Response of Roseau Cane (Phragmites australis) to Two Biotic Stresses: Hyalopterus pruni and Bipolaris yamadae

Heather E. Cizek

Louisiana State University at Baton Rouge

Follow this and additional works at: https://digitalcommons.lsu.edu/gradschool_theses

Part of the Biodiversity Commons, Entomology Commons, and the Plant Pathology Commons

Recommended Citation
https://digitalcommons.lsu.edu/gradschool_theses/5462

This Thesis is brought to you for free and open access by the Graduate School at LSU Digital Commons. It has been accepted for inclusion in LSU Master's Theses by an authorized graduate school editor of LSU Digital Commons. For more information, please contact gradetd@lsu.edu.
RESPONSE OF ROSEAU CANE (PHRAGMITES AUSTRALIS) TO TWO BIOTIC STRESSES: HYALOPTERUS PRUNI AND BIPOLARIS YAMADAЕ

A Thesis

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

in

The Department of Plant Pathology and Crop Physiology

by.
Heather Eileen Cizek
B.S. Natural Resource Ecology and Management, Louisiana State University, May 2019
December 2021
ACKNOWLEDGEMENTS

I would like to thank my major advisor Dr. Rodrigo Valverde for being very helpful throughout my whole masters. He is very knowledgeable in plant virology and has taught me a lot of useful information.

I would also like to thank Drs. Michael Kaller and Sara Thomas-Sharma for being on my graduate committee. They have given me good advice and have helped me to succeed in my masters. I would also like to thank Dr. Kaller for all the help he gave me during my bachelor’s degree.

Thank to David Galo and César Escalante Guardado for being great lab mates and teaching me useful things that helped me with my master’s project and learning about virology in general. They are both really nice and intelligent people. I appreciate all the help that they have given me.

I would like to thank Drs. James Cronin and Rodrigo Diaz of the Biological Sciences and Entomology departments, Louisiana State University, respectively for providing materials and coordinating field trips for this investigation. Also, thanks to D. Galo and R. Valverde for providing some photographs of the experiments.
I would also like to thank the U.S. Department of Agriculture Animal and Plant Health Inspection Service (APHIS), and Agricultural Research Service (ARS) for providing a grant to fund this investigation.

I want to thank my wonderful pets for being there for me during my masters. That includes my guinea pigs (Zoey, Morgan and Elly), my fish (Sophia and Bubbles) and my dog (Allie).

Finally, I would like to thank my mother, Julia Cizek and father, Brian Cizek for supporting me throughout my masters.
# TABLE OF CONTENTS

ACKNOWLEDGEMENTS .................................................................................................................. II

ABSTRACT ...................................................................................................................................... VI

CHAPTER I. LITERATURE REVIEW .............................................................................................. 1
  1.1. ROSEAU CANE ..................................................................................................................... 1
  1.2. ROSEAU CANE DIE-OFF ..................................................................................................... 3
  1.3. MEALY PLUM APHIDS ....................................................................................................... 6
  1.4. BIPOLARIS LEAF SPOT ..................................................................................................... 8
  1.5. APHID-FUNGAL INTERACTIONS ....................................................................................... 9
  1.6. OBJECTIVES ...................................................................................................................... 11

CHAPTER II. COLLECTION, IDENTIFICATION AND ESTABLISHMENT OF A COLONY OF THE MEALY PLUM APHID (HYALOPTERUS PRUNI) ......................................................... 13
  2.1. INTRODUCTION ................................................................................................................ 13
  2.2. OBJECTIVES ................................................................................................................... 15
  2.3. MATERIALS AND METHODS .......................................................................................... 16
  2.4. RESULTS .......................................................................................................................... 22
  2.5. DISCUSSION ..................................................................................................................... 31

CHAPTER III. FEEDING EXPERIMENTS TO DETERMINE THE DAMAGE CAUSED TO ROSEAU CANE BY HYALOPTERUS PRUNI .......................................................................................... 33
  3.1. INTRODUCTION ................................................................................................................ 33
  3.2. OBJECTIVE ...................................................................................................................... 35
  3.3. MATERIALS AND METHODS .......................................................................................... 35
  3.4. RESULTS .......................................................................................................................... 40
  3.5. DISCUSSION ..................................................................................................................... 44

CHAPTER IV. INOCULATION EXPERIMENTS TO DETERMINE THE DAMAGE CAUSED TO ROSEAU CANE BY BIPOLARIS YAMADAE ................................................................................. 46
  4.1. INTRODUCTION ................................................................................................................ 46
  4.3. MATERIALS AND METHODS .......................................................................................... 49
  4.4. RESULTS .......................................................................................................................... 53
  4.5. DISCUSSION ..................................................................................................................... 56
CHAPTER V. DAMAGE CAUSED TO ROSEAU CANE BY THE SIMULTANEOUS INFESTATION AND INFECTION OF HYALOPTERUS PRUNI AND BIPOLARIS YAMADAE RESPECTIVELY ........................................................................................................58

5.1. INTRODUCTION ........................................................................................................58
5.2. OBJECTIVE ..............................................................................................................61
5.3. MATERIALS AND METHODS ..................................................................................62
5.4. RESULTS ..................................................................................................................66
5.5. DISCUSSION ............................................................................................................72

CONCLUSIONS ................................................................................................................74

APPENDIX A. PRELIMINARY RESEARCH ON GROWING ROSEAU CANE IN THE GREENHOUSE AND THE LABORATORY ................................................................76

APPENDIX B. SEQUENCES OF PCR PRODUCTS OBTAINED USING DNA FROM APHIDS AND TWO SETS OF PRIMERS ..................................................................................80

APPENDIX C. RESULTS OF PCR EXPERIMENTS USING BUSH16S1F AND BUSH16S1R PRIMERS ........................................................................................................83

LITERATURE CITED .......................................................................................................85

VITA .................................................................................................................................91
ABSTRACT

Roseau cane (*Phragmites australis*) is considered an invasive plant because of its ability to replace native plant species. However, in Louisiana it plays an important role protecting coastal infrastructure and being part of the marsh ecosystem in the lower Mississippi River Delta. In recent years, Roseau cane has been affected by a die-off, a problem that has also been reported in some European countries. Possible biotic and abiotic factors that have been associated with the die-off include scale insects, climate change, pollution, salinity levels, and pathogens.

In this research, the individual and combined effect of a foliar disease and an insect herbivore on Roseau cane was investigated on two different lineages of Roseau cane (Delta and European). A foliar disease caused by the fungus *Bipolaris yamadae* and the feeding damage caused by the mealy plum aphid (*Hyalopterus pruni*) were evaluated. A colony of aphids used in feeding experiments was established and the aphid’s species identified. Fungal inoculations using spore suspensions from cultures of *B. yamadae* and insect infestations were conducted.

At the end of the aphid feeding experiments, the number of mealy plum aphids was higher on the Delta lineage than the European lineage. Moreover, the Delta lineage had higher aphid damage ratings than the European lineage. The heights of plants with aphids were not statistically significantly different from the heights of plants without aphids. This was also true for the heights of the plants simultaneously infected and infested with *B. yamadae* and *H. pruni* respectively. In inoculation experiments with *B. yamadae* alone, the number of leaf lesions on the Delta lineage was statistically significantly higher than the number of leaf lesions on the European lineage. In contrast, in experiments when the mealy plum aphids were also included,
the number of leaf lesions caused by *B. yamadae* was not statistically different. Both, the mealy plum aphid, and *B. yamadae* were able to reproduce simultaneously on Roseau cane and caused foliar symptoms; however, their interactions appear to be limited.
CHAPTER I. LITERATURE REVIEW

1.1. Roseau Cane

Roseau cane, *Phragmites australis* (Cav.) Trin. ex Steud., also known as common reed, is a perennial grass found in temperate regions worldwide and is one of the most common and dominant species in wetlands (Gigante *et al.*, 2014). Roseau cane spreads by rhizomes and seeds (Ilbagi, 2006). It can survive in flooded areas, riverbanks, littoral zones of lakes, salt marshes, ditches, and bogs; however, it does best in nutrient rich sites, but can grow in oligotrophic to eutrophic conditions (Gigante *et al.*, 2014). When it is not wanted, it can be controlled by cutting, burning, covering with plastics, or applying herbicides (Ilbagi, 2006). Because Roseau cane can survive through harsh conditions and pollutants, in some areas, it has been used to treat agricultural and industrial wastewaters and remove harmful microorganisms (Gigante *et al.*, 2014). In Louisiana, Roseau cane decreases erosion and helps maintain and create marsh sediment (LSU AgCenter, 2019). Roseau cane has been used as food for animals, habitat for wildlife, material to make musical instruments, and baskets (Ilbagi, 2006). Roseau cane also protects marshes from waves and storms (Knight *et al.*, 2018). Therefore, Roseau cane stands are very important for the Louisiana coastal areas when affected by hurricanes and for fish and wildlife.

In the United States, multiple phylogeographical lineages of *P. australis* can be found. They include European, Gulf, Delta, and Greeny. The non-native Greeny and European lineages occur
throughout the United States. Plants of the Gulf coast lineage occur throughout the east coast, and those of the Delta lineage in the Mississippi River Delta (Warwick et al., 2020). The European lineage tend to be invasive and is found, together with the Delta and the Gulf coast lineage, in the Delta of the Mississippi River (LSU AgCenter, 2019). Plants of these four Roseau cane lineages (Delta, Gulf, European, and Greeny) are currently found in Louisiana. The most common lineage of Roseau cane in the lower Mississippi River Delta is the Delta lineage. The Delta lineage is known to be the tallest of the lineages reaching a height of up to five meters. The shoots and ligules of this lineage have hairs and gold to brown flowers. Patches of the European and Greeny lineages can be found in the Mississippi River Delta. Both lineages are known to grow to a height of three to four meters and both have hairless shoots and ligules. The European lineage has flowers, which are initially purple but later turn gold (Figure 1.1).

Figure 1.1. European lineage of Roseau cane. Immature (A) and mature flowers (B).
The Greeny lineage has a bluish tint on the leaves. The Gulf lineage is found more inland than the other lineages. This lineage has hairless leaves and branching stems and can grow three and a half to four and a half meters (LSU AgCenter, 2017). In summary, there are four Roseau cane lineages within Louisiana with each having multiple haplotypes within the lineage (J. Cronin, personal communication).

1.2. Roseau cane die-off

In many parts of the world, Roseau cane has been declining significantly. This decline has been called reed die-back syndrome (a group of symptoms that consistently occur together) (Coppi et al., 2018), Roseau cane die-back or Roseau cane die-off (Knight et al., 2018). It was first noticed in Central European countries, but now it is happening to Roseau cane in many other regions, including the Delta of the Mississippi River (Knight et al., 2018) (Figure 1.2).

![Figure 1.2. Roseau cane in the Mississippi River Delta showing die-off.](image)
After it was found throughout central Europe, the Roseau cane die-off problem was noticed in Italy. In Italy when Roseau cane die-off occurred, biodiversity and water quality in the area declined (Gigante et al., 2014). In United States, die-off was first reported in the Great Lakes in the early 1950’s. Roseau cane die-off was noticed in the state of Louisiana in the Mississippi River Delta in 2016 (Cronin et al., 2020). In Louisiana, symptoms of die-off include large amounts of dead and dying stems, stunted stems, and many stands with low stem density (Knight et al., 2018).

The Roseau cane die-off can cause problems with Louisiana fisheries and oil and gas resources (LSU AgCenter, 2019). Because Roseau cane is the dominant emergent vegetation in the lower Mississippi River Delta, losing Roseau cane can cause sediment to fill in navigation channels (Knight et al., 2018). The Louisiana Department of Wildlife and Fisheries, the Louisiana Department of Agriculture and Forestry, the Coastal Protection and Restoration Authority, the U.S. Army Corps of Engineers, the U.S. Fish and Wildlife Service, and the LSU AgCenter are working together to find a solution to the Roseau cane die-off (LSU AgCenter, 2019).

Alligators are economically important wildlife in Louisiana, and the American Alligator (Alligator mississippiensis) is known to nest in Roseau cane (Merchant et al., 2018). Taking away an animal’s nesting plant away can be detrimental to that species. White-tailed deer (Odocoileus virginianus) at the Pass-a-Loutre Wildlife Management Area in Louisiana used Roseau cane for habitat cover (Baker et al., 2018). Losing a plant that these deer hide in can be harmful to their population size, which ultimately would cause issues in the hunting industry. Migratory birds are also important wildlife to hunter and just like white-tailed deer; migratory
birds use Roseau cane as habitat (Cronin, 2020). The fishing industry would also be impacted by losing Roseau cane because prey fish species use Roseau cane as habitat (Cronin, 2020). The Mississippi River Delta has an extremely high level of biodiversity, which makes it habitat for animals that usually would not be found together (Hester et al., 2005). Roseau cane is listed as a dominant plant species in this area, meaning losing this species can largely change the habitat and animals that survive there (Hester et al., 2005).

Pollution and climate change impacts on Roseau cane have not been widely researched by many scientists yet. It is known that saltwater intrusion has been occurring due to sea level rise and weather changes from climate changes. High salinity (over 20 ppm) has been reported to have caused Roseau cane die-off in Europe (Knight et al., 2020). Stunted Roseau cane growth has been associated with high salinity levels or long exposure to salinity. Pollution and climate change are known to change soil chemistry. In Europe, high organic matter and high concentration of organic acids and sulfides have also been associated with Roseau cane die-off (Khan and Ansari, 2005). It is possible that similar problems are occurring in Louisiana. A scale insect, *Nipponaclerda biwakoensis*, which feeds on Roseau cane has been found in Louisiana and it has been associated with Roseau cane die-off (Cronin et al., 2020; Knight et al., 2018).

Plant pathogens, such as the oomycetes *Pythiogeton* spp. and *Pythium* spp., have been isolated from Roseau cane affected by die-off (Cerri et al., 2017; Croker et al., 2015; Nechwatal et al., 2008). In a study by Allen et al., (2020), 84 foliar fungal taxa were isolated from *P. australis*. In their investigation, the European lineage was the least susceptible to three out of the four pathogens that they tested, but they noted that in their common garden experiment [(experiments
where organisms from different areas are grown together in the same environmental conditions (Berend et al., 2019)], the damage by fungal pathogens in the field was similar between all lineages. In Louisiana, isolates of *Alternaria alternata, Bipolaris yamadae, Fusarium coicis, F. sulawense,* and *Nigrospora osmanthi* have been isolated from foliar tissues of Roseau cane (Galo, 2021). These fungi caused disease symptoms on Roseau cane in detached leaf experiments (Galo, 2021).

### 1.3. Mealy plum aphids

The mealy plum aphid (*Hyalopterus pruni*) occurs throughout the United States (Krause, 1996). The genus *Hyalopterus* contains two recognized species: *H. amygdali* and *H. pruni.*

Morphological means of distinguishing these species have been difficult and identifications have often been based on host plant data rather than structural characters (Lozier et al., 2008). Nevertheless, DNA sequencing of selected genes has been used to identify *Hyaloperus* species (Lozier et al., 2008). Genes include the mitochondrial Cytochrome Oxidase I (COI) gene and 16SrDNA (Lozier et al., 2007). These aphids overwinter in the egg stage on *Prunus* trees and migrate to their secondary host, *P. australis,* in the late spring and summer (Smith, 1935). The mealy plum aphid is known to feed on Roseau cane and many *Prunus* species such as plums, peaches, and apricots (Basky, 2005). Like all aphids, this aphid reproduces via parthenogenesis and develop into adults in about 9.4 days after birth (Ozgokce and Atlihan, 2005). Over their life, each aphid can produce about 48 offspring. When the host dies or becomes too overcrowded with aphids, the aphids develop wings and move to other plants. The mealy plum aphid has
deciduous host plants during the winter and then in the spring feeds on Roseau cane (Park and Blossey, 2008).

Aphids have been known to decrease the health of many plant species (Watanabe et al., 2018). Aphids feed through the phloem of the plant and while feeding, they can spread plant pathogens from plant to plant (Will et al., 2013; Park and Blossey, 2008). The mealy plum aphid is known to be a vector of some plant viruses, including plum pox virus (Cambra and Vidal, 2017). This virus causes a damaging disease to Prunus species and has been known to cause significant economic losses to the stone fruit industry (Garcia et al., 2014). Although, plants resistant to aphids has been reported in many crop species such as soybean, lettuce, melon, tomato, wheat, barley, maize, legumes, and fruit trees, aphids have overcome host resistance by developing new biotypes (Jaouannet et al., 2014).

Aphids cause damage to Roseau cane (Lambert and Casagrande, 2007), and in Louisiana, native Roseau cane has been observed to have more aphids present on it than on non-native Roseau cane (Figure 1.3) (R. A. Valverde, personal communication). Higher aphid densities have been reported in Roseau cane growing in wet areas than in dry areas (Mook and Wiegers, 1999). Yellowing, curling, and wilting have been reported on native Roseau cane plants with high aphid densities. These symptoms have rarely been noticed on non-native Roseau cane plants (Lambert and Casagrande, 2007).
Figure 1.3. Mealy plum aphid (*Hyalopterus pruni*) colonizing Roseau cane.

### 1.4. *Bipolaris* leaf spot

Fungal species of the *Bipolaris* genus are known to be distributed worldwide. Species in this genus have been associated with leaf spots, leaf blights, melting outs, root rots, and foot rots in plants in the family *Poaceae* (Manamgoda *et al.*, 2014). Losses due to pathogens in the genus *Bipolaris* have occurred in economically important crops such as rice, maize, wheat, and sorghum (Manamgoda *et al.*, 2014). In potato dextrose agar at 23 °C, *Bipolaris* colonies grow relatively fast and are a grey to brownish black color. The conidia are known to be curved,
canoe-shaped or obclavate (Ellis, 2016). The pigmentation of the conidia is hyaline to dark greenish yellow (Ellis, 2016). Conidia are also reported to be brown in color, smooth, two to four septate, cylindrical, rounded at both ends and measuring 14.0-30.0 x 6.5-12.0 μm in size (Lin et al., 2012). Bipolaris bicolor produces phytotoxins that are known to affect plants and cause leaf blight. This fungus is known to inhibit the growth of rice seedlings (Miyagawa et al., 1994).

Sugarcane is another economically important crop that is affected by a Bipolaris species (B. spicifera) that causes leaf spot disease (Lin et al., 2012). Interestingly, the same species that infects sugar cane has also been reported to infect immunocompromised humans (Buzina et al., 2003). In Roseau cane, Bipolaris species have been reported to cause leaf lesions. A study on mature leaf and seedling leaf assays concluded that B. sorokiniana is a pathogen that can infect Roseau cane (Devries et al., 2020). In Louisiana, B. yamadae has been identified as a fungus causing leaf spots in Roseau cane (Galo, 2021). This fungus species was first known to cause leaf spots on Guinea grass in Florida (Adhikari et al., 2021). On Guinea grass, the lesions are oblong to irregular shaped and have brownish to dark grayish centers with pale yellowish to brownish black outer margins. On Panicum sp. leaf spots caused by B. yamadae are known to be ovoid or oblong shaped and a brown color with an irregular concentric zone (Manamgoda et al., 2014).

1.5. Aphid-fungal interactions

It has been reported that fungi and aphids may affect each other while on the same host plant (Wilkinson et al., 2019). In a study conducted on aphids’ influence on soil fungal communities, aphids increased the evenness and abundance of fungi (Wilkinson et al., 2019). In another study,
the beech aphid (*Grylloprociphilus imbricator*) decreased the health of trees from its interaction with sooty mold (caused by several ascomycete fungi) since it weakens the leaf function (Cook-Patton *et al*., 2014). Arbuscular mycorrhizal fungi are known to increase the plant growth and fecundity because they change the shape of the phloem sieves, improving the nutrition of the plant (Babikova *et al*., 2014). Effector proteins in the saliva of aphids are known to alter aphid plant interactions. An effector protein that causes a plant to release salicylic acid when aphids feed, was found to decrease the disease caused by the pathogen *Pseudomonas syringae* (Cui *et al*., 2019). Green peach aphids, *Myzus persicae*, on plants without the plant pathogen *Botrytis cinerea* had lower fecundity, were smaller, and had a lower survival time than aphids on plants with *B. cinerea* (Ngah *et al*., 2018). Pea aphids, *Acythosiphon pisum*, also benefitted from being on a host plant infected with *B. cinerea*, whereas the black bean aphid, *Aphis fabae* had reduced fecundity, a lower population growth rate, were smaller, and had a lower survival time on *B. cinerea* infected plants (Srisakrapikoop *et al*., 2021).

Fungi can be detrimental to aphids. Some fungi, in the order Entomophthorales, are pathogenic to aphids. *Pandora neoaphidis* is known to be a fungal pathogen that infects soybean aphids, *A. glycines*, and causes death at times (Noma and Brewer, 2007). The fungi *Beauveria bassiana* and *Metarhizium brunneum* are both known to decrease the number of the green peach aphid, *M. persicae*, when applied to pepper (*Capsicum annum*) plants (Jaber and Araj, 2018).
1.6. Objectives

Pathogens and insect herbivores may play a role in the Roseau cane die-off; therefore, it is important to evaluate the effects that a pathogen and insect herbivore alone and in combination have on the health of Roseau cane. As addressed earlier, the mealy plum aphid is known to cause health problems to several plant species (Watanabe et al., 2018) and this aphid has been reported in Roseau cane (Lambert and Casagrande, 2007). Several fungi have been reported to infect Roseau cane (Devries et al., 2020) and Roseau cane in die-off areas of the Mississippi River Delta often show leaf spot symptoms (Figure 1.4) (R. A. Valverde, personal communication).

![Figure 1.4. Roseau cane growing in a die-off area of the Mississippi River Delta showing leaf spots.](image-url)
Bipolaris yamadae has been reported to cause leaf spots on Roseau cane (Galo, 2021).

Determining the effects of the mealy plum aphid and B. yamadae, alone and together could provide information in the quest to unravel the Roseau cane die-off problem. The objectives of this investigation were:

1. Collect aphids from Roseau cane, identify them, and establish a colony of the mealy plum aphid in the laboratory.

2. Conduct laboratory feeding experiments to determine the damage caused to Roseau cane by H. pruni infestations.

3. Conduct laboratory inoculation experiments to determine the damage caused to Roseau cane by B. yamadae.

4. Evaluate the damage caused to Roseau cane by the simultaneous infestation and infection of H. pruni and B. yamadae respectively.
CHAPTER II. COLLECTION, IDENTIFICATION AND ESTABLISHMENT OF A COLONY OF THE MEALY PLUM APHID
(HYALOPTERUS PRUNI)

2.1. Introduction

Roseau cane (*Phragmites australis*) is a perennial plant in the family Poaceae. In some areas, this plant is considered invasive; however, in Louisiana, it protects marshes from waves and storms and stops sediment from filling navigation channels and therefore it is an essential part of the marsh (Knight *et al*., 2018). This plant helps stop the expansion of invasive plants such as alligator weed and elephant ear (LSU AgCenter, 2019). Because Roseau cane can survive through harsh conditions and pollutants, in some areas, it is used to treat agricultural and industrial wastewaters and remove harmful microorganisms (Gigante *et al*., 2014). Roseau cane has been used as food for animals, habitat for wildlife, material to make musical instruments, and baskets (Ilbagi, 2006).

Alligators are economically important wildlife in Louisiana, and the American Alligator (*Alligator mississippiensis*) is known to nest in Roseau cane (Merchant *et al*., 2018). Taking away an animal’s nesting plant away can be detrimental to that species. White-tailed deer (*Odocoileus virginianus*) at the Pass-a-Loutre Wildlife Management Area in Louisiana used Roseau cane for habitat cover (Baker *et al*., 2018). Losing a plant that these deer hide in can be harmful to their population size, which ultimately would cause issues in the hunting industry. Migratory birds are also important wildlife to hunter and just like white-tailed deer; migratory birds use Roseau cane as habitat (Cronin, 2020). The fishing industry would also be impacted by losing Roseau cane because prey fish species use Roseau cane as habitat (Cronin, 2020). The
Mississippi River Delta has an extremely high level of biodiversity, which makes it habitat for animals that usually would not be found together (Hester et al., 2005). Roseau cane is listed as a dominant plant species in this area, meaning losing this species can largely change the habitat and animals that survive there (Hester et al., 2005).

In many parts of the world, Roseau cane has been declining significantly. This decline has been called reed die-back syndrome (Coppi, 2018), Roseau cane die-back or Roseau cane die-off (Knight et al., 2018). It was first noticed in Central European countries, but now it is happening to Roseau cane in many other regions, including the Delta of the Mississippi River (Cronin et al., 2020; Knight et al., 2018). Roseau cane die-off was noticed in the state of Louisiana in the Mississippi River Delta in 2016 (Cronin et al., 2020). Symptoms of die-off include large amounts of dead and dying stems, stunted stems, and many stands with low stem density (Knight et al., 2018). A scale insect, Nipponaclerda biwakoensis, which feeds on Roseau cane has been found in Louisiana and it has been associated with Roseau cane die-off (Cronin et al., 2020; Knight et al., 2018). Because the scale insect has been associated with the die-off, it is possible that other insects may impact the health of Roseau cane.

Aphids have been known to decrease the health of many plants (Watanabe et al., 2018). It has been reported that aphids may be causing health problems to Roseau cane (Lambert and Casagrande, 2007). The mealy plum aphid, H. pruni, is known to feed on Roseau cane. This aphid species develops into an adult in about 9.4 days after birth and over their life, each aphid can produce about 48 offspring. Like all aphids, the mealy plum aphid reproduces via parthenogenesis (Ozgokce and Atlihan, 2005). The genus Hyalopterus contains two recognized
species: *H. amygdali* and *H. pruni*. Morphological means of distinguishing these species have been difficult and identifications have often been based on host plant specificity rather than structural characters. Nevertheless, DNA sequencing of selected genes has been used to identify *Hyaloperus* species (Lozier et al., 2008).

In Louisiana, four different lineages of Roseau cane can be found (LSU AgCenter, 2017). Delta is the most common lineage in the Mississippi River Delta. The European lineage has been reported to be resistant to the Roseau cane scale insect (Cronin *et al*., 2020). Determining the level of tolerance of the Delta and the European lineages to *H. pruni* will provide important information that will complement what we know of their tolerance to the scale insect. Although, *H. pruni* is the most common aphid found infesting Roseau cane, other aphid species may colonize this plant. Therefore, it is important to determine the aphid identity by methods other than morphological characters or host preference. An aphid colony must be established to conduct experiments under greenhouse or laboratory conditions. To minimize genetic variations, the colony should be started from a single aphid that can reproduce via parthenogenesis (Dedryver *et al*., 2013).

### 2.2. Objectives

The objectives of this investigation were to determine the identity of an aphid collection from Roseau cane and to establish a colony of the mealy plum aphid under laboratory conditions.
2.3. Materials and Methods

2.3.1. Source of Roseau cane plants

Roseau cane plants of the Delta lineage were provided by R. Diaz (Entomology Department, Louisiana State University) and used in preliminary experiments to develop practical methods to grow Roseau cane in the greenhouse and laboratory for experimental use (Appendix A). Additionally, haplotypes of the Delta, Gulf and European lineages of Roseau cane were provided by J. Cronin (Department of Biological Sciences, Louisiana State University). The haplotypes EARL and ECM were from the Delta of the Mississippi River; haplotypes ARM and WS9 from Europe; and haplotypes ANZ, and ICI from the United States Gulf coast. EARL and ECM originated from a dredge island and East Cameron parish respectively, Louisiana; ANZ from Texas; ICI from Vermillion parish, Louisiana; ARM from Little Rock, Arkansas; and W96 from the Salinas River, California.

2.3.2. Growing Roseau cane lineages in the greenhouse and the laboratory and evaluation of their growth

Roseau cane haplotypes (ANZ, ARM, EARL, ECM, ICI, and W96) from the Delta, European, and Gulf lineages were grown from root cuttings (rhizomes). Root cuttings (4.0-8.0 cm) were placed in 10.16 x 10.16 cm plastic pots containing a soil mixture that consisted of soil, sand, and potting mix (Scotts Miracle-Gro Company®, Marysville, OH, USA) at a ratio of 2:1:0.5. Each plant was fertilized once with 12 g of Osmocote® (15-9-12 NPK, Scotts Miracle-Gro Company®). Pots placed on flooded plastic trays in a greenhouse located at the Plant Materials
Center, Central Research Station, Baton Rouge with day/night temperatures averaging 25/18 ºC respectively (Figure 2.1).

![Figure 2.1. Greenhouse propagation of Roseau cane. Stock plants (A), rhizome cuttings (B), and young plants (C).](image)

Pots were kept in the greenhouse until plants grew to a height between 25 to 28 cm. Plants were transferred to the laboratory and pots placed on water-flooded plastic trays (Figure 2.2) and grown under fluorescent lights (54W/120V 60Hz/4.0A Lamps at 17 h light and 7 h dark) with an average temperature of 23ºC.
Ten plants of each of the six Roseau cane haplotypes were grown initially in the greenhouse, then transferred to the laboratory, and visually evaluated weekly for their vegetative growth.

2.3.3. Establishing a colony of the mealy plum aphid

Aphids were collected from Roseau cane growing in an experimental plot at the Louisiana Business and Technology Center, Louisiana State University, Baton Rouge. An aphid colony was established in the laboratory on Roseau cane plants kept in a 39.9 x 39.9 x 59.9 cm screen cages (Restcloud, Chengdu YiShouWeiSheng Technology Co., Ltd, China) (Figure 2.3). To
establish the colony, a single aphid was randomly selected and placed on a Roseau cane plant of the Delta lineage. Cages were kept under the same laboratory conditions described above.

Figure 2.3. Screen cage used to isolate an aphid colony.

2.3.4. Aphid identification

Examination of the morphological characteristics of aphids collected from Roseau cane and using keys from Lozier et al., (2008) and Rakauskas et al., (2013), suggested that they belonged to the Hyalopterus genus. This was supported by reports that Roseau cane is an alternate host for H. pruni (Park and Blossey, 2008). However, to confirm the aphid identity, polymerase chain reaction (PCR) experiments were conducted using species-specific primers reported by Loizer et al., (2007) and Simon et al., (2006). DNA was extracted from 30 mg of aphids using the Zymo
Research Quick DNA/ Insect Miniprep kit (Zymo Research, Ivrine, CA, USA) following the manufacturer instructions. DNA was also extracted from aphids collected from pepper (\textit{Capsicum annuum}) growing in a home garden. In brief, frozen aphids were added to a Zymo Research bashing bead lysis tube (2.0 ml) containing 750 µl of BashingBead Buffer and vortexed for 10 min. After centrifugation, genomic lysis buffer was added to the supernatant and applied to a spin column then washed, and DNA eluted in 100 ul of elution buffer.

The PCR reactions were carried out using the GoTaq® Green Master Mix kit (Promega Corporation, Madison, WI, USA) following the manufacturer’s recommendations. GoTaq® Green Master Mix kit (Promega Corporation) was used to complete the PCR reactions. Two sets of primers were used. Primers C1-J-1718 (GGAGGATTGGAAAAATTGATTAGTTCC) and C1-N-2191 (CCCGGTAAAATTAAAATATAAACTTC) were for the amplification of 343 bp of the mitochondrial cytochrome oxidase subunit 1 (COI) gene and primers Buch16S1F (GAGCTTGCTCTTTGTCGGCAA) and Buch16S1R (CTTCTGCGGGTAACGTCACGAA) for the amplification of 393 bp ribosomal DNA (rDNA) of an endosymbiont of aphids (Amini and Hosseini 2016; Lozier et al., 2007; Simon et al., 2006). Amplifications were performed in a MiniAmp™ Thermal Cycler (ThermoFisher Scientific, Waltham, MA, USA). For each 25 ul reaction, 1 µl of aphid DNA (10-30 ng) and 1 µl of each primer (10 µM) were used. The PCR cycles for reactions using primers C1-J-1718 and C1-N-2191 consisted of initial denaturation at 94°C for 2 min, followed by 36 cycles of denaturation for 30 s at 94°C, annealing 30 s at 53°C, and extension for 30 s at 72°C, and a final extension of 7 min at 72°C (Lozier et al., 2007). The PCR cycles for reactions using primers Buch16S1F and Buch16S1R consisted of initial denaturation at 94°C for 2 min, followed by 36 cycles of denaturation for 30 s at 94°C, annealing
for 30 sec at 55°C, extension for 30 sec at 72°C, and a final extension of 7 min at 72°C (Lozier et al., 2007). Three independent DNA extractions and PCR experiments were conducted for the aphids collected from both, Roseau cane and pepper plants. The PCR products were resolved in agarose (1.2%) gel electrophoresis at 70 V for 1.45 h. GelRed (Biotium, Hayward, CA, USA) was used to stain the DNA. A 100 bp molecular ladder (Promega Corporation) was used to estimate the size of the amplified product. The PCR products were purified using the StrataPrep PCR Purification Kit (Agilent Scientific, Santa Clara, CA, USA) and sent to Psomagen laboratory (Rockville, MD, USA) for sequencing in both directions. Obtained nucleotide sequences were compared with available sequences in GenBank (Sayers et al., 2020) using Basic Local Alignment Search Tool (BLASTn) program (Altschul et al., 1990), searching for highly similar sequences.

2.3.5. Evaluation of Roseau cane haplotypes as feeding plants for mealy plum aphids

Single mealy plum aphids were placed on each one of the six Roseau cane haplotypes to evaluate, under laboratory conditions, the damage to the plants caused by aphid feeding. Plants were placed in screen cages and visually evaluated weekly over a period of six months. Damage was recorded as percentage compared to similar plants without aphids. The percent was assigned by examining the plant and estimating what percent of the whole plant was not green. Aphids were from the same colony. Two plants of each haplotype were evaluated in three independent experiments. A logit-beta generalized linear mixed model was performed in SAS to compare the percent damage to the different lineages of Roseau cane. A Tukey-Kramer difference of means test was used to determine what lineages had significant differences from each other.
2.4. Results

2.4.1 Growing Roseau cane lineages in the greenhouse and the laboratory and evaluation of their growth

Using the procedure described in the Materials and Methods section (detailed in Appendix 1), Roseau cane haplotypes belonging to three lineages were successfully grown in both the laboratory and the greenhouse. Examination and measurements were conducted on 10 four-month-old plants of each haplotype that were grown in the greenhouse. Haplotypes of the Gulf lineage (Figure 2.4 A and B) exhibited thicker leaves than those of the other lineages. Haplotypes of the Delta lineage (Figure 2.4 C and D) had longer and wider leaves than haplotypes of the Gulf lineage but exhibited thinner stems than the other lineages. Haplotypes of the European lineage (Figure 2.4 E and F) had broader leaves and stems than those of the other lineages.
Figure 2.4. Four-month-old Roseau cane plants. Gulf lineage (haplotype ICI) (A), Gulf lineage (haplotype ANZ) (B), Delta lineage (haplotype EARL) (C), Delta lineage (haplotype ECM) (D), European lineage (haplotype W96), (E) and European lineage (haplotype ARM) (F).

2.4.2. Establishing a colony of the mealy plum aphid

An aphid colony was successfully established in the laboratory (Figure 2.5).
Under laboratory conditions, *H. pruni* population increased substantially when new plants were provided. The feeding plants in the aphid colony were changed when about half the plant exhibited foliar damage.

### 2.4.3. Aphid identification

Amplification of DNA was successfully obtained with both, the COI gene primers (Figure 2.6) and the rDNA primers. Sequencing amplified COI gene DNA and sequence comparisons by BLASTn from the established aphid colony confirmed that they were *H. pruni* (Table 2.1). Moreover, sequencing of the PCR products obtained from the aphids collected from pepper revealed that they were *Myzus persicae*, the green peach aphid (Table 2.1). DNA sequences are shown in Appendix B. The sequences of PCR products obtained with primers Buch16S1F and
Buch16S1R which amplified rDNA revealed the presence of *Buchnera aphidicola*, an endosymbiont that is often reported to be associated with aphids (Lozier et al., 2007) (Appendix B and C).

Figure 2.6. Agarose gel showing PCR products amplified using DNA extracted from aphids. Lanes 1, 2, 3 and 5 were amplified using DNA from the mealy plum aphid and COI gene primers. Lanes 4, 6, and 7 were amplified using DNA from an unknown aphid infesting pepper.
Table 2.1. Results of PCR experiments aimed at the identification of aphids collected from Roseau cane and pepper using COI primers C1-J-1718 and C1-N-2191.

<table>
<thead>
<tr>
<th>Plant Source</th>
<th>Aphid Species</th>
<th>Gene(^1)</th>
<th>Sample</th>
<th>Percent Identity(^2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pepper</td>
<td><em>Myzus persicae</em></td>
<td>COI</td>
<td>0010-F</td>
<td>99.14</td>
</tr>
<tr>
<td>Pepper</td>
<td><em>Myzus persicae</em></td>
<td>COI</td>
<td>0010-R</td>
<td>99.13</td>
</tr>
<tr>
<td>Pepper</td>
<td><em>Myzus persicae</em></td>
<td>COI</td>
<td>0011-F</td>
<td>99.78</td>
</tr>
<tr>
<td>Pepper</td>
<td><em>Myzus persicae</em></td>
<td>COI</td>
<td>0011-R</td>
<td>99.56</td>
</tr>
<tr>
<td>Pepper</td>
<td><em>Myzus persicae</em></td>
<td>COI</td>
<td>0012-F</td>
<td>99.78</td>
</tr>
<tr>
<td>Pepper</td>
<td><em>Myzus persicae</em></td>
<td>COI</td>
<td>0012-R</td>
<td>99.57</td>
</tr>
<tr>
<td>Roseau cane</td>
<td><em>Hyalopterus pruni</em></td>
<td>COI</td>
<td>0004-F</td>
<td>100</td>
</tr>
<tr>
<td>Roseau cane</td>
<td><em>Hyalopterus pruni</em></td>
<td>COI</td>
<td>0004-R</td>
<td>99.78</td>
</tr>
<tr>
<td>Roseau cane</td>
<td><em>Hyalopterus pruni</em></td>
<td>COI</td>
<td>0005-F</td>
<td>100</td>
</tr>
<tr>
<td>Roseau cane</td>
<td><em>Hyalopterus pruni</em></td>
<td>COI</td>
<td>0005-R</td>
<td>99.56</td>
</tr>
<tr>
<td>Roseau cane</td>
<td><em>Hyalopterus pruni</em></td>
<td>COI</td>
<td>0006-F</td>
<td>100</td>
</tr>
<tr>
<td>Roseau cane</td>
<td><em>Hyalopterus pruni</em></td>
<td>COI</td>
<td>0006-R</td>
<td>99.78</td>
</tr>
</tbody>
</table>

\(^1\)COI = Cytochrome Oxidase I.
\(^2\)Percent identity to the reference species in the GenBank.

2.4.4. Evaluation of Roseau cane haplotypes as feeding plants to mealy plum aphids

Haplotypes of the Gulf and Delta lineages were severely damaged after one month of aphid feeding. By the end of the third month, all the Delta lineage haplotypes and most of the Gulf lineage haplotypes were severely damaged. Haplotypes of the European lineage showed less damage than the other lineages. After six months of aphid feeding, haplotypes of all the lineages
showed severe damage. Figure 2.7 shows some examples of aphid damage on different Roseau cane lineages. Table 2.2 shows the percent damage by month for each haplotype as the average of three experiments. The Tukey-Kramer difference of means test determined that all three lineages had statistically significant differences from each other. The p-value between the European and Delta lineage was less than 0.0001 and the standard error was 0.3437. The p-value between the Delta and Gulf lineage was 0.0294 and the standard error was 0.2472. The p-value between the European and Gulf lineage was less than 0.0001 and the standard error was 0.3214.
Figure 2.5. Damage to Roseau cane plants caused by aphid feeding. Gulf lineage (haplotype ICI) three months after exposure to aphid feeding (A), Delta lineage (haplotype EARL) three months after exposure to aphid feeding (B), European lineage (haplotype W96) three months after
exposure to aphid feeding (C), and European lineage (haplotype W96) six months after exposure to aphid feeding (D).

Table 2.2. Results of mealy plum aphid feeding experiments on different Roseau cane haplotypes. Percent damage is the average of three experiments.

<table>
<thead>
<tr>
<th>Month</th>
<th>Lineage</th>
<th>Haplotype</th>
<th>Percent Damage $^1$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Delta</td>
<td>EARL</td>
<td>45.00</td>
</tr>
<tr>
<td>1</td>
<td>Delta</td>
<td>ECM</td>
<td>44.17</td>
</tr>
<tr>
<td>1</td>
<td>Gulf</td>
<td>ANZ</td>
<td>32.50</td>
</tr>
<tr>
<td>1</td>
<td>Gulf</td>
<td>ICI</td>
<td>36.67</td>
</tr>
<tr>
<td>1</td>
<td>European</td>
<td>ARM</td>
<td>4.17</td>
</tr>
<tr>
<td>1</td>
<td>European</td>
<td>W96</td>
<td>3.33</td>
</tr>
<tr>
<td>2</td>
<td>Delta</td>
<td>EARL</td>
<td>66.67</td>
</tr>
<tr>
<td>2</td>
<td>Delta</td>
<td>ECM</td>
<td>65.00</td>
</tr>
<tr>
<td>2</td>
<td>Gulf</td>
<td>ANZ</td>
<td>47.50</td>
</tr>
<tr>
<td>2</td>
<td>Gulf</td>
<td>ICI</td>
<td>47.50</td>
</tr>
<tr>
<td>2</td>
<td>European</td>
<td>ARM</td>
<td>7.50</td>
</tr>
<tr>
<td>2</td>
<td>European</td>
<td>W96</td>
<td>5.00</td>
</tr>
<tr>
<td>3</td>
<td>Delta</td>
<td>EARL</td>
<td>98.33</td>
</tr>
<tr>
<td>3</td>
<td>Delta</td>
<td>ECM</td>
<td>95.83</td>
</tr>
<tr>
<td>3</td>
<td>Gulf</td>
<td>ANZ</td>
<td>92.50</td>
</tr>
<tr>
<td>3</td>
<td>Gulf</td>
<td>ICI</td>
<td>82.50</td>
</tr>
<tr>
<td>3</td>
<td>European</td>
<td>ARM</td>
<td>15.00</td>
</tr>
</tbody>
</table>

(table cont’d.)
<table>
<thead>
<tr>
<th>Month</th>
<th>Lineage</th>
<th>Haplotype</th>
<th>Percent Damage</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>European</td>
<td>W96</td>
<td>13.33</td>
</tr>
<tr>
<td>4</td>
<td>Delta</td>
<td>EARL</td>
<td>100</td>
</tr>
<tr>
<td>4</td>
<td>Delta</td>
<td>ECM</td>
<td>98.33</td>
</tr>
<tr>
<td>4</td>
<td>Gulf</td>
<td>ANZ</td>
<td>100</td>
</tr>
<tr>
<td>4</td>
<td>Gulf</td>
<td>ICI</td>
<td>97.50</td>
</tr>
<tr>
<td>4</td>
<td>European</td>
<td>ARM</td>
<td>32.50</td>
</tr>
<tr>
<td>4</td>
<td>European</td>
<td>W96</td>
<td>29.17</td>
</tr>
<tr>
<td>5</td>
<td>Delta</td>
<td>EARL</td>
<td>100</td>
</tr>
<tr>
<td>5</td>
<td>Delta</td>
<td>ECM</td>
<td>100</td>
</tr>
<tr>
<td>5</td>
<td>Gulf</td>
<td>ANZ</td>
<td>100</td>
</tr>
<tr>
<td>5</td>
<td>Gulf</td>
<td>ICI</td>
<td>100</td>
</tr>
<tr>
<td>5</td>
<td>European</td>
<td>ARM</td>
<td>70.00</td>
</tr>
<tr>
<td>5</td>
<td>European</td>
<td>W96</td>
<td>69.17</td>
</tr>
<tr>
<td>6</td>
<td>Delta</td>
<td>EARL</td>
<td>100</td>
</tr>
<tr>
<td>6</td>
<td>Delta</td>
<td>ECM</td>
<td>100</td>
</tr>
<tr>
<td>6</td>
<td>Gulf</td>
<td>ANZ</td>
<td>100</td>
</tr>
<tr>
<td>6</td>
<td>Gulf</td>
<td>ICI</td>
<td>100</td>
</tr>
<tr>
<td>6</td>
<td>European</td>
<td>ARM</td>
<td>99.17</td>
</tr>
<tr>
<td>6</td>
<td>European</td>
<td>W96</td>
<td>96.67</td>
</tr>
</tbody>
</table>

<sup>1</sup>Percent damage is the percentage of non-green tissue on the whole plants compared to similar plants without aphids.
2.5. Discussion

The use of non-cultivated plants in scientific experiments is often hindered by the lack of success of growing them under controlled conditions. In the literature, methods for growing Roseau cane in the greenhouse or laboratory are lacking. Therefore, preliminary experiments, using several haplotypes of three Roseau cane lineages, were conducted to develop a practical growing method. In the greenhouse, haplotypes of the European and Delta lineages grew faster and more robust than haplotypes of the Gulf lineage. The trays where the potted plants were placed were flooded with water, which is more consistent with the environment in which haplotypes of the Delta and European lineages are naturally found (LSU AgCenter 2017, online). Haplotypes of the Gulf lineage are found more inland than those of the other two lineages and that could be a factor on their performance under flooded conditions.

The proper establishment and identification of an insect colony is essential for research on their effect on plants. In the case of aphids, determining the most suitable host for reproduction and survival is an important aspect that needs to be addressed when establishing colonies. Results from this investigation suggest that the Roseau cane plant lineage used for aphid feeding can play an important role in establishing a successful colony. In this investigation, Roseau cane haplotypes of the European lineage were less preferred feeding plants to aphids, however, when these plants were exposed simultaneously with haplotypes of other lineages, aphids fed only when the other lineages were severely damaged. This suggested that aphids survive and reproduce better on haplotypes of the Delta and Gulf lineages. Aphids are not the only insects that have been found more plentiful on haplotypes of the Delta lineage of Roseau cane when
compared with haplotypes of the European lineage. In a study by Cronin et al. (2020), haplotypes of the European lineage were found to be more resistant to Roseau cane scales (*N. biwakoensis*) than those of other lineages.

DNA sequence information obtained from PCR amplifications confirmed that the aphids collected from Roseau cane and used to establish a colony were mealy plum aphids. This was not surprising because that is the only aphid reported to occur on Roseau cane. Furthermore, morphological examinations suggested that aphids belonged to the *Hyalopterous* genus. PCR amplifications using *H. pruni* DNA resulted in the detection of an obligate primary endosymbiont, *Buchnera aphidicola*. Lozier et al., (2007) previously reported the identification of *B. aphidicola* in *H. pruni*. This endosymbiont was also detected in DNA from the green peach aphid collected from pepper.

In conclusion, procedures to grow Roseau cane lineages under controlled conditions and determining the best feeding lineages were developed. A colony of the mealy plum aphid was established in the laboratory and the aphid species identity confirmed.
CHAPTER III. FEEDING EXPERIMENTS TO DETERMINE THE DAMAGE CAUSED TO ROSEAU CANE BY *HYALOPTERUS PRUNI*

3.1. Introduction

Roseau cane (*Phragmites australis*) is a perennial plant in the family Poaceae. In some areas, this plant is considered invasive; however, in Louisiana, it protects marshes from waves and storms and stops sediment from filling navigation channels and therefore it is an essential part of the marsh (Knight *et al.*, 2018). This plant helps stop the expansion of invasive plants such as alligator weed and elephant ear (LSU AgCenter, 2019). Because Roseau cane can survive through harsh conditions and pollutants, in some areas, it is used to treat agricultural and industrial wastewaters and remove harmful microorganisms (Gigante *et al.*, 2014). Roseau cane has been used as food for animals, habitat for wildlife, material to make musical instruments, and baskets (Ilbagi, 2006). In Louisiana different lineages of Roseau cane can be found but the Delta is the most common in the Mississippi River Delta (LSU AgCenter, 2017).

Alligators are economically important wildlife in Louisiana, and the American Alligator (*Alligator mississippiensis*) is known to nest in Roseau cane (Merchant *et al.*, 2018). Taking away an animal’s nesting plant away can be detrimental to that species. White-tailed deer (*Odocoileus virginianus*) at the Pass-a-Loutre Wildlife Management Area in Louisiana used Roseau cane for habitat cover (Baker *et al.*, 2018). Losing a plant that these deer hide in can be harmful to their population size, which ultimately would cause issues in the hunting industry. Migratory birds are also important wildlife to hunter and just like white-tailed deer; migratory birds use Roseau cane as habitat (Cronin, 2020). The fishing industry would also be impacted by losing Roseau cane because prey fish species use Roseau cane as habitat (Cronin, 2020). The
Mississippi River Delta has an extremely high level of biodiversity, which makes it habitat for animals that usually would not be found together (Hester et al., 2005). Roseau cane is listed as a dominant plant species in this area, meaning losing this species can largely change the habitat and animals that survive there (Hester et al., 2005).

In many parts of the world, Roseau cane has been declining significantly. This decline has been called reed die-back syndrome (Coppi, 2018), Roseau cane die-back or Roseau cane die-off (Knight et al., 2018). It was first noticed in Central European countries, but now it is happening to Roseau cane in many other regions, including the Delta of the Mississippi River (Cronin et al., 2020; Knight et al., 2018). Roseau cane die-off was noticed in the state of Louisiana in Mississippi River Delta in 2016 (Cronin et al., 2020). Symptoms of die-off include large amounts of dead and dying stems, stunted stems, and many stands with low stem density (Knight et al., 2018). A scale insect, *Nipponaclerda biwakoensis*, which feeds on Roseau cane has been found in Louisiana and has been associated with Roseau cane die-off (Cronin et al., 2020; Knight et al., 2018). Because the scale insect has been associated with die-off, it is possible that other insects affect the health of Roseau cane. The European lineage is known to be the most resistant to the Roseau cane scales (Cronin et al., 2020).

When feeding, aphids have been known to decrease the health of many plant species (Watanabe et al., 2018). Aphid infestations are reported to cause damage to numerous types of plants and these damages have led to water stress, reduced plant growth, and wilting (Jaouannet et al., 2014). In a study, on the effect of aphids on okra (*Abelmoschus esculentus*) plants, aphids were found to negatively affect plant performance when present at a high density (Singh et al., 2021).
The mealy plum aphid, *Hyalopterous pruni*, has been reported to feed on Roseau cane and may affects its health (Lambert and Casagrande, 2007). Yellosing, curling, and wilting have been noticed on native Roseau cane plants with high aphid densities; however, these symptoms have rarely been noticed on non-native Roseau cane plants (Lambert and Casagrande, 2007).

Studies on the effect of the mealy plum aphid to Roseau cane have not been conducted. Because aphids are known to decrease the health of plants and have been observed on Roseau cane in the field, it is important to determine the damage they can cause and the possibility of being a contributing factor to die-off. Moreover, determining the level of tolerance of the Delta and the European lineages to *H. pruni* is important information that will complement what we know of their tolerance to the scale insect.

### 3.2. Objective

The objective of this research was to conduct laboratory-feeding experiments to determine the ability of *H. pruni* infestations to reproduce and cause damage to two Roseau cane lineages.

### 3.3. Materials and Methods

Two lineages of Roseau cane were used, Delta (haplotype EARL) and European (haplotype W96). Stock plants were provided by J. Cronin (Department of Biological Sciences, Louisiana State University). Roseau cane plants approximately 18.0 cm in height, propagated and grown in
a greenhouse (as described in Chapter 2 and Appendix A) were brought to the laboratory and used in aphid infestation experiments. Pots placed on water-flooded plastic trays and plants were grown under fluorescent lights (54W/120V 60Hz/4.0A Lamps at 17 h light and 7 h dark) with an average temperature of 23°C.

Twenty aphids were placed on several leaves of each Roseau cane lineage. The damage due to aphid infestation and aphid reproduction was recorded weekly during a five-week period. Five plants of each of these lineages were infested with aphids and five used as controls. Four trials were conducted for each lineage. The damage caused by the aphid on the leaves was evaluated by recording the percentage of browning/yellowing according to a scale reported by Heng-Moss et al., (2004). Damage was recorded once a week on a rating scale on 1-5; 1 = 10% or less browning/yellowing, 2 = 11 to 30% browning/yellowing, 3 = 31 to 50% browning/ yellowing, 4 = 51 to 70% browning/yellowing, and 5 = 71% or more browning/yellowing. To prevent aphids moving from plant to plant, a cage consisting of clear tubes, 8.5 cm in diameter and ranging from 46.0 to 80.0 cm in height was placed on each plant. Plastic tops with small holes were used to cover the tops on the tubes (Figure 3.1). Throughout the experiment, the number of aphids per plant and plant height was recorded. Graphs of the results were made in Microsoft Excel (Microsoft Corporation, 2018).
Figure 3.1. Roseau cane plants (with aphids) growing in pots placed on water-flooded plastic trays. Plants are covered with plastic cages to keep aphids restricted to their specific plant.

**Statistical analyses**

The following research hypotheses were proposed for this investigation:

1. The heights of plants without aphids differs from the heights of plants with aphids within the Delta lineage by the last week of the experiment.
2. The heights of plants without aphids differs from the heights of plants with aphids within the European lineage by the last week of the experiment.
3. The number of aphids on the plants has an impact on the height of the plants within the Delta lineage by the last week of the experiment.
4. The number of aphids on the plants has an impact on the height of the plants within the European lineage by the last week of the experiment.
5. The number of aphids on the plants affects the damage severity rating of the plants within the Delta lineage by the last week of the experiment.

6. The number of aphids on the plants affects the damage severity rating of the plants within the European lineage by the last week of the experiment.

7. The number of aphids on the plant is the same for the European lineage and the Delta lineage.

Data Analysis

All model significance was evaluated at the 0.05 alpha level. All models were fitted on R (R core team, 2021), using base R packages. The European and Delta lineage had separate models since other scientists have determined that there is variation in growth of Roseau cane by lineage (Knight et al., 2018). Separating the lineages can help to determine if one lineage may be affected by the predictor variables and the other may not be affected.

For hypotheses, one and two, t-tests were used to evaluate if there was a significant difference in the response variable, height, and the predictor variable, presence, or absence of aphids, at the end of the fifth week. This was done with the Delta lineage for hypothesis one and the European lineage for hypothesis two. The residuals were examined using histograms and scatterplots for each lineage and the linear model did not fit well across all values of the data. T-tests were selected because the response variable, height, follows a normal distribution and the predictor variable, presence, or absence of aphids, is categorical.
For hypotheses three and four, linear models were used to see if the predictor variable, number of aphids on the plant, had an impact on the response variable, height, by the end of the experiment. Since height was the dependent variable and it was normally distributed, a linear model was used. The residuals were examined using histograms and scatterplots for each lineage. Model residuals were examined, and the linear model fit well across all values of the data. This model was also selected because the predictor variable, number of aphids, is a quantitative variable.

For hypotheses five and six, a linear model for the predictor variable, number of aphids, and response variable, damage severity rating, at the end of week six was considered, but neither of them followed a normal distribution, so generalized linear models were considered. Since the response variable, damage severity rating, can only be the values 1, 2, 3, 4 or 5, it is count data with only whole numbers and no negative values. A Poisson generalized linear model would be a good model for this data. A Poisson generalized linear model was used to see if the predictor variable, number of aphids, effected the response variable, damage severity rating, by the last week. This was done for each lineage separately with the Delta lineage for hypothesis five and the European lineage for hypothesis six.

For hypothesis seven, a t-test was used to evaluate if there was a significant difference in the response variable, aphid count, and the predictor variable, lineage, at the end of the fifth week. T-tests were selected because the predictor variable was categorical.
3.4. Results

Roseau cane plants of both lineages showed damage in the form of foliar browning or yellowing (Figure 3.2). Some leaves became partially brown, usually starting from the tips of the leaf (Figure 3.2A). Some plants had some leaves turn completely yellow (Figure 3.3A) or brown (Figure 3.3B). Some of the Delta lineage plants showed a completely brown foliage by the end of the experiments (Figure 3.3C). By the end of the experiments, the Delta lineage with aphids exhibited more severe damage than the European lineage (Figure 3.4). On the Delta lineage, aphids reproduced more efficiently resulting in significant higher number when compared to the European lineage of Roseau cane (Figure 3.5).

Figure 3.2. Roseau cane plant leaves showing typical browning (A) and chlorosis (B) caused by mealy plum aphid feeding.
Figure 3.3. Roseau cane plants showing damage from mealy plum aphid feeding. Plant showing yellowing of the leaves (A), plant showing limited browning (B), and plant completely brown (C).

For both the Delta (p = 0.7975, t-statistic = -0.2586) and European (p = 0.2296, t-statistic = -1.2212) lineage there was not a statistically significant difference between the heights of plants with and without aphids (Figure 3.4). The number of aphids on the plant did not have a statistically significant difference for either lineage by the last week (fifth week) of the experiments (For Delta lineage p = 0.2274, F-statistic = 1.562 and for European lineage p = 0.2136, F-statistic = 1.662) (Figure 3.5). Similarly, there was no statistically significant effect of the number of aphids on damage severity rating of the plants within the European lineage (p = 0.5172, Standard error = 0.0335) or the Delta lineage (p = 0.7050, Standard error = 0.0039) by the last week of the experiments. The number of aphids on the European lineage was statistically
significantly \((p= 5.119 \times 10^{-5}, t\text{-statistic}= 5.1064)\) lower than the number of aphids on the Delta lineage on the last week of the experiments. The mean of the damage at the end of the fifth week for the Delta lineage plants with aphids was 3.35 with a standard deviation of 1.4965, whereas the mean of the damage for the European lineage plants with aphids was 1.85 with a standard deviation of 0.5871 (Figure 3.6). The mean of the damage at the end of the fifth week for the Delta lineage plants without aphids was 1.65 and whereas the mean of the damage for the European lineage plants without aphids was 1.5. Overall, the Delta lineage had more aphids each week (after the first week) than the European lineage. Damage was more severe on the Delta lineage than the European lineage. In addition, the Delta lineage had faster aphid population increase than the European lineage. The heights of the plants were not affected by the presence or absence of aphids because the height differences were not statistically significant from the control plants (Figure 3.4).
Figure 3.4. Weekly height of two Roseau cane lineages, with and without mealy plum aphids. Height is the average of four experiments, each with five plants per treatment and control. Error bars represent the calculated standard error.

Figure 3.5. Weekly count of mealy plum aphids on two Roseau cane lineages after initially placing 20 aphids. Aphid number is the average of four experiments. Each week was statistically analyzed separately with t-tests. There is a statistically significant difference between a and b. There is no statistically significant difference between same letters on the same week. Aphid count is the average of four experiments, each with five plants per treatment and control. Error bars represent the calculated standard error.
Figure 3.6. Damage (percent of browning/yellowing) to two Roseau cane lineages (Delta and European) caused by mealy plum aphid feeding during a five-week period. Damage is the average of four experiments, each with five plants per treatment and control. Damage was measured on a rating scale of 1-5; 1 = 10% or less browning/yellowing, 2 = 11 to 30% browning/yellowing, 3 = 31 to 50% browning/yellowing, 4 = 51 to 70% browning/yellowing, and 5 = 71% or more browning/yellowing. Error bars represent the calculated standard error.

3.5. Discussion

Aphid reproduction and feeding experiments were conducted using two lineages of Roseau cane. Overall, aphid reproduction was better when they fed on the Delta lineage and caused more damage to this lineage. By the end of the experiments, the number of aphids in the Delta lineage was statistically significantly higher than the number of aphids on the European lineage. This explains why the Delta lineage plants had higher damage severity ratings than the European lineage. In a study conducted by Cronin et al., (2020), the European lineage of Roseau cane was found to be more resistant to the insect scales than other lineages tested. Results of the feeding
experiments using the mealy plum aphid suggest that the European lineage is also more resistant to aphids. Lambert and Casagrande (2007) noticed foliage yellowing, curling, and wilting on native Roseau cane plants with high aphid densities, but rarely noticed that on non-native Roseau cane plants. These observations are consistent with the European lineage being more resistant to the mealy plum aphid. The height of plants with aphids for both lineages was not statistically significant when compared with plants without aphids. This result is surprising because in general, insect herbivore damage tends to slow the plant growth.

There have been several studies on the factors associated with Roseau cane die-off in Louisiana (Cronin et al., 2020; Knight et al., 2018). Some scientists have suggested replacing the Delta lineage with the European lineage since it is more resistant to scales, which is an insect pest that has been associated with the Roseau cane die-off (Cronin et al., 2020). Nevertheless, in many crops, resistant genes to aphids have been used but new aphid biotypes have developed and overcome the resistance (Jaouannet et al., 2014). Similarly, even if the European lineage of Roseau cane is more resistant to aphids now, it is possible that aphids will adapt to this lineage and therefore render it more susceptible to mealy plum aphid damage.
CHAPTER IV. INOCULATION EXPERIMENTS TO DETERMINE THE DAMAGE CAUSED TO ROSEAU CANE BY *BIPOLARIS YAMADAE*

4.1. Introduction

Roseau cane (*Phragmites australis*) is a perennial plant in the family Poaceae. In some areas, this plant is considered invasive; however, in Louisiana, it protects marshes from waves and storms and stops sediment from filling navigation channels and therefore it is an essential part of the marsh (Knight *et al.*, 2018). This plant helps stop the expansion of invasive plants such as alligator weed and elephant ear (LSU AgCenter, 2019). Because Roseau cane can survive through harsh conditions and pollutants, in some areas, it is used to treat agricultural and industrial wastewaters and remove harmful microorganisms (Gigante *et al.*, 2014). Roseau cane has been used as food for animals, habitat for wildlife, material to make musical instruments, and baskets (Ilbagi, 2006).

Alligators are economically important wildlife in Louisiana, and the American Alligator (*Alligator mississippiensis*) is known to nest in Roseau cane (Merchant *et al.*, 2018). Taking away an animal’s nesting plant away can be detrimental to that species. White-tailed deer (*Odocoileus virginianus*) at the Pass-a-Loutre Wildlife Management Area in Louisiana used Roseau cane for habitat cover (Baker *et al.*, 2018). Losing a plant that these deer hide in can be harmful to their population size, which ultimately would cause issues in the hunting industry. Migratory birds are also important wildlife to hunter and just like white-tailed deer; migratory birds use Roseau cane as habitat (Cronin, 2020). The fishing industry would also be impacted by losing Roseau cane because prey fish species use Roseau cane as habitat (Cronin, 2020). The Mississippi River Delta has an extremely high level of biodiversity, which makes it habitat for
animals that usually would not be found together (Hester et al., 2005). Roseau cane is listed as a dominant plant species in this area, meaning losing this species can largely change the habitat and animals that survive there (Hester et al., 2005).

In many parts of the world, Roseau cane has been declining significantly. This decline has been called reed die-back syndrome (Coppi, 2018), Roseau cane die-back or Roseau cane die-off (Knight et al., 2018). It was first noticed in Central European countries, but now it is happening to Roseau cane in many other regions, including the Delta of the Mississippi River (Cronin et al., 2020; Knight et al., 2018). Roseau cane die-off was noticed in the state of Louisiana in Mississippi River Delta in 2016 (Cronin et al., 2020). Symptoms of die-off include large amounts of dead and dying stems, stunted stems, and many stands with low stem density (Knight et al., 2018). A scale insect, Nipponaclerda biwakoensis, which feeds on Roseau cane, has been found in Louisiana and it has been associated with Roseau cane die-off (Cronin et al., 2020; Knight et al., 2018). Because the scale insect has been associated with the die-off, it is possible that other insects will affect the health of Roseau cane.

Plant pathogens, such as the oomycetes Pythiogeton spp. and Pythium spp., have been isolated from Roseau cane affected by die-off (Cerri et al., 2017; Croker et al., 2015; Nechwatal et al., 2008). In a study by Allen et al. (2020), 84 foliar fungal taxa were detected from P. australis. In their investigation, the European lineage was the least susceptible to three out of the four pathogens that they tested, but they noted that in their common garden experiment, the damage by fungal pathogens in the field was similar between all lineages. In Louisiana, isolates of Alternaria alternata, Bipolaris yamadae, Fusarium coicus, Fusarium sulawense and Nigrospora
osmanthi have been obtained from foliar tissues of Roseau cane (Galo, 2021). These fungi caused disease to Roseau cane in detached leaf experiments (Galo, 2021).

Fungal species of the Bipolaris genus are known to be distributed worldwide. Species in this genus have been associated with leaf spots, leaf blights, melting outs, root rots, and foot rots in plants in the family Poaceae (Manamgoda et al., 2014). Losses due to pathogens in the genus Biopolaris have occurred in economically important crops such as rice, maize, wheat, and sorghum. (Manamgoda et al., 2014). In potato dextrose agar at 23 ºC, Bipolaris colonies grow relatively fast and are a grey to brownish black color. The conidia are known to be curved, canoe-shaped or obclavate (Ellis, 2016). The pigmentation of the conidia is hyaline to dark greenish yellow (Ellis, 2016). Conidia are also reported to be brown in color, smooth, two to four septate, cylindrical, rounded at both ends and measuring 14.0-30.0 x 6.5-12.0 μm in size (Lin et al., 2012). Bipolaris bicolor produces phytotoxins that are known to affect plants and cause leaf blight. This fungus is known to inhibit the growth of rice seedlings (Miyagawa et al., 1994). Sugarcane is another economically important crop that is affected by a Bipolaris species, B. spicifera that causes leaf spot disease (Lin et al., 2012). Interestingly, the same species that infect sugar cane has also been reported to infect immunocompromised humans causing fungus balls in the sinuses (Buzina et al., 2003). In Roseau cane, several Bipolaris species have been reported to cause leaf lesions. A study on mature leaf and seedling leaf assays concluded that B. sorokiniana is a pathogen that can infect Roseau cane (Devries et al., 2020). This fungus species causes leaf spots on Guinea grass in Florida (Adhikari et al., 2021). On this plant the lesions are oblong to irregular shaped and have brownish to dark grayish centers with pale yellowish to brownish black outside margins. On Panicum sp. leaf spots caused by B. yamadae are known to be ovoid
or oblong shaped and a brown color with an irregular concentric zone (Manamgoda et al., 2014). Recently, in Louisiana, *B. yamadae* has been identified as a fungus causing leaf spots in Roseau cane (Galo, 2021).

### 4.2. Objective

The objective of this investigation was to conduct laboratory inoculation experiments to determine the damage caused to Roseau cane by the fungus *B. yamadae*.

### 4.3. Materials and Methods

#### 4.3.1. Plant materials

In this investigation, two lineages of Roseau cane were used, Delta (haplotype EARL) and European (haplotype W96). Plants were provided by J. Cronin (Department of Biological Sciences, Louisiana State University). Roseau cane plants approximately 18.0 cm in height, propagated and grown in a greenhouse (as described in Chapter 2 and Appendix A) were brought to the laboratory and used in aphid infestation experiments. Pots placed on water-flooded plastic trays and plants were grown under fluorescent lights (54W/120V 60Hz/4.0A Lamps at 17 h light and 7 h dark) with an average temperature of 23°C.

#### 4.3.2. Isolation of fungi

A Roseau cane isolate of *B. yamadae* kindly provided by D. Galo (Department of Plant Pathology and Crop Physiology, Louisiana State University) was used in this investigation. This isolate was collected from Roseau cane Delta lineage plants showing symptoms that were found
in Plaquemine, Louisiana. The fungus was increased by sub-culturing in potato dextrose agar (PDA) plates (8.5 cm x 8.5 cm). Plates were incubated at room temperature (21 ºC) under 14-hour light conditions and inspected for mycelial growth and spore production. For long term storage, the fungus was stored in 30% glycerol at -70 ºC. Spores from colonies (Figure 4.1) were used in plant inoculations.

Figure 4.1. Colony of *Bipolaris yamadae* growing on a petri dish containing potato dextrose agar after one month.

**4.3.3. Inoculation of *Bipolaris yamadae***

Roseau cane plants of approximately 18.0 cm in height were used in the experiments. Seven to 10 days-old *B. yamadae* colonies in petri dishes containing sporulating fungi were filled with autoclaved water and a microscope slide was used to release the *B. yamadae* spores into the water. Two layers of cheesecloth (Veratec, Inc. Walpole, MA) was used to filter the spore
suspension and a hemocytometer was used to quantify the spores. Inoculum was diluted to a final concentration of $1 \times 10^6$ spores/ml, and tween 20 (0.01% vol/vol) was added to the spore suspensions (Mould et al., 1991). Spores were sprayed on the foliage of 20 Roseau cane plants of each lineage (Figure 4.2). Control plants were sprayed with water. Plastic bags were placed over the plants for two days to provide a humid environment (Figure 4.3). Damage was evaluated weekly over a five-week period by recording the number and size of the leaf lesions. Leaf lesions under 1.0 mm were recorded as small leaf lesions and counted per leaf. All other leaf lesions were measured and recorded. Whole plants were evaluated, but the data recording was separated by leaf starting at the top leaf and ending at the bottom leaf. The numbers and color of lesions and the presence of yellow margins were recorded. Four trials were conducted. After the lesions were present, re-isolations were conducted. Colony morphology was examined to confirm the fungus on the plant was still *B. yamadae*.

Figure 4.2. Spray bottle containing a spore suspension of *Bipolaris yamadae* (A) and *B. yamadae* spores observed with a light microscope (B).
The following research hypothesis was proposed for this investigation:

The fungus *B. yamadae* causes the same number of leaf lesions on the European and Delta lineages.

All model significance was evaluated at the 0.05 alpha level. All models were fit in R (R core team, 2021), using base R packages. For the hypothesis, a t-test was used to evaluate if there was a significant difference in the response variable, leaf lesion count, and the predictor variable, lineage, at the end of the fifth week. T-tests were selected because predictor variable is categorical. The data was skewed right.
4.4. Results

The foliage of most of the plants of both lineages inoculated with *B. yamadae* exhibited brownish/black leaf lesions (Figure 4.4B) which appeared between two to four days after spraying. As infection progressed, a yellow margin developed around the lesions (Figure 4.4C). During later stages of the infection, the lesions increased in size and sometimes coalesced (Figure 4.4C). Both lineages developed similar symptoms. There was a statistically significant (p= 2.5 e-06, t-statistic= 5.5973) difference between the Delta and European lineage in the number of leaf lesions. The Delta lineage had more lesions than the European lineage (Figure 4.5). When separated by size of the lesion, the Delta lineage had more leaf lesions under 2.0 mm than the European, but the number of larger lesions in both lineages was similar (Figure 4.6).
Figure 4.4. Symptoms caused by *Bipolaris yamadae* on the foliage of Roseau cane plants. Healthy leaf (A), small brownish/black leaf lesions (B), and leaf with coalesced brown leaf lesions and a yellow margin (C).
Figure 4.5. Effect of Bipolaris yamadae on two lineages of Roseau cane. Numbers are the average of lesions caused by the fungus in four trials at the end of the experiments. There is a statistically significant difference between a and b. Lesion count is the average of four experiments, each with five plants per treatment and control. Error bars represent the calculated standard error.
Figure 4.6. Size and number of lesions caused by Bipolaris yamadae on leaves of two lineages of Roseau cane. Results are the average of lesion size at the end of the experiments in four trials. Each size category was statistically analyzed separately with t-tests. There is a statistically significant difference between a and b. There is no statistically significant difference between same letters on the same week. Lesion count is the average of four experiments, each with five plants per treatment and control. Error bars represent the calculated standard error.

4.5. Discussion

In this investigation, two Roseau cane lineages were evaluated for their response to infection by B. yamadae. The number of lesions and lesion size were recorded. There was statistically significant difference between the number of leaf lesions on the European versus the Delta lineage. More leaf lesion damage was obtained on the European lineage. Galo (2021) isolated and identified B. yamadae from Roseau cane growing in die-off areas of the Mississippi River Delta and used it in inoculation experiments. The results of this investigation are similar to the
results of \textit{B. yamadae}-inoculation experiments conducted by Galo, (2021). In Galo’s investigation, the Delta and Gulf lineages exhibited more disease severity than the European lineage.

In some plant hosts, \textit{B. yamadae} can cause lesions that are oblong to irregular shaped and have brownish to dark grayish centers with pale yellowish to brownish black outside margins (Adhikari \textit{et al.}, 2021). The lesions observed in this investigation were brown in color and sometimes had yellowish margins. On \textit{Pancium} species, leaf spots caused by \textit{B. yamadae} are reported to be ovoid or oblong shaped and a brown color with an irregular concentric zone (Manamgoda \textit{et al.}, 2014). This is consistent with the brown ovoid and oblong lesions formed in this experiment. In Allen \textit{et al.}, (2020) inoculated Roseau cane lineages with four plant pathogens and the European lineage was the least susceptible of the three pathogens they tested. That is similar to the results of this investigation, because \textit{B. yamadae} has more lesions on the Delta lineage than the European lineage.

For smaller lesions size categories (less than 1 mm and 1-1.99 mm) there was a significant difference between the Delta and European lineage, but in larger lesion size categories (2-2.99 mm and over 3 mm) there was not. Since both lineages were to be susceptible to large lesions this means both are impacted by large areas of damage.
CHAPTER V. DAMAGE CAUSED TO ROSEAU CANE BY THE SIMULTANEOUS INFESTATION AND INFECTION OF HYALOPTERUS PRUNI AND BIPOLARIS YAMADAE RESPECTIVELY

5.1. Introduction

Roseau cane (*Phragmites australis*) is a perennial plant in the family Poaceae. In some areas, this plant is considered invasive; however, in Louisiana, it protects marshes from waves and storms and stops sediment from filling navigation channels and therefore it is an essential part of the marsh (Knight *et al.*, 2018). This plant helps stop the expansion of invasive plants such as alligator weed and elephant ear (LSU AgCenter, 2019). Because Roseau cane can survive through harsh conditions and pollutants, in some areas, it is used to treat agricultural and industrial wastewaters and remove harmful microorganisms (Gigante *et al.*, 2014). Roseau cane has been used as food for animals, habitat for wildlife, material to make musical instruments, and baskets (Ilbagi, 2006).

Alligators are economically important wildlife in Louisiana, and the American Alligator (*Alligator mississippiensis*) is known to nest in Roseau cane (Merchant *et al.*, 2018). Taking away an animal’s nesting plant away can be detrimental to that species. White-tailed deer (*Odocoileus virginianus*) at the Pass-a-Loutre Wildlife Management Area in Louisiana used Roseau cane for habitat cover (Baker *et al.*, 2018). Losing a plant that these deer hide in can be harmful to their population size, which ultimately would cause issues in the hunting industry.

Migratory birds are also important wildlife to hunter and just like white-tailed deer; migratory birds use Roseau cane as habitat (Cronin, 2020). The fishing industry would also be impacted by losing Roseau cane because prey fish species use Roseau cane as habitat (Cronin, 2020).
Mississippi River Delta has an extremely high level of biodiversity, which makes it habitat for animals that usually would not be found together (Hester et al., 2005). Roseau cane is listed as a dominant plant species in this area, meaning losing this species can largely change the habitat and animals that survive there (Hester et al., 2005).

In many parts of the world, Roseau cane has been declining significantly. This decline has been called reed die-back syndrome (Coppi, 2018), Roseau cane die-back or Roseau cane die-off (Knight et al., 2018). It was first noticed in Central European countries, but now it is happening to Roseau cane in many other regions, including the Delta of the Mississippi River (Cronin et al., 2020; Knight et al., 2018). Roseau cane die-off was noticed in the state of Louisiana in Mississippi River Delta in 2016 (Cronin et al., 2020). Symptoms of die-off include large amounts of dead and dying stems, stunted stems, and many stands with low stem density (Knight et al., 2018). A scale insect, Nipponaclerda biwakoensis, which feeds on Roseau cane has been found in Louisiana and it has been associated with Roseau cane die-off (Cronin et al., 2020; Knight et al., 2018). Because the scale insect has been associated with the die-off, it is possible that other insects will affect the health of Roseau cane.

When feeding, aphids have been known to decrease the health of many plant species (Watanabe et al., 2018). Aphid infestations are reported to cause damage to numerous types of plants and these damages may lead to water stress, reduced plant growth, and wilting (Jaouannet et al., 2014). In a study, on the effect of aphids on okra (Abelmoschus esculentus) plants, aphids were found to negatively impact plant performance when present at a high density (Singh et al., 2021). The mealy plum aphid, Hyalopterous pruni, has been reported to feed on Roseau cane and
therefore it may be causing health problems (Lambert and Casagrande, 2007). Yellowing, curling, and wilting have been noticed on native Roseau cane plants with high aphid densities; however, these symptoms have rarely been noticed on non-native Roseau cane plants (Lambert and Casagrande, 2007).

Fungal species of the *Bipolaris* genus are known to be distributed worldwide. Species in this genus have been associated with leaf spots, leaf blights, melting outs, root rots, and foot rots in plants in the family *Poaceae* (Manamgoda *et al*., 2014). Losses due to pathogens in the genus *Bipolaris* have occurred in economically important crops such as rice, maize, wheat, and sorghum. (Manamgoda *et al*., 2014). In potato dextrose agar at 23 °C, *Bipolaris* colonies grow relatively fast and are a grey to brownish black color. The conidia are known to be curved, canoe-shaped or obclavate (Ellis, 2016). The pigmentation of the conidia is hyaline to dark greenish yellow (Ellis, 2016). Conidia are also reported to be brown in color, smooth, two to four septate, cylindrical, rounded at both ends and measuring 14.0-30.0 x 6.5-12.0 μm in size (Lin *et al*., 2012). *Bipolaris bicolor* produces phytotoxins that are known to affect plants and cause leaf blight. This fungus is known to inhibit the growth of rice seedlings (Miyagawa *et al*., 1994). Sugarcane is another economically important crop that is affected by a *Bipolaris* species, *B. spicifera*, that causes leaf spot disease (Lin *et al*., 2012). Interestingly, the same species that infect sugar cane has also been reported to infect immunocompromised humans causing fungus balls in the sinuses (Buzina *et al*., 2003). In Roseau cane, several *Bipolaris* species have been reported to cause leaf lesions. A study on mature leaf and seedling leaf assays concluded that *B. sorokiniana* is a pathogen that can infect Roseau cane (Devries *et al*., 2020). This fungus species causes leaf spots on Guinea grass in Florida (Adhikari *et al*., 2021). On this plant the lesions are
oblong to irregular shaped and have brownish to dark grayish centers with pale yellowish to brownish black outside margins. On Panicum sp. leaf spots caused by B. yamadae are known to be ovoid or oblong shaped and a brown color with an irregular concentric zone (Manamgoda et al., 2014). Recently, in Louisiana, B. yamadae has been identified as a fungus causing leaf spots in Roseau cane (Galo, 2021).

It has been reported that fungi and aphids may impact each other while on the same host plant. In a study conducted on aphids influence on soil fungal communities, aphids increased the evenness and abundance of fungi (Wilkinson et al., 2019). In another study, the beech blight aphid (Grylloprociphilus imbricator) decreased the health of trees from its interaction with sooty mold (caused by several ascomycete fungi) by weakening the leaf function (Cook-Patton et al., 2014). An interest of this study was to see if aphids and a fungus interact on Roseau cane. Bipolaris yamadae was chosen as the fungus because it has defined lesions that are distinguishable from the mealy plum aphid damage. From results obtained in Chapters 3 and 4, both H. pruni and B. yamadae alone can cause foliar symptoms on Roseau cane. Determining their combined effect by placing them both on the same plant should be investigated. It is possible that this will enhance or decrease the impacts that they alone have on the plant.

5.2. Objective

The objective of this research was to evaluate the damage caused to Roseau cane by the simultaneous infection and infestation of B. yamadae and the mealy plum aphid respectively.
5.3. Materials and Methods

Two lineages of Roseau cane were used, Delta (haplotype EARL) and European (haplotype W96). Stock plants were provided by J. Cronin (Department of Biological Sciences, Louisiana State University). Roseau cane plants approximately 18.0 cm in height, propagated and grown in a greenhouse (as described in Chapter 2 and Appendix A) were brought to the laboratory and used in aphid infestation experiments. Pots placed on water-flooded plastic trays and plants were grown under fluorescent lights (54W/120V 60Hz/4.0A Lamps at 17 h light and 7 h dark) with an average temperature of 23°C.

Seven to 10 days-old *B. yamadae* colonies in petri dishes containing sporulating fungi were filled with autoclaved water. A microscope slide was used to rub on the *B. yamadae* colonies to release the spores into the water. Two layers of cheesecloth (Veratec, Inc. Walpole, MA.) was used to cover a beaker and the spores in the petri dish poured in the beaker. A hemocytometer was used to obtain a spore count. Inoculum was diluted to a final concentration of 1x10^6 spores/ml, and tween 20 (0.01% vol/vol) was added to the spore suspensions (Mould *et al.*, 1991). Spores were sprayed on the foliage of 20 Roseau cane plants of each lineage, with 20 plants were sprayed with water as control. Plastic bags were placed over the plants for two days to provide a humid environment. Damage was evaluated weekly over a five-week period by recording the number and size of the leaf lesions. Leaf lesions under 1.0 mm were recorded as small leaf lesions and counted per leaf. All other leaf lesions were measured and recorded. Whole plants were evaluated, but the data recording was separated by leaf starting at the top leaf and ending at the
bottom leaf. The numbers and color of lesions and the presence of yellow margins were recorded. Four trials were conducted

Two days after the *B. yamadae* spores were sprayed on the leaves of twenty total plants (Figure 5.1A), 20 aphids were placed on the leaves of each of the EARL and W96 haplotypes of Roseau cane. The aphids were placed after the spores were sprayed because placing aphid first and then applying the fungus spores may affect the aphid behavior or even may kill the aphids. To prevent aphids moving from plant to plant, clear tubes (8.5 cm diameter and ranging from 46.0 to 80.0 cm in height) were placed on each plant (as described in Chapter 3, Figure 5.1B). The damage caused by the aphid on the leaves was evaluated by recording browning/yellowing damage. Aphids were not placed in control plants. Damage was analyzed once a week on a scale on 1-5; 1 = 10% or less browning/yellowing, 2 = 11 to 30% browning/yellowing, 3 = 31 to 50% browning/yellowing, 4 = 51 to 70% browning/yellowing, and 5 = 71% or more browning/yellowing. The damage by *B. yamadae* was obtained by recording the number and size of the leaf lesions. Leaf lesions under 1.0 mm by 1.0 mm were recorded as small leaf lesions and counted per leaf. All other leaf lesions were measured and recorded. All the larger leaf lesions were then recorded in the categories 1.0 mm-1.99 mm, 2.0 mm- 2.99 mm and or over 3.0 mm. Five plants of each of the lineages contained the aphids and fungus infections and five were used as controls. Four trials for each lineage were conducted. Graphs of the results were made in Microsoft Excel (Microsoft Corporation, 2018). The results of the damage over a five-week period were compared to the damage caused by the aphids and fungus alone.
Figure 5.1. Images showing *Bipolaris yamadae* inoculation (A) and subsequent addition of mealy plum aphids contained with cages (B).

**Research hypothesis and statistical analyses**

The following research hypotheses were proposed for this investigation

1. The heights of plants without aphids differs from the heights of plants with aphids within the Delta lineage by the last week of the experiment.

2. The heights of plants without aphids differs from the heights of plants with aphids within the European lineage by the last week of the experiment.

3. The number of aphids is the same for both the European and Delta lineage.

4. The number of leaf lesions is the same for both the European and Delta lineage.

5. The number of aphids in the Delta lineage in experiments with aphids alone is the same as the number of aphids in the Delta lineage in the experiments with aphids plus the fungus.

6. The number of aphids in the European lineage in experiments with aphids alone is the same as the number of aphids in the European lineage in the experiments with aphids plus the fungus.
7. The number of leaf lesions in the Delta lineage in experiments with the fungus alone is the same as the number of leaf lesions in the Delta lineage in the experiments with aphids plus the fungus.

8. The number of leaf lesions in the European lineage in experiments with the fungus alone is the same as the number of leaf lesions in the European lineage in the experiments with aphids plus the fungus.

All model significance was evaluated at the 0.05 alpha level. All models were fit in R (R core team, 2021). The European and Delta lineage had separate models for heights since other scientists have determined that there is variation in growth of Roseau cane by lineage (Knight et al., 2018). Separating the lineages can help to determine if one lineage may be affected by the predictor variables and the other may not be affected.

For hypotheses one and two, $t$-tests were used to evaluate if there was a significant difference in the response variable, height, and the predictor variable, presence, or absence of aphids, at the end of the fifth week. This was done with the Delta lineage for hypothesis one and the European lineage for hypothesis two. $t$-tests were selected because the response variable, height, follows a normal distribution and the predictor variable, presence, or absence of aphids, is categorical.

For hypothesis three and four $t$-tests were used to evaluate if there was a significant difference in the response variable, aphid count or leaf lesion count, and the predictor variable, lineage, at the end of the fifth week. $T$-tests were selected because predictor variable is categorical. For hypothesis five, six, seven and eight four $t$-tests were used to evaluate if there was a significant
5.4. Results

The damage caused to Roseau cane plants by simultaneous infection and infestation by *B. yamadae* and the mealy plum aphid respectively was like the damage caused by aphids and the fungus respectively described in the result sections of Chapters 3 and 4. Aphid feeding caused yellowing and browning. Infections by *B. yamadae* caused circular to oval brown leaf lesions with yellow margins. Some of the Roseau cane with aphids and the fungus exhibited more coalescing lesions caused by the fungus and higher number of total leaf lesions compared to the *B. yamadae* alone trials (Figure 5.2). The Delta lineage with aphids and the fungus had more severe damage than the control and the European lineage by the five week of the experiments (Figure 5.3). Throughout weeks 2-5, aphids reproduced statistically significant in higher numbers on the Delta lineage than on the European lineage (Figure 5.4). For both lineages there was not a statistically significant difference in plant heights of the control plants compared to the experimental plants (For the Delta lineage p= 0.1930, t-statistic= -1.3317 and for the European lineage p= 0.2757, t-statistic= -1.1061). The heights of the plants were both lower for plants with aphids and fungi than control plants, but this was not statistically significant (Figure 5.5). The Delta lineage had more leaf lesions than the European lineage, but there was not a statistically significant difference (p= 0.1354, t-statistic= 1.5255) (Figure 5.6). The Delta lineage had more lesions for all size categories (under 1.0 mm, 1.0 mm-1.99 mm, 2.0 mm-2.99 mm and over 3.0 mm) than the European lineage but had the largest difference between lineages in number of
lesions sized 1.0 mm - 1.99 mm (Figure 5.7). For both the Delta (p = 0.8353, t-statistic = 0.2097) and the European (p = 0.1072, t-statistic = -1.6830) lineage, there was not a statistically significant difference in the number of aphids in aphid alone trials compared to the number of aphids in the aphids plus fungus experiments. For the Delta lineage number of leaf spots in the fungus alone compared to the aphids plus fungus experiments was not statistically significant (p = 0.2101, t-statistic = -1.2838). In contrast, for the European lineage the difference of these parameters was statistically significant (p = 0.0175, t-statistic = -2.5537).
Figure 5.2. Roseau cane leaves showing lesions and yellowing/browning caused by *Bipolaris yamadae* infections and *Hyalopterous pruni* infestations respectively.

![Figure 5.2](image)

Figure 5.3. Damage caused by the mealy plum aphid during a five-week period to plants of two lineages of Roseau cane (Delta and European) after inoculation with *Bipolaris yamadae*. Data is the average of four experiments, each with five plants per treatment and control. Damage by the aphid was on a rating scale of 1-5; 1 = 10% or less browning/yellowing, 2 = 11 to 30% browning/yellowing, 3 = 31 to 50% browning/yellowing, 4 = 51 to 70% browning/yellowing, and 5 = 71% or more browning/yellowing. Error bars represent the calculated standard error.

![Figure 5.3](image)
Figure 5.4. Number of mealy plum aphids colonizing two lineages of Roseau cane inoculated with *Bipolaris yamadae*, after a five-week period. Data is the average of four trials. Each week was separately statistically analyzed with t-tests. There is a statistically significant difference between a and b. There is no statistically significant difference between same letters on the same week. Aphid count is the average of four experiments, each with five plants per treatment and control. Error bars represent the calculated standard error.
Figure 5.5. Combined effect of the mealy plum aphid and *Bipolaris yamadae* on the height of two lineages of Roseau cane during a five-week period. Data is the average of four trials. Height is the average of four experiments, each with five plants per treatment and control. Error bars represent the calculated standard error.
Figure 5.6. Average number of lesions caused by *Bipolaris yamadae* to two lineages of Roseau cane (Delta and European) infested with mealy plum aphids, at the end of the experiments. There is no statistically significant difference between same letters. Lesion count is the average of four experiments, each with five plants per treatment and control. Error bars represent the calculated standard error.
Figure 5.7. Lesion number and size caused by *Bipolaris yamadae* to two lineages of Roseau cane infested with mealy plum aphids, at the end of the experiments. Each week was separately statistically analyzed with t-tests. There is a statistically significant difference between a and b. There is no statistically significant difference between same letters on the same week. Lesion count is the average of four experiments, each with five plants per treatment and control. Error bars represent the calculated standard error.

5.5. Discussion

Simultaneous insect herbivore feeding, and fungal infections can cause more severe damage to plants (Wilkinson *et al.*, 2019). In a study conducted on aphids influence on soil fungal communities, aphids increased the evenness and abundance of fungi (Wilkinson *et al.*, 2019). In the *H. pruni* and *B. yamadae* feeding and inoculation combined experiments there were more leaf lesions than in the *B. yamadae* alone trials. One explanation for this is that the aphids may have spread the fungus inoculum like the spread to sooty mold (Cook-Patton *et al.*, 2014). On the other hand, it has been reported that the green peach aphid, *Myzus persicae*, feeding on plants without the plant pathogen *Botrytis cinerea* had lower fecundity, were smaller, and had a lower survival time than aphids on plants with *B. cinerea* (Ngah *et al.*, 2018). Another aphid, the pea aphid, *Acyrthosiphon pisum* also benefitted from being on a host plant infected with *B. cinerea* (Srisakrapikoop *et al.*, 2021). In contrast the black bean aphid, *Aphis fabae* had reduced fecundity, a lower population growth rate, were smaller, and had a lower survival time (Srisakrapikoop *et al.*, 2021). Unlike the relationships of *M. persicae, A. pisum*, and *A. fabae* with *B. cinerea, H. pruni* did not have a statistically significant difference in number of aphids present on either lineage when placed on the plant with *B. yamadae*. 
In inoculation experiments of *B. yamadae* alone and combined with aphid infestation, leaf lesions and yellowing/browning were obtained. The aphid damage caused in the simultaneous fungus infection and aphid infestation experiments was not different to the damage caused by the aphid infestations alone which is described in Chapter 3. Nevertheless, as pointed out above, the number of lesions caused by the fungus was higher when aphids were present. The fact that the Delta lineage had more aphids and more severe aphid damage than the European lineage was not surprising. Similar results were obtained in the experiments of Chapter 3, which consisted of no significant different in the heights of the control plants compared to experimental plants, more aphids reproduced on the Delta lineage and the Delta lineage had more severe damage than the European lineage. Unlike results obtained after inoculating *B. yamadae* alone (Chapter 4), the total number of leaf lesions was not statistically significant between the European and Delta lineage. In results obtained by Allen *et al.*, (2020), the European lineage was the least susceptible to three out of the four pathogens (*Stagonospora* sp., two different *Cladosporium* sp. and *Alternaria alternata*) that they tested. However, they noted that in their common garden experiment, the damage in the field was similar between all lineages. Plants in their common garden experiments may have had similar damage because other factors, such as insects, could have been involved. That would be consistent with this experiment where in the *B. yamadae* alone experiments there was a difference between lineages, but when another factor (the mealy plum aphids) was added then there was not a statistically significant difference between lineages. The results indicate that aphids can coexist with *B. yamadae* and that their combined effect to the two lineages did not increase aphid damage but increased *B. yamadae* lesion count in the European lineage.
CONCLUSIONS

After developing methods for successful Roseau cane cultivation in the greenhouse and laboratory, experiments were conducted to study the effect of two biotic stresses on Roseau cane: the mealy plum aphid (*Hyalopterus pruni*) and the fungus *Bipolaris yamadae*. The individual and combined effect of these two stressors were evaluated.

A mealy plum aphid colony was successfully established in the laboratory, and the aphid species identified. The colony was established by using a single aphid that reproduced via parthenogenesis after being placed on a Roseau cane plant. The identification was conducted by examination of the morphological characteristics of aphids collected from Roseau cane and using a dichotomous key (Rakauskas et al., 2013). Moreover, to confirm the aphid identity, polymerase chain reaction (PCR) experiments were conducted using species-specific primers reported by Loizer et al., (2007) and Simon et al., (2006).

The mealy plum aphid feeding did not affect the height of two lineages of Roseau cane plants. Nevertheless, the feeding damage was more severe on the Delta lineage than the European lineage. Aphids reproduced more efficiently and reached higher numbers when they fed on plants of the Delta lineage than when they fed on the European lineage of Roseau cane.

Inoculations of two Roseau cane with *B. yamadae* caused brown leaf lesions that later developed yellow margins. Although both lineages showed lesions after being inoculated with *B. yamadae*, infections by this fungus caused more lesions in the Delta lineage than in the European lineage.
Both lineages had more smaller-sized lesions than larger-sized ones. Most plants in both lineages displayed lesions on multiple leaves of the plants.

Simultaneous feeding of the mealy plum aphids and infections by *B. yamadae* in two Roseau cane lineages did not affect the height of the plants. In plants infected with *B. yamadae*, the mealy plum aphid reproduced more efficiently and caused more damage to plants of the Delta lineage than to plants of the European lineage. Leaf lesions were more numerous on plants when the mealy plum aphids and *B. yamadae* were simultaneously present than on plants with *B. yamadae* alone. This suggests that the aphids increase the susceptibility of the plant or perhaps, spread the fungal spores. Even though the European lineage was more resistant to mealy plum aphid when combined with *B. yamadae* than the Delta lineage, *B. yamadae* caused similar leaf lesion damage to both lineages when combined with the mealy plum aphids.

Because mealy plum aphids caused browning symptoms similar to those found in die-off impacted Roseau cane, aphid feeding may be another factor involved in the die-off. Furthermore, under experimental conditions, *B. yamadae* caused lesions to Roseau cane like those observed in plants found in die-off areas of the Mississippi River Delta. This raised the possibility that like the mealy plum aphid, *B. yamadae* or other fungi causing leaf lesions may also be a factor in Roseau cane die-off. Nevertheless, the combined effect of aphids, fungi, and other factors such as the scale insect, salinity, pollution, may be involved in the Roseau cane die-off and should be evaluated.
Because of the lack of information on growing Roseau cane under greenhouse and laboratory conditions, several methods were tested in preliminary experiments.

Greenhouse

Root cuttings of two Roseau cane lineages containing six haplotypes (ANZ, ARM, EARL, ECM, ICI, and W96) were obtained from J. Cronin (Department of Biological Sciences, Louisiana State University). The plants were vegetative propagated by cuttings (6.35 cm – 7.62 cm long) of rhizomes, transplanted into plastic pots (10.16 cm x 10.16 cm x 8.89 cm) or clay pots (10.16 cm in diameter) and permanently flooded with water in plastic containers (Figure A.1). The soil mixture consisted of soil, sand, and potting mix (Scotts Miracle-Gro Company®) at a ratio of 2:1:0.5. Plants were fertilized with Osmocote® (15–9–12 NPK, The Scotts Miracle-Gro Company®, Marysville, OH, USA) and grown in a greenhouse located in the LSU AgCenter Central Research Station, Baton Rouge.
Laboratory

In the laboratory, plants were grown with an average temperature of 23 °C and artificial light (54W/120V 60Hz/4.0A Lamps) at 17 h light and 7 h dark. Rooted cuttings from plants grown in the greenhouse were used. The soil mixture prepared to grow plants in the greenhouse was used as soil source for laboratory plant growing experiments. Three growing treatments were tested using 2.5cm x 2.5cm x 7.0cm plastic vials (Figure A.2). The first treatment consisted of one part of soil and one part of water, the second was water only and the third was soil only. Plants grew better on the one part soil one part water treatment.
Figure A.2. Roseau cane cuttings growing in vials in the laboratory. Vial with water only (A), vial with half soil half water (B) and vial with soil only (C).

Another experimental method tested to grow Roseau cane in the laboratory consisted of planting rooted cuttings in individual commercial plastic cells 4.0 cm. x 4.5 cm. x 5.6 cm. placed in a tray with water (Figure A.3). In this method, during the first 24 hours after planting the plants were covered with a clear plastic cover in order to create a humid chamber (Figure A.4). The plastic cell method allowed the plants to grow faster than the vial treatments.
Figure A.3. Roseau cane cuttings growing in plastic cells in the laboratory.

Figure A.4. Newly transplanted Roseau cane cuttings in plastic cells in a humid chamber.
APPENDIX B. SEQUENCES OF PCR PRODUCTS OBTAINED USING DNA FROM APHIDS AND TWO SETS OF PRIMERS

Only the sequences with the highest percentage of identity to the type species are included.

Sample No. 001-F. Forward BLAST results- *Buchnera aphidicola (Hyalopterus sp.)* haplotype 1

Primers Buch16S1F and Buch16S1R

TATATCTGGGGATCTGCCCAAAAGAGGGGATAACTACT
AGAAATGGTGCTAATACCCGATAAAAGTTGAAAAACCAAGTGGGGGACC
TTTATAAGGCCTCATGCTTTTGGATGAACCCAGACAGATTAGCTTTGGT
GTAAGGTAAAGGGTTACCAAGGAACGATCTCTACGTGGCTCTGAGAGGAT
AACCAGGCACACTGGAACACGACGCAGACTCCACCTAGGGGAGCGAG
CAGTGGGGGAATATTTGCACAATGGGAGCAAGCCTGATGCAGCTATGGCG
TTGTATGAAGAGGCTTAGGGTTGTAAGGTACTTTCAAGCGAGGAGGAAA
AGATAATATAATAATATTTACGTAGCAGCCNNCCGCAGAAGAA

Sample No. 001-R. Reverse BLAST results- *Buchnera aphidicola (Hyalopterus sp.)* haplotype 1

Primers Buch16S1F and Buch16S1R

ATCTTTTCTCTCTGCTGAAATCTTTTACAACCCCTAGAG
CTTCTTCATACACCGGCCATAGCTGCACTACAGCTTTCCACATTGTGCA
TATTTGCACTCGCTGCCTCCCGTAAAGGTCTGTGACGCTGCTCAGCTCCA
GTTGCTGCTGTTATCTCTACTCCAGACAGCTAGAGAGATCGTGCTGCTTGTA
AGCCTTTACCTACCAAAAGCTATCTCTGTGCTGCTGCTGCTGCTGCTGCTG
AGGCCTTTATAAAAGGCTCCCACCTTTGCTTTTCAACTTTATGCAGCTATA
GCCACCATTTCTAGTTATCCTCCCCTCTCTTTTGGGGAGACCTCCCAGATAT
TACTCACCAGTTTGGCCGAGACAAANAGNGNCAAAGCTCC

Sample No. 004-F. Forward BLAST results- *Hyalopterus pruni*

Primers C1-J-1718 and C1-N-2191

TCGATTTAAATAATATT
AGATTTTGTATTACTCCCTTCTCCTTTAAATAATAATAATCTGTAGATTTAT
AATTAAATAACGGAAACAGGAACAGGATGAAACAAATATTATCCACCATTATCTA
ATAATTTGCACATAAATAATATTTTCAAGTTGATTTAACAATTTTTTCACTT
CATTTAGCAGGAATCTCATCAATTITTAGGAAGCAATTAAATTTTATTTTGCAC
AATTTAAATTATAATACCTAAATACCAAAAAATTAAATCAAATTTTCTTTAT
TTCCATGATCAATTATTTAATTACAGCTACCTTTATTTATTTATCACTCCCA
GTGGTAGCTGGAGCTATTACTATTATTATTAAACTGATCGTAAAATTAC
ATACTTTTTTGATCCTGCTGGAGGAGGAGATCCAATTCTTTACCAACACT
TATTCGATTGTTTGTACCCCTGAAAGTTTATATTTATTTTTTTTACCGG
GA

Sample No. 004-R. Reverse BLAST results- *Hyalopterus pruni*

Primers C1-J-1718 and C1-N-2191

GAATTGGATCTCCTC
CTCCAGCAGGTCAAAAAATGTGTTATTTAAATTACGATCAGTTAATAAT
ATAGTAATAGCTCCAGCTAAAACGAGGTGATAAAAATTAATAAGGTAGC
TGTAATTAAAATGGATCATGGAAAAATGAAATTGATTTAATTATATGT
TATTAGGTattaATTATTTAAAATGTTGCAAAATATTAATTGCTCCTAAA
ATTGATGAGATCTCTGCAAATTGAAGTGAAAAATATTGAAAATCAACTGA
AATATATTATGTGCAATTATTAGATAATTGTTGGATAAAATTGTTCATC
CTGTTCCCTGTCCGTATATATATAATATACAGATTATTATTAAAG
GAAGGGGGGGATGAAATCAATATTATTTAATCGAGGGAAAGATAT
CGTATTCCATCCTATTATTATAGGAACATCAATT

Sample No. 007-F. Forward BLAST results- *Buchnera aphidicola* (*Myzus persicae*)

Primers Buch16S1F and Buch16S1R

ATATCTGGGGATCTACCCAAAAGAGGGGGATAAC
TACTAGAAATGCTAAATACCCGCAAATATGTTGAAAATCAAAAGTGGGG
GACCTTTTAGGGCTACTGCTTTTGGATGAACCCAGACGAGATTAGCTTTGT
TGTAAGGTAAATGCTTACCAAAGCTACGATCTCTAGCTTGGGAGAG
ATAACCAGCCACACTGGAAACTGAGACACGGTCCACGACTCCAGGGA
AGCAGTGGGGAAATTTGCACAAATGGGCGAAAGCCTGATGCGCTTGCCG
CGTGTATGAAGGAGCCCTTAGGGGTGTAAGGTACTTTTCCAGCGGGAAA
AGAAATAAAAATTTATTATTTTCGTCGTACGTTCCCGCCAGAG

Sample No. 007-R. Reverse BLAST results- *Buchnera aphidicola* (*Myzus persicae*)

Primers Buch16S1F and Buch16S1R

TTTATTTCTTCNCCTGCTGAAGTACTTTTACACCAACCAAGG
CCTTCTCTACACGGGGCTAGCTCAGCTACGAGGGTCTTCGCCCTAGTGCA
ATATTCCCCACTGCTGCCTCCCGTAGGAGTCTGGACCGTGTCTCAGTTCC
AGTGTGGCTGTTATCTCCTCAGACCAGCTAGAGATCGTACGCTTGGTAA
GCCATTACCTTACCAAAAGCTAATCTCGTCTGGGTCCATCCAAAAGCAT
GAGGCCTAAAGGCTCCCCACCTTTAGTTTTTCAACATTATGCAGGTATTAG
CTACCCATTCTAGTATCTATCCCTTCTTTTGGGTAGATCCCGAGATATT
ACTCACCCTTTGCCGCTTGCCCGACAAAGCNGCNAGCTCACA

Sample No. 0012-F. Forward BLAST results- Myzus persicae
Primers C1-J-1718 and C1-N-2191
GCAGATTTAATAACAT
TAGATTTCTGATTATTACCACCCCTCATTAATAAATAAATTTGTAGTTTTT
TAAATTAATAATGGAACAGGAACAGGTGAACTATTTACCCACCCCTTATCA
AATAATATTGCACATAATAATAATTACGTTTTAACTATTTTTTTCTATT
ACATTTAGCAGGAATTTTCTACAATTAAAATTGAGCAATTTAAATTGTA
CAATCTAAAATATACACCCACCAATATATAATAATAATACCAATCTCTTTA
TTCCCATGTATCAATTATTTGACTTTTTATAATTTTTTATTTTACC
TTTTCTAGCAGGTCTATTACAATATTATAACTGATCGTAATTAAAAATAA
CTTCTTTTTTGACCCAGCACGGGGAAGGTGACCCAAATCTTGAATCAACAT
TTATTTTGATTTTTTGACGACATCCTATCCCTGGAAGTCTATTTTTATTTTACCGG

Sample No. 0012-R. Reverse BLAST results- Myzus persicae
Primers C1-J-1718 and C1-N-2191
ATTGGGTGTCACCTCCCC
TGCTGGTCTAAAAATGAAAGTATTAAAAATTACGATCAGTTAATAATATTG
TAATAGCACCCTGCTAGAACAGGTAAAGATAAATATAAAATAATAGCTGTA
ATTAAATGTATCGAATAAAAGGGATTTGTTTTAATTTTATATTGTT
TGGTATTATTATTAGGATGTTACAAATAAAATTAAAAATTGCTCTAAAAATTG
ATGAAATTCTCGCTAAATGTAATAGAAAAATAAGTTAAATCAACTGAAATA
TTATTTATGTGCAATTATTATTGATAAGGTTGGGTAATAATAGTTCATCCTCTG
TCCTGTCCATTATTAAAAACTACAATAATTATATTATTATTATAGG
GTGGTAAATATCAGAATCTAATGTATTATTTAATCCTGTTGGAAAGATATATCA
GGACATCTATTATTAGGAACTAAATCAATTTCCAAATCCTCC
APPENDIX C. RESULTS OF PCR EXPERIMENTS USING BUSH16S1F AND BUSH16S1R PRIMERS

Table C.1. Results of PCR experiments aimed at the identification of aphids collected from Roseau cane and pepper using primers Buch16S1F and Buch16S1R.

<table>
<thead>
<tr>
<th>Organism</th>
<th>Plant Source</th>
<th>Species</th>
<th>Amplified gene</th>
<th>Sample ID</th>
<th>% Nucleotide Identity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacterium Roseau cane</td>
<td>Buchnera aphidicola (Hyalopterus sp.)</td>
<td>rDNA</td>
<td>0001-F</td>
<td>99.73</td>
<td></td>
</tr>
<tr>
<td>Bacterium Roseau cane</td>
<td>Buchnera aphidicola (Hyalopterus sp.)</td>
<td>rDNA</td>
<td>0001-R</td>
<td>98.18</td>
<td></td>
</tr>
<tr>
<td>Bacterium Roseau cane</td>
<td>Buchnera aphidicola (Hyalopterus sp.)</td>
<td>rDNA</td>
<td>0002-F</td>
<td>98.65</td>
<td></td>
</tr>
<tr>
<td>Bacterium Roseau cane</td>
<td>Buchnera aphidicola (Hyalopterus sp.)</td>
<td>rDNA</td>
<td>0002-R</td>
<td>98.43</td>
<td></td>
</tr>
<tr>
<td>Bacterium Roseau cane</td>
<td>Buchnera aphidicola (Hyalopterus sp.)</td>
<td>rDNA</td>
<td>0003-F</td>
<td>96.00</td>
<td></td>
</tr>
<tr>
<td>Bacterium Roseau cane</td>
<td>Buchnera aphidicola (Hyalopterus sp.)</td>
<td>rDNA</td>
<td>0003-R</td>
<td>96.00</td>
<td></td>
</tr>
<tr>
<td>Bacterium Pepper</td>
<td>Buchnera aphidicola (Myzus persicae)</td>
<td>rDNA</td>
<td>0007-F</td>
<td>99.74</td>
<td></td>
</tr>
<tr>
<td>Bacterium Pepper</td>
<td>Buchnera aphidicola (Myzus persicae)</td>
<td>rDNA</td>
<td>0007-R</td>
<td>98.18</td>
<td></td>
</tr>
</tbody>
</table>

(table cont’d.)
<table>
<thead>
<tr>
<th>Organism</th>
<th>Plant Source</th>
<th>Species</th>
<th>Amplified gene</th>
<th>Sample ID</th>
<th>% Nucleotide Identity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacterium Pepper</td>
<td><em>Buchnera aphidicola</em> <em>(Myzus persicae)</em></td>
<td>rDNA</td>
<td>0008-F</td>
<td>96.85</td>
<td></td>
</tr>
<tr>
<td>Bacterium Pepper</td>
<td><em>Buchnera aphidicola</em> <em>(Myzus persicae)</em></td>
<td>rDNA</td>
<td>0008-R</td>
<td>97.83</td>
<td></td>
</tr>
<tr>
<td>Bacterium Pepper</td>
<td><em>Buchnera aphidicola</em> <em>(Myzus persicae)</em></td>
<td>rDNA</td>
<td>0009-F</td>
<td>98.61</td>
<td></td>
</tr>
<tr>
<td>Bacterium Pepper</td>
<td><em>Buchnera aphidicola</em> <em>(Myzus persicae)</em></td>
<td>rDNA</td>
<td>0009-R</td>
<td>97.55</td>
<td></td>
</tr>
</tbody>
</table>
LITERATURE CITED


Gigante, D., C. Angiolini, F. Landucci, F. Maneli, B. Nisi, O. Vaselli, R. Venanzoni, and L.


88
https://doi.org/10.3389/fpls.2014.00663


https://doi.org/10.1371/journal.pone.0202411


VITA

Heather Eileen Cizek grew up in Elk Grove Village, Illinois. She attended James B. Conant high school in Schaumburg, Illinois and graduated in 2016. She started her Bachelors in August of 2016 at Louisiana State University. In May of 2019, she completed her Bachelor of Science in Natural Resource Ecology and Management focusing on fisheries with a minor in wildlife ecology. During her undergraduate studies, she ran both cross country and track for Louisiana State University. During that time, she also had an internship at the Louisiana State University’s School of Renewable Natural Resources where she assisted in a project involving different herbicide level impacts on respiration of Bluegill- Green Sunfish hybrids. She also had an internship at the New Orleans aquarium where she helped with husbandry along with research involving Tequila Splitfin breeding. In August of 2019, Heather started her Masters, having Dr. Rodrigo Valverde as her advisor. The project she worked on involved the Roseau cane die-off. Heather developed methods to grow Roseau cane in the greenhouse and the laboratory. She researched the effects of the mealy plum aphid and the fungus *B. yamadae* individually and simultaneously on Roseau cane. She plans to receive her Masters in December 2021.