sulphoric acid, 1.0 g of sodium sulfite and 30.0 g of sodium bisulfite). Later on, all samples were diluted with 20% of acetic acid until a final volume of 30 ml was obtained. The absorbance reading was quantified at 630 nm by using DR5000 UV-visible spectrophotometer (Hach®, Loveland, CO). Different concentrations of standard series at rates of 0, 0.4, 0.8, 1.6, 3.2, 4.8, and 6.4 $\mu\text{g ml}^{-1}$ of silicon, as well as references, and blank samples were tested. Silicon content of the samples was determined by replacing the absorbance into this formula:

$$\text{SI Content} = \frac{(\text{Abs}_{\text{sample}} - \text{Abs}_{\text{blk}}) - \text{Cfi}}{\text{Cfs}} \times \frac{Vd \times CV}{Swt \quad VA}$$

Where:

$\text{Abs}_{\text{sample}}$ = absorbance reading of sample

Abs_{blk} = absorbance reading of reagent blank

Cfi = $\mu\text{g Si g}^{-1}$ when absorbance is zero (derived from the standard curve or intercept)

Cfs = $\mu\text{g Si g}^{-1}$ per unit of absorbance (derived from the standard curve)

Vd = final digest volume (mL)

Swt = oven-dry equivalent weight of digested sample (g)

Vc = final colorimetric volume (mL)

Va = volume of aliquot used for colorimetric analysis (mL)

3.2.6. Data analysis

Means of live *N. biwakoensis* per m of stem on *P. australis* lineages was Poisson distributed. The means were transform to a normal distribution by using the natural logarithm function LOG in SAS (SAS Institute 2009). Therefore, for the comparison of *N. biwakoensis* growth rate on different *P. australis* genotypes, we used mixed model analysis of variance (PROC MIXED, SAS Institute 2009). Year variable was not an independent variable because plant genotypes in experiment in 2018 and 2019 were the same plant material. Thus, year was treated as a random effect and the population source were arranged per lineages. Effects were considered significant when p-values were less than 0.05. Means with significant effects were compared and arranged in groups to determine the difference between them by using Tukey's post-hoc test. Data from silicon and phenolic content of each lineages were transform to a normal distribution by using the natural logarithm function LOG in SAS (SAS Institute 2009). Means of both variables were analyzed by using mixed model analysis of variance (PROC MIXED, SAS Institute 2009). The population source were arranged per lineages. Lineages means were compared using the Tukey test for any significant effect detected at $P < 0.05$.

3.4. Results

3.4.1. Comparison of *N. biwakoensis* growth rate on different *P. australis* lineages

There was a significant difference on the total number of live *N. biwakoensis* among genotypes ($F_{3, 115} = 5.57$; $P = 0.0013$). Densities of *N. biwakoensis* were lower on European,

Gulf, and Native genotypes compared with Delta genotype (Figure 3.2). European, Gulf and Native genotypes (57.58 ± 6.94 , mean \pm SE) had 1.45-times less live scales compared with Delta genotype (111.23 ± 12.53 , mean \pm SE).

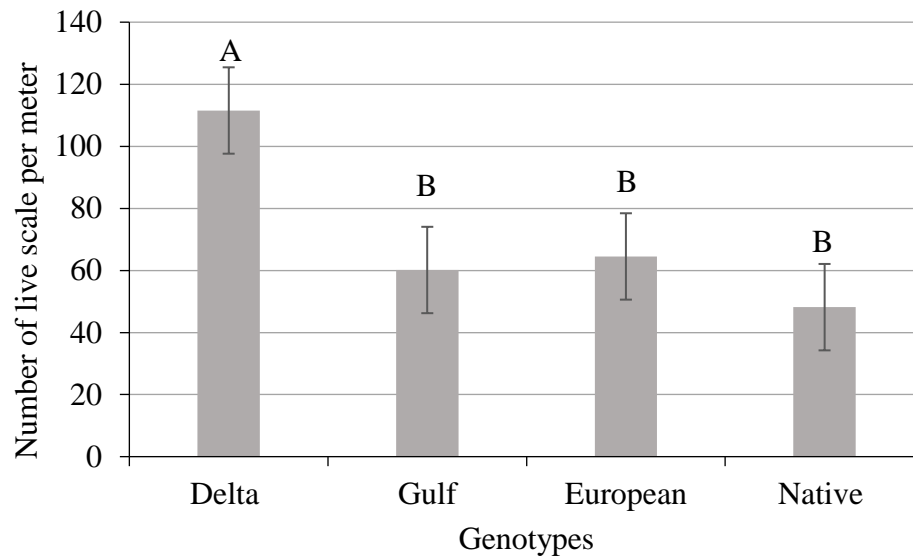


Figure 3.2. Means of total live *N. biwakoensis* among *P. australis* lineages on summer 2018 and 2019 ($F_{3,115} = 5.57$; $P = 0.0013$). Columns with different letter are significantly different ($P < 0.05$)

3.4.2. Quantification of total phenolic content of *P. australis* lineages

There was a significant difference on the total phenolic content among *P. australis* lineages ($F_{3,63} = 15.21$; $P = 0.001$, Figure 3.3). The total phenolic content of the European (106.35 ± 2.27 , Nmol/g) had 1.39-times more than Delta (93.43 ± 4.40 , Nmol/g) lineages.

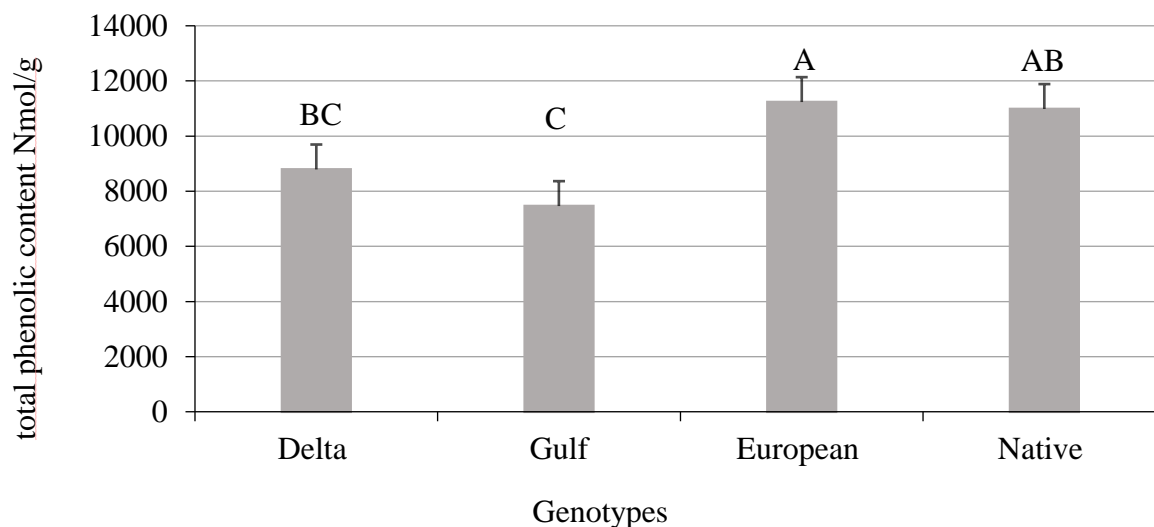


Figure 3.3. Means of the total phenolic content (Nmol/g) of *P. australis* lineages ($F_{3, 63} = 15.21$; $P = < 0.001$. Columns with different letter are significantly different ($P < 0.05$).

3.4.3. Quantification of silicon content of *P. australis* lineages

There was a significant difference on the amount of silicon content *P. australis* lineages ($F_{3, 54} = 7.90$; $P = 0.0002$, Figure 3.4). The amount of silicon content in *P. australis* lineages was greater in the European ($164.58 \pm 4.26 \mu\text{g/g}$) and Native ($157.16 \pm 9.32 \mu\text{g/g}$) compared with Gulf ($140.95 \pm 4.45 \mu\text{g/g}$) and Delta ($137.95 \pm 7.37 \mu\text{g/g}$) genotype which contained lower quantities of silicon content. In addition, the European genotype had 1.19-times more silicon content compared with Delta.

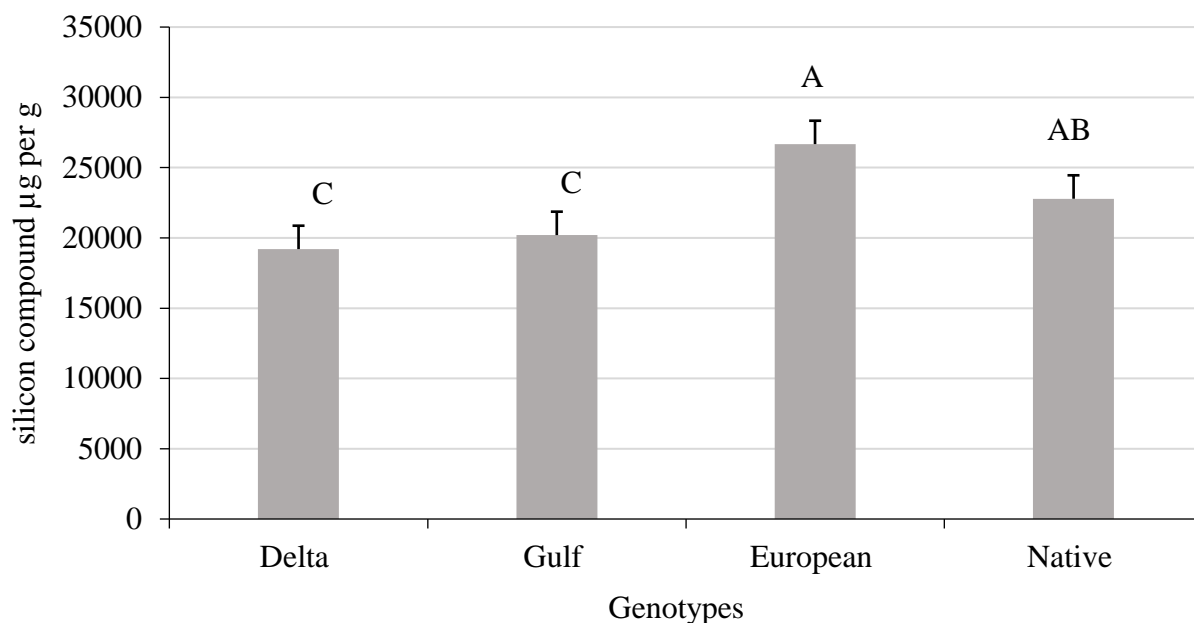


Figure 3.4. Mean of silicon content ($\mu\text{g/g}$) among *P. australis* lineages ($F_{3, 54} = 7.90$; $P = 0.0002$). Columns with different letter are significantly different ($P < 0.05$).

3.4. Discussion

Results from the population growth of *N. biwakoensis* among *P. australis* lineages during summer 2018 and 2019 revealed that Delta lineages had 1.45-times more scale insects compared with European, Native, and Gulf. These outcomes are in agreement with field data at the MRD, where the Delta lineages has 2- to 7-times greater number live scale compared to European lineages. Consequently, Delta was considered more susceptible to *N. biwakoensis* (Knight *et al.* 2020). Previous studies of European lineages had demonstrated fewer occurrences of herbivores insects such as stem-feeders, leaf-miners, leaf-chewers and aphids (Cronin *et al.* 2015; Bhattarai *et al.* 2017). Therefore, the European lineages could have defense traits against *N. biwakoensis* at the MRD.

Morphology and chemical defenses traits of plants could affect the incidence of phytophagous insects (Floyd *et al.* 1994). According to Morgan 1984, the different levels of total phenolic content may increase or decrease the number of phytophagous insects (Morgan 1984). In other plant systems, several studies had demonstrated that phenolic content could differ between genotypes of the same species. Similarly, six genotypes of *Gossypium hirsutum* (L.) were reported with high levels of phenolic content suppressing the infestation of *Tetranychus urticae* (Kock) (Lege *et al.* 1995). In this study, the concentration of total phenolics (ug/ ml) varied among *P. australis* genotypes. European and Native genotypes had higher levels of total phenolics content compared with Gulf and Delta genotypes. Although, previous studies demonstrated that the European lineages of *P. australis* contained 34% higher total phenolic content than Native lineages (Cronin *et al.* 2016; Bhattarai *et al.* 2017). To my knowledge, this study is the first to compare the total phenolic content of Gulf and Delta lineages.

Poaceae plants have a significant silicon concentration in their plant tissue, and it is considered important in the plant defenses against insects (Reynolds *et al.* 2016). Several studies demonstrated that some genotypes are better at accumulating silicon content than others (Majumder *et al.* 1985). According to Deren 2001, one genotype of *Oryza sativa* (L.) contained more silicon content than three other lineages. Besides, forty-two out of fifty-two lineages of *Saccharum officinarum* (L.) had large silicon content (Deren 2001). In this study, European and Native lineages had higher silicon content compared with Gulf and Delta lineages. Consequently, this study suggests that the high silicon content of European lineages might confer defenses traits against *N. biwakoensis* compared with Delta lineages.

Several mechanisms have been described in which silicon and phenolics enhanced pest resistance. According to Dykes *et al.* 2005, total phenolic content on *Sorghum bicolor* (L.) varies

among 13 genotypes due to the level of tannin (Dykes et al. 2005). Similarly, one study reveals that silicon content can differ vastly between plants, and genotypes of the same species by increasing or decreasing amount of lignin, phenolic content, and chitinases (Deren 1992; Porter and Lawlor 199; Epstein 1999). Although it is unknown the mechanism in which *P. australis* used these chemical defenses traits. Future research using metabolomics could revealed greater variation on the primary and secondary chemistry of *P. australis* genotypes.

In conclusion, the European genotype of *P. australis* may confer more defense traits against *N. biwakoensis* than Delta genotype. These variations in the chemical defenses traits could explain in part the difference in scale densities between these genotypes. European genotype could be considered to be part of the restoration project at Mississippi River Delta because it the most resistant genotype at the MRD. In addition, European genotype could recover areas that have been affected by *N. biwakoensis*, and prevent negative ecological consequences at the Mississippi River Delta

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Chapter 4. Conclusions and recommendations

Common reed, *P. australis*, is the most dominant plant in the Mississippi River Delta (MRD), Louisiana. In the fall of 2016, common reed stand die-back was reported at the MRD, and was associated with an outbreak of an Asian invasive scale [*Nipponaclerda biwakoensis* (Kuwana), Hemiptera: Aclerdidae]. In Asia, *N. biwakoensis* was studied only to understand and obtain data about its parasitoids (Kaneko 2004; Kaneko 2005). When *N. biwakoensis* was detected at MRD in Louisiana, there was a limited knowledge of its biology and ecology. Particularly, scientists and stakeholders wanted to understand if *N. biwakoensis* could affect other crops and marsh grasses of the same family of *P. australis*, and its impacts among *P. australis* lineages. In my research, I have demonstrated that the scale is effectively host specific to *P. australis* in Louisiana; and showed that the European lineages might have defenses traits against *N. biwakoensis*.

At the beginning of my research, the non-choice experiment showed that nymphs of *N. biwakoensis* could establish on *Schoenoplectus californicus*, *Spartina alterniflora*, *Sorghum bicolor*, and the control, *P. australis*. However, the greenhouse experiment revealed that the nymphs of *N. biwakoensis* could develop until its mature stage only on *Phragmites australis*, validating the information that *N. biwakoensis* do not develop on other plants. My study concluded that *N. biwakoensis* is a specialist insect on *P. australis* and is unlikely to threaten other grasses with agronomic or environmental importance in Louisiana.

There are five lineages of *P. australis* present in Unites States: European, Delta, Gulf, Greeny, and Native. European and Delta are the only two lineages at the MRD, and Delta lineages is the dominant vegetation. A previous study evaluated the density of herbivores between European and Native; they found that European lineages had a fewer amount of

herbivores compared with Native lineages (Bhattarai *et al.* 2017). My study was the first research that compared *N. biwakoensis* density among all the lineages of *P. australis* except Greeny because of its limited distribution. The population growth of the scale among *P. australis* lineages varied among lineages, and the European lineages contained lower scale density than the Delta lineages. Besides, my study shows that phenolics and silicon content varied among lineages. European and Native lineages contained a large amount of phenolics and silicon content compared with Gulf and Delta. When comparing the lineages present at the MRD, European lineages had a higher level of total phenolic and silicon content than those of the Delta lineages. Several studies have demonstrated that some plants suppress phytophagous insects by inducing defenses traits like phenolics and silicon content (Floyd *et al.* 1994; Reynolds *et al.* 2016). Furthermore, some studies demonstrated that phenolics and silicon could differ between lineages of the same species (Morgan 1984; Majumder *et al.* 1985; Deren 1992; Epstein 1999; Porter and Lawlor 1999). These studies are in agreement with our results. Therefore, this study concludes that European lineages may confer resistance traits against *N. biwakoensis*. This information will help when selecting a *P. australis* lineages during the implementation of the restoration projects in the MRD.

Future studies should examine the parasitism levels among *P. australis* lineages and their role in the difference in densities of *N. biwakoensis* among lineages. In addition, it is essential to determine the specific type and the amount of phenolic content present among *P. australis* lineages. In the past, several studies have demonstrated that poaceae grasses had different amount and types of phenolics content between lineages. Three genotypes of *Gossypium hirsutum* (L.) present more amount of syringic acid and p-coumaric acid, types of phenols, compared with other three lineages (Lege *et al.* 1995). Similarly, six lineages of *Triticum*

aestivum (L.) have different type of phenolics compound such as 2'-hydroxychalcone, 2,2'-dihydroxychalcone, 3,4-dihydroxychalcone compared with 3',4',7'-trihydroxyflavone and fustin-3-O-glucoside found in others 12 lineages (Kharazian and Rahiminejad 2009).

Furthermore, it is also important to assess the mechanism in which silicon content enhances resistance against *N. biwakoensis*. Several studies demonstrated that silicon increases resistance against insect by silicon deposition resulting in plant tissue hardness (Reynolds *et al.* 2009; Liang *et al.* 2015), increasing defense enzymes, such as polyphenol oxidase, peroxidase, phenylalanine ammonia-lyase in leaves, increasing the production of defensive chemicals, such as tannins, lignin and phenols (Reynolds *et al.* 2009; Liang *et al.* 2015), and attracting natural enemies (Kvedaras *et al.* 2010).

Further studies on the physical barriers like cuticular waxes among genotypes should be evaluated. Several studies showed that physical barriers like waxes deter insect in Poaceae grasses. Higher concentration of triterpenols in leaf tissues of *Sorghum bicolor* (L.) enhanced resistance against *Rhopalosiphum maidis* (Fitch) (Heupel 1985). Higher concentration of waxes on *Triticum aestivum* (L.) reduced the feeding of *Chorthippus parallelus* (Zetterstedt) (Bernays and Chapman 1975). Resistant lineages of *Oryza sativa* (L.) against *Nilaparvata lugens* (Stål) was attributed to large concentration of waxes (Woodhead and Padgham 1988). In addition, one study revealed different wax structure in the leaf surface of three different ecotypes of *P. australis* (Liu *et al.* 2012); consequently, there is a probability that waxes in the leaf surface could differ among *P. australis* lineages. Furthermore, it is essential to evaluate *P. australis* genotypes' metabolomics since different metabolites were detected in Poaceae grasses. 114 different metabolites were found on *Saccharum officinarum* (L.), in which 56 were identified including 19 phenolics and 25 flavones, and 12 isomeric flavone C-glycosides (Coutinho *et al.*

2016). Similarly, different metabolites were found between the lineages of *Oryza sativa* and *Triticum aestivum* (Mochida *et al.* 2008). Therefore, metabolomics analysis will help to a better understanding of the whole mechanism in which *P. australis* used its defense traits against *N. biwakoensis*.

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Appendix A. Supplemental Tables

Table A.1. List of the crops and marsh grasses including in the inoculation of preliminary experiment of summer of 2017

Plant species	Total live scales
<i>Zea Mays</i>	0 ± 0
<i>Schoenoplectus californicus</i>	0 ± 0
<i>Phragmites australis</i>	3.0 ± 2.9
<i>Oryza sativa</i>	0 ± 0
<i>Saccharum officinarum</i>	0 ± 0
<i>Sorghum bicolor</i>	2.1 ± 4.2
<i>Spartina alterniflora</i>	0 ± 0

Table A.2. List of the origin and lineages of *Phragmites australis* population assessed in the *Nipponaclerda biwakoensis* population growth rate experiment on summer 2018 and 2019

year	Lineages	Population	Site name	State	Latitude	Longitude
2018	Delta	BUR	Buras	Louisiana	29.37	-89.60
2018	Delta	CJM	Cameron Jetty	Louisiana	29.77	-93.34
2018	Delta	CRM	Creole 2, Cameron	Louisiana	29.88	-93.07
2018	Delta	DOG	Targa refiner	Louisiana	29.23	-89.38
2018	Delta	EAR1	Dredge island	Louisiana	29.19	-89.29
2018	Delta	ECM	East Cameron	Louisiana	29.77	-93.29
2018	Delta	PLM	Pass-A-Loutre	Louisiana	42.53	-87.95
2018	Delta	RRM	Rockefeller Road, Cameron Bonnet Carre	Louisiana	29.68	-92.81
2018	Gulf	BCI	Spillway	Louisiana	30.05	-90.37
2018	Gulf	DEL	Delacroix	Louisiana	29.79	-89.76
2018	Gulf	FBI	Fontainebleau State Park	Louisiana	30.33	-90.04
2018	Gulf	GL1	Grand Isle 1	Louisiana	29.23	-90.00
2018	Gulf	HOP	Hopedale	Louisiana	29.81	-89.61
2018	Gulf	ICI	Intracoastal city	Louisiana	29.78	-92.20
2018	Gulf	MOB1	Mobile Bay	Alabama	30.66	-87.92
2018	Gulf	OBI	Okeehlee Boat Ramp	Florida	26.65	-80.16
2018	Gulf	PCB	Panama City Beach	Florida	30.26	-85.97
2018	Gulf	PIT	Ironton	Louisiana	29.64	-89.96
2018	Gulf	PONT7	Pontchartrain 7	Louisiana	30.33	-90.27

(table cont'd.)

year	Lineages	Population	Site name	State	Latitude	Longitude
2018	Gulf	PR1	Pearl River site 1 Rockefeller Boat	Louisiana	30.23	-89.61
2018	Gulf	RBI	Launch	Louisiana	30.18	-93.26
2018	Gulf	SHR	Shreveport	Louisiana	32.53	-93.82
2018	Gulf	FLI	John Prince Park	Florida	26.65	-80.16
2018	Gulf	GMI	Golden Meadow	Louisiana	29.46	-90.23
2018	Gulf	PCI	Pointe-Aux-Chenes	Louisiana	29.45	-90.46
2018	Gulf	WS7	Salt Creek Greeson Wash	California	33.44	-115.84
2018	Gulf	WS4	Patch	California	32.67	-115.61
2018	Gulf	CRI	Creole 1, Cameron Anzalduas County	Louisiana	29.83	-93.11
2018	Gulf	ANZ1	Park	Texas	26.14	-98.32
2018	Gulf	SAU	Bayou Sauvage	Louisiana	30.06	-89.82
2018	European	140M	I-40	Arizona	34.71	-114.48
2018	European	APM	Appoquinimink	Delaware	39.45	-75.64
2018	European	ARMI-1	Little Rock	Arkansas	34.69	-92.29
2018	European	EAR2	water's edge	Louisiana	29.17	-89.28
2018	European	ESM	East Sandwich	Massachusetts	41.74	-70.43
2018	European	FPM	Falmouth	Massachusetts	41.55	-70.6
2018	European	LNM	Lincoln 1	Illinois	40.22	-89.27
2018	European	NYM	Montezuma	New York	43	-76.7
2018	European	RIM	Galilee	Rhode Island	41.37	-71.50
2018	European	SALM	Salinas River	California	35.49	-120.65
2018	European	SCM	Georgetown Sherman Marsh	South Carolina	33.35	-79.26
2018	European	SMM	Exotic	Maine	44.02	-69.58
2018	European	WS2	Jim Andre Patch	California	32.21	-117.98
2018	European	BSCM	Bath	Maine	44.51	-70.35
2018	European	TCM	Choptank	Maryland	38.77	-75.95
2018	European	CTM	Ragged Rock Agua Caliente	Connecticut	41.30	-72.35
2018	European	WS5	Patch	California	32.94	-116.30
2018	European	NJM	Estell Manor	New Jersey	39.41	-74.73
2018	European	WHM	Winthrop Harbor 1	Wisconsin	42.47	-87.84
2018	European	OGM	Oglesby 1	Illinois	41.29	-89.08
2018	European	ZWM	Zion 1	Wisconsin	42.49	-87.90

(Table cont'd.)

year	Lineages	Population	Site name	State	Latitude	Longitude
2018	European	WS9	Salinas River	California	35.49	-120.65
2018	European	LITM	Litchfield 1	Illinois	39.14	-89.66
2018	European	TELM	Telegraph 1	Missouri	38.42	-90.34
2018	European	GCM	Granite City 1	Illinois	38.66	-90.09
2018	European	PPM	Pleasant Prairie 1	Minnesota	42.53	-87.95
2018	Native	FOXN	Fox River Wetland	Wisconsin	42.92	-88.28
2018	Native	FPN	Falmouth	Massachusetts	41.55	-70.60
2018	Native	GRN1	Pass-A-Loutre	Louisiana	29.21	-89.30
2018	Native	ITAM	Near Lake Itasca	Minnesota	47.46	-93.50
2018	Native	NCN	Mackay Island	North Carolina	36.51	-75.95
2018	Native	PCN	Palm Canyon Road	California	33.82	-116.31
2018	Native	PORN	Port Orford	Oregon	42.75	-124.50
2018	Native	ROD1	South of Venice	Louisiana	29.12	-89.28
2018	Native	SCRN	Santa Clara River	California	34.35	-119.00
			Tappahannock			
2018	Native	VAN	Native	Virginia	37.91	-76.85
2018	Native	SCN	Squaw Creek	Missouri	40.06	-95.24
2018	Native	USGN	Utah St. George	Utah	37.09	-113.56
			Little Caliente Hot			
2018	Native	LCN	Springs	California	34.54	-119.62
2019	Delta	BUR	Buras	Louisiana	29.37	-89.60
2019	Delta	DOG	Targa refiner	Louisiana	29.23	-89.38
			Bonnet Carre			
2019	Gulf	BCI	Spillway	Louisiana	30.05	-90.37
2019	Gulf	CRI	Creole 1, Cameron	Louisiana	29.83	-93.11
2019	Gulf	DEL	Delacroix	Louisiana	29.79	-89.76
			Fontainebleau State			
2019	Gulf	FBI	Park	Louisiana	30.33	-90.04
2019	Gulf	FLI	John Prince Park	Florida	26.65	-80.16
2019	Gulf	GII	Grand Isle 1	Louisiana	29.23	-90.00
2019	Gulf	GMI	Golden Meadow	Louisiana	29.46	-90.23
2019	Gulf	HOP	Hopedale	Louisiana	29.81	-89.61
2019	Gulf	MOB1	Mobile Bay	Alabama	30.66	-87.92
2019	Gulf	PCB	Panama City Beach	Florida	30.26	-85.97
2019	Gulf	PCI	Pointe-Aux-Chenes	Louisiana	29.45	-90.46
2019	Gulf	PIT	Ironton	Louisiana	29.64	-89.96

(Table cont'd.)

year	Lineages	Population	Site name	State	Latitude	Longitude
2019	Gulf	Pont 7	Pontchartrain 7	Louisiana	30.33	-90.27
2019	Gulf	PR1	Pearl River site 1	Louisiana	30.23	-89.61
			Rockefeller Boat			
2019	Gulf	RBI	Launch Bayou S	Louisiana	30.18	-93.26
2019	Gulf	SAU	Greeson Wash	Louisiana	30.06	-89.82
2019	Gulf	WS4	Patch	California	32.67	-115.61
2019	Gulf	WS7	Salt Creek	California	33.44	-115.84
			Anzalduas County			
2019	Gulf	ANZ1	Park	Texas	26.14	-98.32
2019	European	140M	I-40	Arizona	34.71	-114.48
2019	European	APM	Appoquinimink	Delaware	39.45	-75.64
2019	European	ARMI	Little Rock	Arkansas	34.69	-92.29
2019	European	BSCM	Bath	Maine	44.51	-70.35
2019	European	CTM	Ragged Rock	Connecticut	41.30	-72.35
2019	European	ESM	East Sandwich	Massachusetts	41.74	-70.43
2019	European	FPM	Falmouth	Massachusetts	41.55	-70.60
2019	European	GCM	Granite City 1	Illinois	38.66	-90.09
2019	European	LITM	Litchfield 2	Illinois	39.14	-89.66
2019	European	LNM	Lincoln 1	Illinois	40.22	-89.27
2019	European	NJM	Estell Manor	New Jersey	39.41	-74.73
2019	European	NYM	Montezuma	New York	43.00	-76.70
2019	European	OGM	Oglesby 1	Illinois	41.29	-89.08
2019	European	PPM	Pleasant Prairie 1	Minnesota	42.53	-87.95
2019	European	RIM	Galilee	Rhode Island	41.37	-71.50
2019	European	SALM	Salinas River	California	35.49	-120.65
			Sherman Marsh			
2019	European	SMM	Exotic	Maine	44.02	-69.58
2019	European	TCM	Choptank	Maryland	38.77	-75.95
2019	European	TELM	Telegraph 1	Missouri	38.42	-90.34
2019	European	WHM	Winthrop Harbor 1	Wisconsin	42.47	-87.84
2019	European	WS2	Jim Andre Patch	California	32.213	-117.98
			Agua Caliente			
2019	European	WS5	Patch	California	32.94	-116.30
2019	European	WS9	Salinas River	California	35.49	-120.65
2019	European	ZWM	Zion 1	Wisconsin	42.49	-87.90

(Table cont'd)

year	Lineages	Population	Site name	State	Latitude	Longitude
2019	Native	FLIN	Forney Lake	Iowa	40.85	-95.77
2019	Native	FPN	Falmouth	Massachusetts	41.55	-70.60
2019	Native	LCN	Little Caliente Hot Springs	California	34.54	-119.62
2019	Native	NCN	Mackay Island	North Carolina	36.51	-75.95
2019	Native	SCN	Squaw Creek	Missouri	40.06	-95.24
2019	Native	USGN-1	Utah St. George	Utah	37.09	-113.56

Table A.3. List of the origin and lineages of *Phragmites australis* populations tested in the phenolic content quantification experiment on summer 2018

Population	Lineages	Site name	State	Latitude	Longitude
BUR	Delta	Buras	Louisiana	29.37	-89.60
CJM	Delta	Cameron Jetty	Louisiana	29.77	-93.34
CRM	Delta	Creole 2, Cameron	Louisiana	29.88	-93.07
DOG	Delta	Targa refiner	Louisiana	29.23	-89.38
EARI	Delta	Dredge island	Louisiana	29.19	-89.29
ECM	Delta	East Cameron	Louisiana	29.77	-93.29
PLM	Delta	Pass-A-Loutre	Louisiana	42.53	-87.95
RRM	Delta	Rockefeller Road, Cameron	Louisiana	29.68	-92.81
ANZ1-1	Gulf	Anzalduas County Park	Texas	26.14	-98.32
BCI	Gulf	Bonnet Carre Spillway	Louisiana	30.05	-90.37
CRI	Gulf	Creole 1, Cameron	Louisiana	29.83	-93.11
DEL	Gulf	Delacroix	Louisiana	29.79	-89.76
FBI	Gulf	Fontainbleau State Park	Louisiana	30.33	-90.04
FLI	Gulf	John Prince Park	Florida	26.65	-80.16
GL1	Gulf	Grand Isle 1	Louisiana	29.23	-90.00
GMI	Gulf	Golden Meadow	Louisiana	29.46	-90.23
HOP	Gulf	Hopedale	Louisiana	29.81	-89.61
ICI	Gulf	Intracoastal city	Louisiana	29.78	-92.20
MOB1	Gulf	Mobile Bay	Alabama	30.66	-87.92
OBI	Gulf	Okeeheelee Boat Ramp	Florida	26.65	-80.16
PCB	Gulf	Panama City Beach	Florida	30.26	-85.97
PCI	Gulf	Pointe-Aux-Chenes	Louisiana	29.45	-90.46
PIT	Gulf	Ironton	Louisiana	29.64	-89.96
PONT7	Gulf	Pontchartrain 7	Louisiana	30.33	-90.27
PR1	Gulf	Pearl River site 1	Louisiana	30.23	-89.61
RBI	Gulf	Rockefeller Boat Launch	Louisiana	30.18	-93.26
SAU	Gulf	Bayou Sauvage	Louisiana	30.06	-89.82
SHR	Gulf	Rockefeller Boat Launch	Louisiana	32.53	-93.82
WS4	Gulf	Salt Creek	California	32.67	-115.61
WS7	Gulf	Salt Creek	California	33.44	-115.84
140M	European	I-40	Arizona	34.71	-114.48
APM	European	Appoquinimink	Delaware	39.45	-75.64
ARMI	European	Little Rock	Arkansas	34.69	-92.29
BSCM	European	Bath	Maine	44.51	-70.35
CTM	European	Ragged Rock	Connecticut	41.30	-72.35
EAR2	European	water's edge	Louisiana	29.17	-89.28
ESM	European	East Sandwich	Massachusetts	41.74	-70.43

(Table cont'd)

Population	Lineages	Site name	State	Latitude	Longitude
FPM	European	Falmouth	Massachusetts	41.55	-70.60
GCM	European	Granite City 1	Illinois	38.66	-90.09
LITM	European	Litchfield 1	Illinois	39.14	-89.66
NJM	European	Estell Manor	New Jersey	39.41	-74.73
NYM	European	Montezuma	New York	43.00	-76.70
OGM	European	Oglesby 1	Illinois	41.29	-89.08
PPM	European	Pleasant Prairie 1	Minnesota	42.53	-87.95
RIM	European	Galilee	Rhode Island	41.37	-71.50
SALM	European	Salinas River	California	35.49	-120.65
SCM	European	Georgetown	South Carolina	33.35	-79.26
SMM	European	Sherman Marsh Exotic	Maine	44.02	-69.58
TCM	European	Choptank	Maryland	38.77	-75.95
TELM	European	Telegraph 1	Missouri	38.42	-90.34
WHM	European	Winthrop Harbor 1	Wisconsin	42.47	-87.84
WS2	European	Jim Andre Patch	California	32.21	-117.98
WS5	European	Agua Caliente Patch	California	32.94	-116.30
WS9	European	Salinas River	California	35.49	-120.65
ZWM	European	Zion 1	Wisconsin	42.49	-87.90
FOXM	Native	Fox River Wetland	Wisconsin	42.92	-88.28
FPN	Native	Falmouth	Massachusetts	41.55	-70.60
GRN1	Native	Pass-A-Loutre	Louisiana	29.21	-89.30
ITAM	Native	Near Lake Itasca	Minnesota	47.46	-93.50
LCN	Native	Little Caliente Hot Springs	California	34.54	-119.62
NCN	Native	Mackay Island	North Carolina	36.51	-75.95
PCN	Native	Palm Canyon Road	California	33.82	-116.31
PORN	Native	Port Orford	Oregon	42.75	-124.50
SCRN	Native	Santa Clara River	California	34.35	-119.00
USGN-1	Native	Utah St. George	Utah	37.09	-113.56
VAN	Native	Tappahannock Native	Virginia	37.91	-76.85
SCN	Native	Squaw Creek	Missouri	40.06	-95.24

Table A.4. List of *Phragmites australis* lineages tested in the silicon content quantification experiment on summer 2018

Population	Lineages	Site name	State	Latitude	Longitude
BUR	Delta	Buras	Louisiana	29.37	-89.60
CJM	Delta	Cameron Jetty	Louisiana	29.77	-93.34
CRM	Delta	Creole 2, Cameron	Louisiana	29.88	-93.07
DOG	Delta	Targa refiner	Louisiana	29.23	-89.38
EAR1	Delta	Dredge island	Louisiana	29.19	-89.29
ECM	Delta	East Cameron	Louisiana	29.77	-93.29
PLM	Delta	Pass-A-Loutre	Louisiana	42.53	-87.95
RRM	Delta	Rockefeller Road, Cameron	Louisiana	29.68	-92.81
ANZ1	Gulf	Anzalduas County Park	Texas	26.14	-98.32
BCI	Gulf	Bonnet Carre Spillway	Louisiana	30.05	-90.37
CRI	Gulf	Creole 1, Cameron	Louisiana	29.83	-93.11
DEL	Gulf	Delacroix	Louisiana	29.79	-89.76
FBI	Gulf	Fontainbleau State Park	Louisiana	30.33	-90.04
FL1-1	Gulf	John Prince Park	Florida	26.65	-80.16
GL1	Gulf	Grand Isle 1	Louisiana	29.23	-90.00
GMI-1	Gulf	Golden Meadow	Louisiana	29.46	-90.23
HOP	Gulf	Hopedale	Louisiana	29.81	-89.61
ICI	Gulf	Intracoastal city	Louisiana	29.78	-92.20
MOB1	Gulf	Mobile Bay	Alabama	30.66	-87.92
OBI	Gulf	Okeeheelee Boat Ramp	Florida	26.65	-80.16
PCB	Gulf	Panama City Beach	Florida	30.26	-85.97
PCI	Gulf	Pointe-Aux-Chenes	Louisiana	29.45	-90.46
PIT	Gulf	Ironton	Louisiana	29.64	-89.96
PONT	Gulf	Pontchartrain 7	Louisiana	30.33	-90.27
PRI	Gulf	Pearl River site 1	Louisiana	30.23	-89.61
RBI	Gulf	Rockefeller Boat Launch	Louisiana	30.18	-93.26
SAU	Gulf	Bayou Sauvage	Louisiana	30.06	-89.82
SHR	Gulf	Rockefeller Boat Launch	Louisiana	32.53	-93.82
WS4	Gulf	Greeson Wash Patch	California	32.67	-115.61
WS7	Gulf	Salt Creek	California	33.44	-115.84
140M	European	I-40	Arizona	34.71	-114.48
ARM1	European	Little Rock	Arkansas	34.69	-92.29
BSCM	European	Bath	Maine	44.51	-70.35
ESM	European	East Sandwich	Massachusetts	41.74	-70.43
GCM	European	Granite City 1	Illinois	38.66	-90.09
LITM	European	Litchfield 1	Illinois	39.14	-89.66
NJM	European	Estell Manor	New Jersey	39.41	-74.73

(Table cont'd)

Population	Lineages	Site name	State	Latitude	Longitude
OGM	European	Oglesby 1	Illinois	41.29	-89.08
PPM	European	Pleasant Prairie 1	Minnesota	42.53	-87.95
SALM	European	Salinas River	California	35.49	-120.65
SCM	European	Georgetown	South Carolina	33.35	-79.26
SMM	European	Sherman Marsh Exotic	Maine	44.02	-69.58
TCM	European	Choptank	Maryland	38.77	-75.95
TELM	European	Telegraph 1	Missouri	38.42	-90.34
WHM	European	Winthrop Harbor 1	Wisconsin	42.47	-87.84
WS2	European	Jim Andre Patch	California	32.21	-117.98
WS5	European	Agua Caliente Patch	California	32.94	-116.30
WS9	European	Salinas River	California	35.49	-120.65
ZWM	European	Zion 1	Wisconsin	42.49	-87.90
APM	European	Appoquinimink	Delaware	39.45	-75.64
RIM	European	Galilee	Rhode Island	41.37	-71.50
EAR2	European	water's edge	Louisiana	29.17	-89.28
CTM	European	Ragged Rock	Connecticut	41.30	-72.35
WS9	European	Salinas River	California	35.49	-120.65
FOXM	Native	Fox River Wetland	Wisconsin	42.92	-88.28
FPN	Native	Falmouth	Massachusetts	41.55	-70.60
LCN	Native	Little Caliente Hot Springs	California	34.54	-119.62
UGSN	Native	Utah St. George	Utah	37.09	-113.56
VAN	Native	Tappahannock Native	Virginia	37.91	-76.85

Vita

Leslie Alejandra Aviles Lopez grew up in San Salvador, El Salvador. She got her bachelor's degree in Agronomy at Zamorano University, Honduras. She did an internship in biological control at the National Agricultural and Forestry Technology Center in 2014 in La Libertad, El Salvador. As her interests in entomology and biological control expanded, she decided to join the Department of Entomology at Louisiana State University to pursue her master's degree with Dr. Rodrigo Diaz. She plans to receive her masters on august 2020. Upon conclusion of her master's, Leslie Aviles will join the Department of Entomology at Purdue University to work on her doctorate with Dr. Laura Ingwell.