November 2021

Revealing the native status of Conyza bonariensis: Specialization of insect herbivores associated with Conyza and Erigeron spp. (Asteraceae) in Louisiana, Texas, and Mississippi

Carlos Wiggins
LSU Agricultural Center

REVEALING THE NATIVE STATUS OF *CONYZA BONARIENSIS*: SPECIALIZATION OF INSECT HERBIVORES ASSOCIATED WITH *CONYZA* AND *ERIGERON* SPP. (ASTERACEAE) IN LOUISIANA, TEXAS, AND MISSISSIPPI

A Thesis

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

in

The Department of Entomology

by
Carlos Wiggins
B.A., Louisiana State University, Shreveport, 2018
M.S., Louisiana State University, 2021
December 2021

Acknowledgements

I would like to thank the following agents for funding my project: LSU AgCenter and CSIRO-Australia. I would also like to thank the members of my committee for their guidance and assistance: Dr. Rodrigo Diaz, Dr. Michael Kaller, and Dr. Nathan Lord. Additionally, I would like to thank the following people for their help with identification of insect specimens: Victoria Bayless, Dr. Christopher Carlton, Dr. Deborah Matthews, Dr. Scott Schneider, and Laura Miller. I would also like to give additional thanks to the student workers and my fellow laboratory mates for all their assistance throughout my project.

Table of Contents

Abstract

Flaxleaf fleabane [*Conyza bonariensis* (L.) Cronquist] (Asterales: Asteraceae) is an annual herb which grows up to 1.0 m in height and is native to South America. Due to introduction and subsequent development of herbicide resistance, *C. bonariensis* has become a major agricultural weed in Australia. The purpose of this study was to catalog herbivorous insects associated with local populations of *C. bonariensis* in Louisiana, Texas and Mississippi in order to establish comparisons with the Australian fauna and identify potential biological control agents. Leaves, roots, stems, and flowers of *C. bonariensis* were inspected for signs of insects and pathogens. Results revealed the presence of numerous Hemipterans, including *Halticus bractatus* [Say] (Miridae), *Lygus lineolaris* [Palisot de Beauvois], and *Taylorilygus apicalis* [Fieber] attacking the stem, leaves, or pedicel. An agromyzid fly, *Calycomyza humeralis* [von Roser] was also found attacking the leaves. In addition, field surveys included the exploration of close relatives of *C. bonariensis*, including *C. canadensis* [(L.) Cronquist], *Erigeron philadelphicus* (L.), and *E. procumbens* [(Houst. ex Mill.) G.L. Nelson]. These surveys resulted in the finding of several insect species, including *Hypera postica* [Gyllenhal] and *Listroderes difficilis* [Germain] (Coleoptera: Curculionidae). A gall forming midge, *Neolasioptera erigerontis* [Felt 1907] was found associated with *C. canadensis* and seemed to show a high level of host specificity. Outcomes of this survey determined the host specificity, field population densities and type and degree of damage of these insects.

Two types of common garden experiments were conducted during the study. These experiments took place at the LSU, Burden Botanical Museum and Garden in Baton Rouge, Louisiana. The first experiment looked at the community and diversity of

herbivorous insects associated with *C. bonariensis*, *C. canadensis*, *E. Philadelphicus*, as well as how these plant species developed over time. The results of this experiment showed that both *Conyza* spp. had steady growth rates during the early summer months of June and July, when this experiment took place. *E. philadelphicus*, being a species that grows and flowers more during the mid to late spring season, these plants did not show a difference in the rate of growth during this experiment. Additionally, it was seen that more insects were found associated with the two *Conyza* spp. than with the *Erigeron* sp. Both *Conyza* sp. grow larger than the *Erigeron* sp., and unlike the two *Conyza* spp., *E. philadelphicus* does not produce multiple axial branches from its main stem that could provide more food for herbivorous. The second experiment was an exclusion study looking at how the lack of various biotic stressors (pathogens and herbivorous insects) affected the growth and development of *C. bonariensis*. The results of this experiment seem to show that there was no difference in the development and growth of plants whether treated with insecticide, fungicide, a combination an insecticide and fungicide, or treated with a water control. From this experiment, it seemed that plants treated with the water control were healthier than those treated with any other treatment. Despite being attacked by both insects and pathogens throughout the study, most plants treated it with the water control had a darker green coloration, which is seen healthy *C. bonariensis* plants.

Chapter 1. Background and Literature Review

1.1. Flaxleaf Fleabane Distribution, Biology, and Ecology

Flaxleaf fleabane, *Conyza bonariensis* [L.] Cronquist is an annual or short-lived perennial/ biennial weed which is native to South America (Michael 1977). In addition to its native range, this plant is also widespread throughout regions of the southern United States and Central America. *Conyza bonariensis* can grow up to 1m tall, and produce an average of 100,000 seeds per plant, which are spread via the wind on umbrella-like pappus (GRDC 2013). Seeds only germinate in the presence of light, and seedlings emerge only from the top 1 cm of soil, when temperatures are between 10 and 30 °C (50-86 °F), with the optimal temperature being around 20 to 25 °C (68-77 °F) (GRDC 2013). Within southern Louisiana, *C. bonariensis* is found primarily in disturbed habitats of urban and/or suburban environments. *C. bonariensis* is a hardy plant that is densely covered in short trichomes along the stem and leaves (Acedo 2018). This plant tends to grow fairly well along roadsides, sidewalks, railways, and parking lots, but is sensitive to soil disturbance such as uprooted or mowed.

Conyza bonariensis has become a major weed in Australian cropping since the move to no-till farming (GRDC 2013). The wind dispersal method of the seeds of *C. bonariensis* is one of the main reasons this plant has become such a widespread problem within northern New South Wales and southern Queensland. Seeds are easily dispersed, and with no-tillage systems, are easily able to germinate under the right conditions. This weed is estimated to cause revenue losses in excess of $43 million for grain producers (CSIRO 2015). The current method of controlling this weed is the use of

herbicides; however, *C. bonariensis* has developed resistance to a range of herbicides across four different modes of action in eleven countries including Australia (Michael et. al. 2016). Some of the herbicides that flaxleaf fleabane has developed resistance to include glyphosate, paraquat, atrazine, simazine, chlorsulfuron and diquat (Michael et. al. 2016). Herbicide resistant populations are typically managed using control strategies that include rotation of different chemical herbicides integrated with cultural control methods (Cronquist 1980).

There are several *Erigeron* and *Conyza* spp. worldwide, with *C. bonariensis*, *C. canadensis*, and *C. sumatrensis*, native to South America, being some of the most invasive and widespread species (Wang et. al. 2018). Herbicides are used to control each of these plant species and are most effective when applied during their early stages of development; however, each species varies in their levels of susceptibility to different herbicides, making it important to properly identify individuals at an early developmental stage (Alpen et. al. 2014). Unfortunately, before the development of flowers, these species are extremely difficult to distinguish from one another morphologically. In a study focusing on the development of a DNA barcode system for the eight *Conyza* spp. of Australia, diagnostic nucleotide sites were found to exist in the nuclear (ITS) gene regions that could separate all the *Conyza* species observed, except for *C. bonariensis* and *C. bilbaoana* which shared the same nucleotide sites (Alpen et. al. 2014). Utilizing the DNA barcoding system already established can help with the proper species level identification of the three most invasive *Conyza* spp. for adequate early application herbicide treatment; however with the development of herbicide

resistance in these species, they may become increasingly more difficult to treat over time.

1.2. Study Purpose

The purpose of this study was to identify potential biological control agents in Louisiana, Texas and Mississippi that may be beneficial in the management of *C. bonariensis* in Australia. Using several species of closely related plants to *C. bonariensis*, this study analyzed different insects associated with *C. bonariensis* that may be considered either generalists or specialists of this plant. Several insects were found associated with *C. bonariensis*, as well as a few of the closely related plant species. Most of the insect species identified were found to be generalist feeders and are associated with plants other than *C. bonariensis* or its relatives; however, a few insects seemed promising, being found on only *C. bonariensis* or one of its close relatives. Several insect species were found associated with *C. bonariensis* in southern Louisiana, with most species identified as being generalist feeders. A few insects may be specialists of *C. bonariensis* or one of its close relatives, but further studies are needed to identify whether these insects will feed on the other related species. Additionally, an exclusion experiment was conducted within a common garden plot to determine what variables - insects, pathogens, or both - most affected the growth and development of *C. bonariensis.* As various insects and pathogens may have different impacts on plant development, further studies are needed to determine how individual species may affect plant growth under more controlled conditions.

1.3. Previous Work and Present Outlook

Surveys of natural enemies of *C. bonariensis* have been conducted in Argentina, Brazil, Paraguay, and Colombia. From these surveys, it seemed that there was a similar community of insects associated with *C. bonariensis* in these countries, which included roughly 35 species/ morphospecies (CSIRO 2015). These insects are found across 14 families and include primarily root feeders, foliage feeders, stem borers, stem gallers, and phloem feeders (CSIRO 2015). Two of the most promising species found during these surveys was a gall fly, *Trupanea bonariensis* (Brethes) (Diptera: Tephritidae) and the mealy bug *Paracoccus* spp. (Hemiptera: Pseucoccidae). *Trupanea bonariensis* is a stem-gall forming fly in the family Tephritidae, which is only known to attack *Conyza* spp. (McKay et. al. 2001). A lab colony of this gall fly is in the early stages of establishment in Brazil (CSIRO 2015).

Currently, several insect species have been found associated with *C. bonarieneis* during this study within the southern United States. Some of these insects include *Corythucha marmorata* (Uhler) (Hemiptera: Tingidae) (Appendex2: Fig. 18-E), which are phloem feeding insect, that were found feeding on the stem and leaves of *C. bonariensis*, and when in high densities, seems to cause severe stress to plants. This species was found to have host plants of both *Conyza canadensis* and *Erigeron annuus* in North America. It is considered polyphagous, and is a generalist feeder, with hosts found in several different families of plants such as Asteraceae, Lamiaceae, and Solonaceae (Kim 2014). *Synchlora frondaria* (Guenee) (Lepidoptera: Geometridae) was reared from partitioned flower heads of *C. bonariensis* during this study. This species has been found associated with several plants in the family Asteraceae, including plants

in the genus *Bidens* and *Chrysanthemum*, as well as *Pluchea* (Treiber 1979). It has also been found associated with black berries, plants in the family Rosaceae, genus *Rubus* (Beadle 2018). Within the southern United States, *Lioptilodes albistriolatus* (Zeller 1877) (Lepidoptera: Pterophoridae) (Appendex2: Fig. 18-D) has been found associated with feeding on the flowers of both *C. bonariensis* and *C. canadensis* as immatures. These insects feed on the developing seeds and flower of several genera in the family Asteraceae; these include plants in the genera *Erigeron* and *Conyza*, as well as others such as: *Baccharis* (L.), *Solidago* (L.), and *Symphyotrichum* (Ness) (Matthews 2012).

Microtechnites bractatus (Say) (Hemiptera: Miridae) is a phloem feeding insect, that was found feeding on the leaves and upper stem of *C. bonariensis*. This species has a large host range, but is a pest of various crops including, but not limited to, beans, beets, and cabbage. This insect also has hosts of various weeds, including plants in the genera *Bidens, Convolvulus, Malva, Amaranthus, Plantago, Ambrosia, Polygonum, and Carduus,* and other plant species (Capinera 1999). *Notiodes aeratus* (LeConte) (Coleoptera: Brachyceridae) was found associated with the flowers of *C. bonariensis*. As immatures, *N. aeratus* is considered a miner, and is known to develop within the leaves, petioles, and rhizomes of *Marsilea mollis*. Species in the genus Notiodes are associated with two families of plants, Marsileaceae and Cyperceae (O'Brien 2009). *Phenacoccus solani* (Ferris) (Hemiptera: Pseudococcidae) (Appendex2: Fig. 18-C) are phloem feeding insect, that were found associated with the stem and roots of *C. bonariensis*. This is a polyphagous species, which was found to be associated with more than thirty families of plants (Fizdale 2019). It has a host range of roughly thirty-six families, with eighty-nine genera, which includes plants in both genera *Conyza* and

Erigeron. This species is also widely distributed within the U.S., including both Texas and Louisiana.(Ben-Dov 2016). *Calycomyza humeralis* (von Roser 1840) (Diptera: Agromyzidae) (Appendex2: Fig. 18-F) seems to be associated with feeding on leaves of *C. bonariensis* as leaf mining larvae. This is a species most common on Asteraceae and was recorded on ten genera. It is a widely distributed species found throughout North America and has also been known to occur on the families Heliantheae and Madieae (Spencer 1990). Another closely related species, *C. minor* (Spencer) (Diptera: Agromyzidae) is only known to have one host plant, *Conyza canadensis;* it occurs in Florida and South Carolina and has recently been documented on Guadeloupe (Spencer 1990). This species shares the same feeding habits as *C. humeralis*, feeding within the tissues of leaves of their host plants as immatures.

1.4. Proposed Research Hypothesis and Specific Objectives

As *C. bonariensis* is considered an introduced species to the southern United States (Strother 1943) by several sources including the United States Department of Agriculture; it would be expected that most if not all insects associated with this species within Louisiana would be generalist feeders. As an introduced species, insects which are native to the United States would not have had time to coevolve with this species. Without this coevolution, the native insects could not become specialized to feed primarily or solely on *C. bonariensis*. The objectives of the study were 1: to survey populations of *Conyza* and *Erigeron* spp. and document the diversity of insect herbivores associated with any closely related *Conyza* and *Erigeron* spp. within the southern United States, 2: to determine how various biotic stressors affect the growth

and development of *Conyza bonariensis* over time, and 3: to observe the association of various insects between different *Conyza* and *Erigeron* spp. and determine the diversity and abundance of the various feeding guilds of those insects. Additionally, this study looked at the development of these different plant species under similar growth conditions over time; as well as the community of insects associated with each. It also looked towards determining the potential host specificity and damage of putative specialist herbivores associated with *C. bonariensis.*

Chapter 2. Surveys of natural enemies and their associations with *Conyza* and *Erigeron* spp. (Asteraceae) within Louisiana, Texas, and Mississippi

2.1. Introduction

Native to South America, flaxleaf fleabane, *Conyza bonariensis* [L.] Cronquist is a highly invasive weed, spp. in Australia (Aplen et. al. 2014). Due to the invasiveness of this species, this weed has become a major economic pest for various row cropping systems; and for Australia's grain producers alone, there is estimated revenue losses of over $43 million per year as a result of yield loss and management (CSIRO 2015). A single mature *C. bonariensis* plant can produce on average 110,000 seeds that are spread by the wind, which is one of the reasons why this species is so invasive (GRDC 2013). Populations of this weed have developed herbicide resistance to several different types of herbicide; including glyphosate, paraquat, atrazine, simazine, chlorsulfuron and diquat, in eleven countries including Australia (Michael 2016). As a result of the development of herbicide resistance and the economic impact this weed has on Australia's no-till agroecosystem, agencies in Australia, such as Commonwealth Scientific and Industrial Research Organization (CSIRO), have been looking for alternative ways of control this weed. In the search for alternate means of controlling this weed, surveys have been conducted in the native range of *C. bonariensis* within Argentina, Paraguay, Brazil, and Colombia to find potential biological control (CSIRO 2015). In addition to the surveys conducted in South America, surveys for potential agents of this weed have also been conducted in the various regions of the southern

United States, where the climate matched closest to the invasive range of *C. bonariensis* in New South Wales and southern Queensland, Australia.

The enemy release hypothesis is the general idea that invasive species are less impacted by herbivores, pathogens, and other limiting factors in their invasive ranges; due to the invasive species now being separated from the natural enemies they have coevolved with within their native range (Keane et. al. 2002). The lack of natural enemies allows plants to allocate more resources into growth and reproduction, rather than defense; allowing them to often become larger and spread more rapidly than they would in their native range (Middleton 2019) . As invasive plants are no longer being pressured by their natural enemies, they can reallocate the resources used in defense, such as, chemicals and physical barriers- such as thorns and trichomes (Middleton 2019).

There are several *Conyza* and *Erigeron* spp. found throughout the southern United States, with the only known native *Conyza* spp. being *C. canadensis* and *C. ramosisma* (Dolan et al. 2021). For many of the *Conyza* spp. in the Americas, these plants are very difficult to control and are extremely problematic weeds for various crops (Trezzi et. al. 2015). As they have been introduced to many countries around the world, due to the subsequent development of herbicide resistance and a lack of natural enemies in their introduced ranges, three of the most invasive *Conyza* spp. have become serious issues in several countries for more than 40 different types of crop (Holm et. al. 1997; Thébaud et. al. 1995). Though there are several *Erigeron* and *Conyza* spp. worldwide, *C. bonariensis*, *C. canadensis*, and *C. sumatrensis* are some of the most invasive and widespread species (Wang, A. et. al. 2018)

As there have been several herbivorous insect species found associated with both *Conyza* and *Erigeron* species within the southern United States, it is important to both identify the insects associated with those plant species, and to determine the range of hosts that the individual insect species are associated with. When looking for biological control agents, it is important that potential agents have narrow host ranges or are specialized in feeding on a particular host species (Brodeur 2012). As a result, the objective of the study was to survey populations of *Conyza* and *Erigeron* spp. and document the diversity of insect herbivores associated with any closely related *Conyza* and *Erigeron* spp. within the southern United States. In addition, this study looked towards determining the potential host specificity and damage of putative specialist herbivores associated with *C. bonariensis*.

2.2. Materials and Methods

Insects were found in twenty-one sites where *C. bonariensis* and/or closely related species were located, and included fifteen in Louisiana, two in Mississippi, and four in Texas (Figure 1). The other plant species that were found with insects associated with them were: *Erigeron annuus* (L.) Pers., *E. philadelphicus* (L.), *E. procumbens* (Houst. ex Mill.) G.L. Nesom, *C. canadensis* (l.) Cronquist, and a plant that is suspected as *C. sumatrensis* (Retz.) E. Walker. Several insects were also collected from plants that were also in the family Asteraceae. These plants were found within fairly close proximity to either *C. bonariensis* or a related species. The surveys for natural enemies of *C. bonariensis* took place in southeastern regions of Mississippi, Texas, and Louisiana. Sites were originally found based on occurrences of *C.*

bonariensis found within herbarium record including online databases and the LSU herbarium. Once all promising locations were visited based on the herbarium records, attempts were made to explore new areas based on similar habitat descriptions found in the records or from other resources such as the USDA plant database, describing habitats of this species.

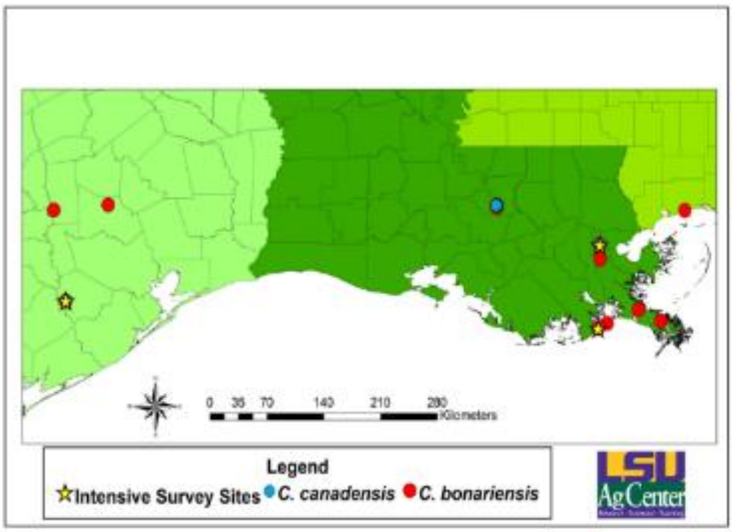

Figure 1. Map indicating sampling sites where insect and plant samples of *C. bonariensis* and *C. canadensis* were collected. General surveys were conducted for 25 different sites and are indicated by the red and blue circles showing sites where both *Conyza* spp. were found. Intensive surveys occurred at 3 of the 25 visited sites; indicated by yellow stars.

Exploratory surveys were surveys conducted with the purpose of finding target plants and properly identifying those plant species. These surveys were conducted intensely from early-February to late-March 2019. During this time, the focus was proper

identification of *Conyza* and *Erigeron* spp. in the field, as there was no prior experience in identifying these plants other than by observing dried herbarium specimens. Using online images for identification of *Conyza* spp. was often misleading, as many of the photos of *C. bonariensis* were misidentified. In addition, there were no sources that included distinguishing physical characteristics of immature *Conyza* spp. in their overwintering or rosette forms. This made field identification of *Conyza* spp. very difficult during the time of these surveys, due to all plants found being in their rosette stages. Additionally, many of the *Erigeron* spp. that were flowering at the time had similar features, such as, flower arrangement, color and shape; leaf shape, and the density of stem hairs. The similarities of these features, along with the lack of pigmentation in herbarium specimens made identifying these plants fairly difficult.

When searching for plants during these surveys, all plants with similar characteristics 9flower and/ or leaf shape, flower color, stem hair density, and rosette leaf arrangement) were collected. As the goal of these surveys were to find the target plants in the genus *Conyza* and *Erigeron*, sampling times were relatively brief, taking only 5 -10 minutes per site; and though these plants were difficult to identify initially, after building confidence in field identification based on their physical characteristics, it was found that both *Conyza* and *Erigeron* spp. were common near and around disturbed areas such as near agricultural fields, along railways, and even within areas of cities with soil exposure (like within the cracks of sidewalks or along the edge of buildings).

General surveys were surveys conducted with the purpose of finding large populations of *C. bonariensis* along with any related *Conyza* or *Erigeron* spp. within an

area. These surveys were conducted from mid-March 2019 and continued through mid-June 2019. This was the time when *C. bonariensis* was found and was able to be more easily identified due to them beginning to flower. During this time plants were inspected in the field, for any insects feeding externally on the plant. Plants were also collected and searched in the lab for all life stages of herbivorous insects of the plant; including roots, stems, leaves, and flowers. Due to *C. bonariensis* being found more commonly in urban and suburban areas, it was not always possible to collect entire plant as most plants in urban areas were found growing out of cracks in concrete sidewalks or building sides. This resulted in several plants being broken off at the roots. Additionally, finding large areas of undisturbed plants or areas not treated with herbicides or insecticides was extremely difficult, as these plants are considered weeds and grow in areas where human traffic is high; causing areas with high densities of target plants to be either sprayed with herbicides, or be inadvertently affected by insecticides such as those used for insects like mosquitos. Overall, there were 25 sites found with large enough populations of *Conyza* and *Erigeron* spp. for repeated sampling to occur.

Intensive surveys were surveys conducted with the purpose of collecting herbivorous insects associated with populations of *Conyza* and/or *Erigeron* spp. for multiple repeated sampling events. These surveys were conducted at 3 of the 25 general survey sites. These surveys were repeated at each site 3 times per year over a 2-year period, which included both insect and plant sample collecting. In the field, 15 plants were initially inspected for signs of herbivorous insect activity and the presence of insects for about 2 minutes per plant. Insects found associated with *C. bonariensis* or any other closely related plant species were collected and cataloged- being placed in

vials 70% ethanol. The areas which composed primarily of a single target plant species were swept for insects for 15 minutes, sweeping areas of roughly 5ft^2 per minute. As to not disturb insects feeding on plants, sweeping areas were estimated based on the size of an area measured nearby and the time it takes to move within the area by step. Following the sweeping, all insects were then collected and placed in Zip-loc bags. Ten plant samples of each target plant species present at a given site were collected at random and placed in individual bags to be transported back to the laboratory for further inspection and processing. The total process of insect and plant sample collection per site took roughly 45 minutes to an hour

In the laboratory, measurements (diameter and height) were taken of each plant collected. Plants were then partitioned into flowers, leaves, stems, and roots and placed into individual containers labeled with the number (1-10) and the plant species within them. Branches with a diameter of more of equal to 0.5 cm were placed with the stem partitions, while leaves were kept attached to smaller branches to reduce the rate of decay/ molding. All containers were them placed in growth chambers set to 25°C, 50-70% RH. These containers were then checked daily for signs of insect activity and/or emergence for a total of 7 days. Containers with stem and root matter are

2.3. Results

From the surveys, a total of 29 morphospecies were found associated with or reared from both *C. bonariensis* and *C. canadensis*. Though several insect species were found associated with *C. bonariensis* or other related plant species; the following

insects seemed to cause the most injury or damage to plants. Larvae of *Lioptilodes albistriolatus* (Zeller 1880) (Lepidoptera: Pteriphoridae), were found feeding on the flower heads of both *Conyza* species. As the larvae fed on multiple flower heads while developing, it resulted in flower head die-off. *Corythucha marmorata* (Uhler1878) (Hemiptera: Tingidae) were found feeding throughout the upper portion of *C. bonariensis* on the stem and leaves and were also found associated with *E. philadelphicus*. These insects are cell lacerators (grouped as phloem feeders), which in high densities caused severe damage to plants. *Mordellistena pustulata* (Melsheimer 1846) (Coleoptera: Mordellidae) were found feeding on the pith of the main stem of both *Conyza* spp. as larvae. The feeding of these larvae resulted in a canal in the main stem, where the pith was eaten. Though the entire pith of the plant could be hollowed out by these insects, this injury did not seem to amount to much overall damage to the plant. Similarly, to the mordellid species, *Hippopsis lemniscate* (Fabricius 1801) (Coleoptera: Cerambycidae) were found feeding on the pith of the main stem of both *C. canadensis* as larvae. Their feeding resulted in similar injury to the plant, with the entire pith of the plant being eaten in some instances. Both *Lygus lineolaris* (Palisot de Beauvois 1818) and *Taylorilygus apicalis* (Fieber 1861) (Hemiptera: Miridae) were found associated with both *Conyza* spp., as well as several *Erigeron* spp. Both insects were found feeding on the petioles of the flowers of their host plants. In many cases, both species were found feeding on the same plant; and though these insects were often found in high densities on a single plant, their feeding did not seem to result in much stress to their host.

In the field, the number of insects associated with both *C. bonariensis* and *C. canadensis* were recorded; the number of insects reared from the plant partitions of

each species were recorded as well. Once identified, insects were grouped based on their feeding guilds: Foliage feeders, phloem feeders (including cell lacerators), stem borers, root borers, and leaf minors. The percentage of insects reared from each partition for either *C. bonariensis* or *C. canadensis* was also recorded. Results showed that when compared among feeding guilds, phloem feeders were the most abundant group on *C. bonariensis*. Most insects associated with this species were found on the flowers (Figure 2-A). For *C. canadensis*, similar results were seen; as among feeding guilds, phloem feeders were also the most abundant group to occur on this plant species. It was also seen that most insects associated with this species were found on the flowers as well (Figure 2-B).

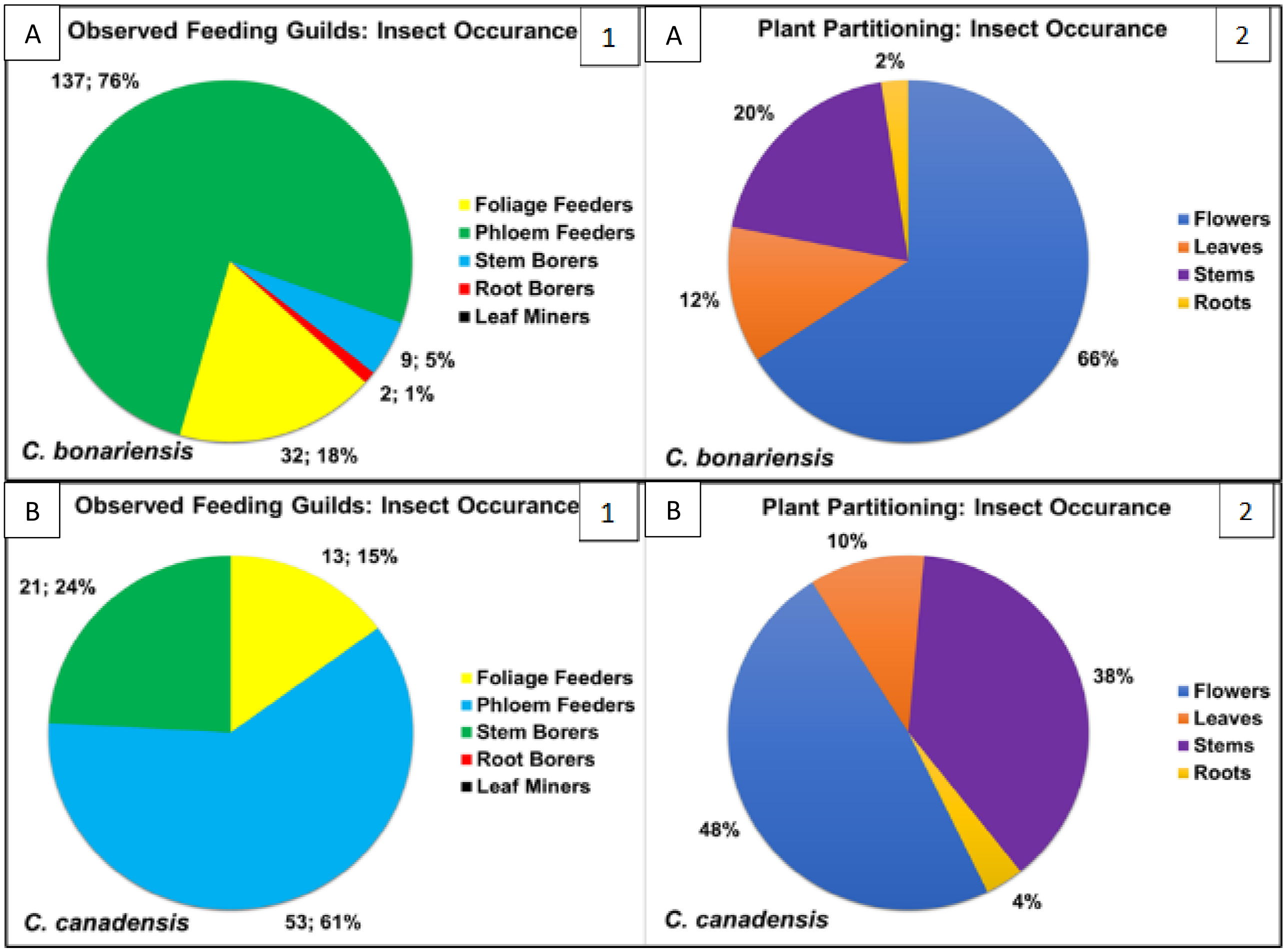

Figure 2. (A-1) Number and percentage of insect occurrences among feeding guilds from plants (*C. bonariensis*) collected during intensive surveys: insect occurrences include insects reared from partitions. (A-2) Percentage of insect occurrences on various parts of the plant (Flowers, Leaves, Stems, and Roots): insect occurrences include insects reared from partitions. (B-1) Number and percentage of insect occurrences among feeding guilds from plants (*C. canadensis*) collected during intensive surveys: insect occurrences include insects reared from partitions. (B-2) Percentage of insect occurrences on various parts of the plant (Flowers, Leaves, Stems, and Roots): insect occurrences include insects reared from partitions.

2.4. Discussion

From other surveys conducted to search for biological control agents of *C. bonariensis* in South America, a total of 19 species were found associated with this weed (CSIRO, 2015-2017). In surveys conducted in Brazil, most insects found on *C. bonariensis* were considered generalist feeders; with most being phloem feeding (or cell lacerating) hemipterans (CSIRO, 2015-2017). One gall forming midge was found to be a specialist of this plant species. With specialist feeders being found associated with this plant species, there is a greater chance that at least one of the insects found associated with this species could be used as a biological control agent. *Conyza bonariensis* would not be the first example of the use of biological control for plants in the family Asteraceae; there have also been examples of the successful use of biological control agents in this family. One example is Rush Skeletonweed: *Chondrilla juncea* L. (Asteraceae); which has had several biological control agents found and used in controlling it (Andreas et. al. 2016).

In the field, many of the insects that were observed feeding on the two *Conyza* spp. seemed to cause what appeared to be severe damage to plant, but ultimately the feedings did not result in much injury or stress to plant. For the stem boring mordellid and cerambyicid larvae, these insects would sometimes consume the entire pit of their host plant, leaving the stem hollow; however, despite this damage plant grew fairly well, and in many cases without any observable physical stress. The pterophorid larvae would, in many cases, consume multiple flower heads of plants; however, as these

plant species produce hundreds of flowers and seeds per plant, the consumption of a few flower heads seemed minuscule in comparison to the overall fecundity and health of plants.

The hemipterans, primarily *Corythucha marmarata*, in many cases caused severe stress to plants in both field and greenhouse settings. In high densities, these insects would often kill plants grown under greenhouse conditions; and even in low densities, the stress induced by the feeding of these insects was visible. Feeding from these insects would cause noticeable dark feeding spots and yellowing or browning of the stem and leaves. As these insects would mostly feed on the upper part of plants, the lower part of the main stem would remain fairly clear of damage. One explanation for the severe stress induced by the feeding of *C. marmarata* could be that these insects may infect their hosts with a potential pathogen during feeding, or they may facilitate a secondary infection as a result of their feeding damage. Two other tingid species, *C. ciliata* (Say, 1832) and *C. arculata* (Say, 1832) are known to be possible vector of both plant and leaf pathogens (Mitchell 2004; Paulin et. al. 2020). Currently, there have been no studies showing that *C. marmorata* is a vector for any known pathogens; however, there is still the possibility that the species may act as a vector for a potential plant pathogen.

During my surveys, a larger percentage and number of insects were found associated with *C. bonariensis* than *C. canadensis*. This could be in part due to the focus of the surveys being primarily on finding insects associated with *Conyza bonariensis*; however, during collecting events, an equal number of both species was collected at a given time, with all samples being checked for signs of insect activity at

once. Another explanation for the larger number of insects found associated with *C. bonariensis* during field survey could be that the secondary chemicals associated with this species are less effective in deterring the native insects that have coevolved and are also associated with *C. canadensis*. In the *C. bonariensis* of South America, there have been three primary compounds found within the oils produced by this species: trans-β-farnesene, trans-ocimene and β-sesquiphellandrene (Araujo et. al. 2013). These three compounds are also found in other plant species and are known to act as deterrents for various herbivores (Gao et. al. 2015; Dhandapani et. al. 2020; Armengol et. al. 2017). In the *C. canadensis* of Turkey, there have been five compounds found within the oils produced by this species: limonene, spathulenol, β-pinene, cis-lachnophyllum ester, and (2Z,8Z) -matricaria ester (Ayaz et. al 2017). These compounds act as feeding deterrents and repellants to insects, and are even known to be toxic to some herbivorous insects (Ayaz et.al. 2017; Erasto et. al. 2008; Ninkuu et. al. 2021; Kumar et. al. 2017; Silva, J. 2004). During the monitoring of plant material for insect emergence, it was noticed that containers with *C. canadensis* material would produce a strong odor after 1-2 days. The leaf and flower material would also lasted longer within growth chambers than *C. bonariensis* material. From these containers, less insects were observed emerging from the *C. canadensis* material; even though in most samples there was more plant material due to this species growing larger than *C. bonariensis*.

In conclusion, the surveys demonstrated a lack of potential biological control agents for *C. bonariensis*. However, based on the observations regarding plant stress made during the surveys of this study, an ideal agent for biological control of this weed

would be an insect which affects the flow of nutrients, disturbs the roots, and/or can either facilitate the infection or is a vector of a plant pathogen. This insect would most likely be a hemipteran, as they are sap feeding insects which can affect nutrient flow with their feeding, some species have been found associated with the roots of plants (Ex. *Phenacoccus solani*), and these insects may act as vectors or facilitate pathogen infection, which could spread rapidly within plants. As the invasive range of the species extends west from California, east to Virginia (USDA 2014a); it may be suggested to conduct further studies to search for potential biological control agents of this weed in other parts of the southern United States with similar climates to Brazil and Argentina or Queensland and New Southern Wales, Australia where C. bonariensis is common. One suggested region of the southern United States could be Florida; as its climate seems to match that of Brisbane, Australia and C. *bonariensis* has been documented in that state (Laidlaw 2011: USDA 2014).

Chapter 3. Feeding guilds of herbivorous insects associated with *Conyza* and *Erigeron* spp. and plant performance of *Conyza bonariensis* under exclusion conditions

3.1. Introduction

Flaxleaf fleabane, *Conyza bonariensis* [L.] Cronquist is a highly invasive weed found throughout the southern United States. Plants in the genus *Conyza* are a very adaptive group of plants which can be found growing in a variety of habitats with different abiotic conditions, such as acidic soil, sandy soil, and water stress (Soares et. al 2017). Native to South America, this weed and several other *Conyza* species are major economic pests of crops in 70 countries around the world, including Australia (Holm et al., 1997; Hao et al., 2009). The earliest known record of *C. bonariensis* in Australia was from herbarium records collected in 1842 in New South Wales (AVH 2021), and though it is unclear when exactly *C. bonariensis* was introduced into Australia, this species was widespread throughout southeastern South Australia during the first botanical collection for that region in 1847 (Wu 2007). In its native range of South America, *C. bonariensis* is known to be the alternate host of several pests for various crop plants; some of these pests include different pathogenic species of *Colletotrichum* in corn and soybean, and several lepidopteran and pentatomid species associated with soybean (Bonacci et. al. 2016; Dalazen et. al. 2016). In addition to being an alternate host of several pest species; *C. bonariensis* also directly impacts the crop plants of fields that it may infest. Glyphosate resistant populations of *C. bonariensis* have been known to reduce the yield of soybean crops, as they can directly compete with the plants for available soil nutrients, water, and light (Agostinetto et. al.

2018). With many populations developing herbicide resistance, insects which feed on crop plants can use *C. bonariensis* as alternate host to maintain their populations while their preferred food sources are unavailable. *Conyza bonariensis* is also a host to several pathogens, such as *Colletotrichum* spp., *Alternaria* spp., and *Septoria* spp.; which species in the latter genus have been used as a biocontrol of *Cirsium arvense* (Bonacci et. al 2018).

Plants rely on a variety of mechanisms to defend against insect herbivory. In *Conyza* spp. some of these mechanisms include physical barriers such as trichomes and secondary chemical defensive compounds. In all three *Conyza* species: *C. bonariensis*, *C. canadensis*, and *C. sumatrensis*; the stems and leaves are either covered in a thin velvety layer of trichomes (*C. bonariensis* and *C. sumatrensis*) or are sparsely covered in long trichomes along the margin of the leaves and stem (*C. canadensis*). The trichomes of *C. bonariensis* and *C. canadensis* have been found to be glandular, producing no chemicals (Perveen., et. al 2016). The dense layer of non-glandular trichomes found in *C. bonariensis* may be used for environmental protection and an additional means to protect the plant from chewing insects, like what is seen in other plant species. Several secondary chemical compounds are produced by *Conyza* spp.; these may include phenols, saponins, alkaloids, and both mono- and sesquiterpenes (Richard et. al 2002). Many of the compounds associated with *Conyza* spp. have been shown to negatively affect herbivores; whether it be repelling/ repulsing or potentially killing a herbivore (Harborne 1993; Kortbeek 2018).

In its native range of South America, there have been one potentially host specific insect and even a host specific pathogen found associated with *Conyza*

bonarienisis: *Trupanea bonariensis* (Brèthes 1908) (Tephritidae), a gall forming fly, and *Puccinia cnici-oleracei* (Pers. 1823) (ex. *Conyza*) (Pucciniaceae), a rust fungus (CSIRO 2015). Many of the gall forming insects have high levels of specificity for their hosts (Santo, 2009). There are many host specific insects associated with other plants in the family Asteraceae; one example is the Rush Skeletonweed, *Chondrilla juncea* (L.) (Asteraceae) which has had two different host specific insect species found associated with it, and even used as biocontrol agents (Milan et. al. 2016). In Australia, the community of insects and pathogens found associated with *C. bonariensis* is very limited; with most being pests of agricultural crops, using *C. bonariensis* as an alternate host (Rafter et.al. 2017). To better understand the community of insects associated with both *Conyza* and *Erigeron* species in the southern United States, one objective of this study was to characterize the insect community associated with *Conyza* and *Erigeron* spp., and to determine the diversity and abundance of the various feeding guilds of those insects. The second objective of this study was to look at the development of these different plant species under similar growth conditions over time, and to observe the community of insects associated with each. Due to the lack of knowledge on the insect and pathogen pressure on *C. bonariensis* in Louisiana, the third objective of this study was to determine how various these stressors affect the growth and development of *C. bonariensis* over the growing season.

3.2. Materials and Methods

Community of herbivores: *Conyza bonariensis*, *C. canadensis*, and *E. philadalphicus*, were in a greenhouse and were transplanted into a common garden plot located at the LSU Burden Botanical Garden and Museum in Baton Rouge, LA (Fig. 3). A total of 36 plants of each species were planted. As a variable, plant height was measured for each plant within the garden plot over a 39-day period, with measurements being recorded twice per week for each plant species grown within the plot. For each week during the sampling period, the mean plant height of all plants within the garden plot for each species was calculated. Data were collected from June 6, 2020 to July 17, 2020. Additionally, the family level diversity of all insects associated with the three plant species for the June-July experiment period was calculated.

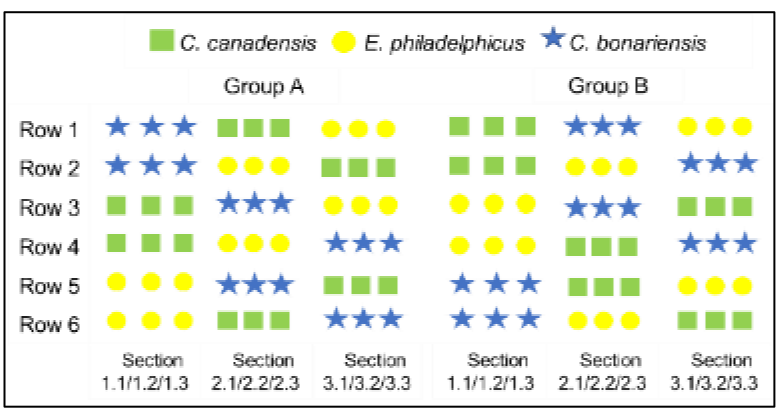

Figure 3. Layout of the plot: the plot was 3.3m long and was divided into 2 groups with 6 rows along its length. Each row had 3 sections; each with three different plant species within them. Each plant species within the plot is represented by a distinct shape and color: *C. canadensis* – green square, *E. philadelphicus*- yellow circle, *C. bonariensis*- blue star.

Data collection and analysis: The most dominant insect feeding guild, total number of insects present per plant, and dominant insect families were recorded. For herbivorous insects, the feeding guilds were classified as foliage feeders, nectar feeders, phloem feeders (including plant-cell lacerators), plant-fluid feeders, pollen feeders, and stem borers. Additionally, the types of damage observed on each plant species was recorded, which included, leaf defoliation, leaf mines, leaf blotch mines, leaf puncture marks, leaf nutrient drain coloration, stem holes, stem puncture marks, stem nutrient drain coloration, flower petiole puncture marks, disk flower damage, flower head holes, seed feeding damage, and pathogen. A Shannon diversity index was also calculated to characterize community species and family diversity for insects associated with each plant species.

Monitoring of plant performance began 1 week prior to the first treatment. Plant measurements (height and diameter), damage observed, developmental stage, and insect/pathogen occurrence were recorded. Each week the garden plot was visited to check for signs of plant damage and to recording the height and diameter of plants. For the first six weeks plans were in their rosette stages, so the diameter of the plants was measured as the diameter of the circumference of the plants at their longest points from the apex of a leaf to the apex of the opposite leaf. A freezing event occurred over a 1 week period in February 2021. During this period temperatures dropped to -7 ^{o}C, and all plants within the plot in a layer of ice for 4 days.

The abundance and diversity of insects per plant species was recorded; which shows how variables such as temperature and humidity changed in regard to date

These data were recorded twice per week from April 20, 2020 until May 29, 2020. Data were analyzed using Program R (R Core Team 2021). The proportion of feeding guilds for *C. bonariensis*, *C. canadensis*, and *E. philadelphicus* were analyzed and the total relative abundance was calculated for each. The number of the different types of herbivorous insects with varying feeding habits, associated with each plant species was also recorded. The change in plant height, family level Shannon diversity, and insect abundance over time, as a linear relationship, mean plant height, and standard error of plant height were generated to explore relationships between plant height with species and sampling date. Then, plant height, diversity, and abundance were analyzed by individual generalized linear models (GLMs) with plant height, diversity, or abundance as the response variable and species and sampling data as fixed effects. For each response variables, several GLMs were compared with different link functions and probability distributions (e.g., log-Poisson, inverse-Gamma, log-Gamma) with the combination with lowest AIC selected for interpretation.

Conyza bonariensis exclusion study: Sixty four plants of similar sizes/ stages of development were grown within biodegradable pots in a greenhouse and were transplanted to common garden plot, which was established adjacent to the initial plot at the LSU Burden Botanical Garden and Museum in Baton Rouge, LA (Fig. 4). For the first six weeks from January 25 – March 1, 2021, plants were in their rosette stages and did not have stems. Biodegradable pots were chosen as to not risk disturbing the roots of plants, which could result in the death of young plants. The mean height of plants at the time of transplanting was approximately 1.5 cm from the ground (soil covering the roots and the pots) to the base of rosette leaves. The mean diameter of plants was

approximately 31 cm and was measured from the tips of opposing rosette leaves. Plants were placed in groups of 4 within a section. Each group was placed 0.3 m away from one another, and each were given a different treatment: systemic fungicide, systemic insecticide, a combination of both systemic fungicide and systemic insecticide, and a water treatment (control). The arrangement of the treatments varied for each row, and each group was treated with their respective treatment options as recommended by the product labels. All treatments were applied at once, every 3 weeks, with the first applications being on January 18, 2021, one week prior to the first recordings. The following products were used for the insecticide and fungicide treatments: insecticides, Prevathon, and BioAdvance: fruit, Citrus and vegetable insect control, and fungicides Dynasty Daconil: vegetable, fruit, flower, shrubs, tree fungicide, and Infuse: disease control. The active ingredients for each product is as followed: Preveton-hlorantraniliprole and rynaxpyr., BioAdvance – imidacloprid, Dynasty- azoxystrobin, Daconil – chlorothalonil, and Infuse – thiophanate – methyl. The dosages for each product are as followed: Preveton- 0.0152 ml/section, BioAdvance – 1.627 ml/section, Dynasty- 0.0080 ml/section, Daconil – 1.627 ml/section, and Infuse – 1.627 ml/section. The dosage for each product was calculated using the mean weight of plants (7.893 g) and the length of each section (1.111 ft^2).

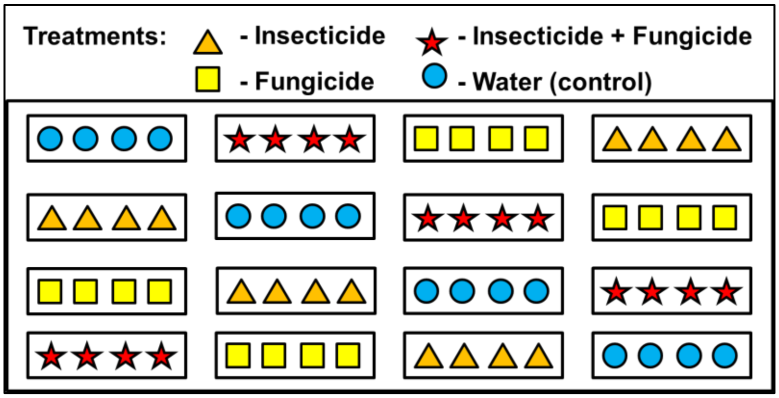

Figure 4. Layout of the plot: the plot was 3.3m long, with 4 rows along its length. Each column contained 4; each with 4 plants in each. Each treatment within the plot is represented by a distinct shape and color: insecticide- orange triangle, fungicide- yellow square, insecticide+fungicide- red star, and the water control- blue circle.

3.3 Results

Community of herbivores: All three plant species grown were put in the garden plot had similar types of damage from both insect herbivores and pathogen. *Conyza canadensis* differed from *C. bonariensis* in that no stem holes were observed. In *E. philadelphicus*, stem holes, stem puncture marks, and flowerhead holes were not observed. Of the 13 types of damage observed all were seen in *C. bonariensis*, 12 out of 13 were seen in *C. canadensis*, and 10 out of 13 were seen in *E. philadelphicus*. During the period of the study, *C. bonariensis* and *C. canadensis* had a steady increase in height, while *E. philadelphicus* had no significant change in plant height (Fig. 5).

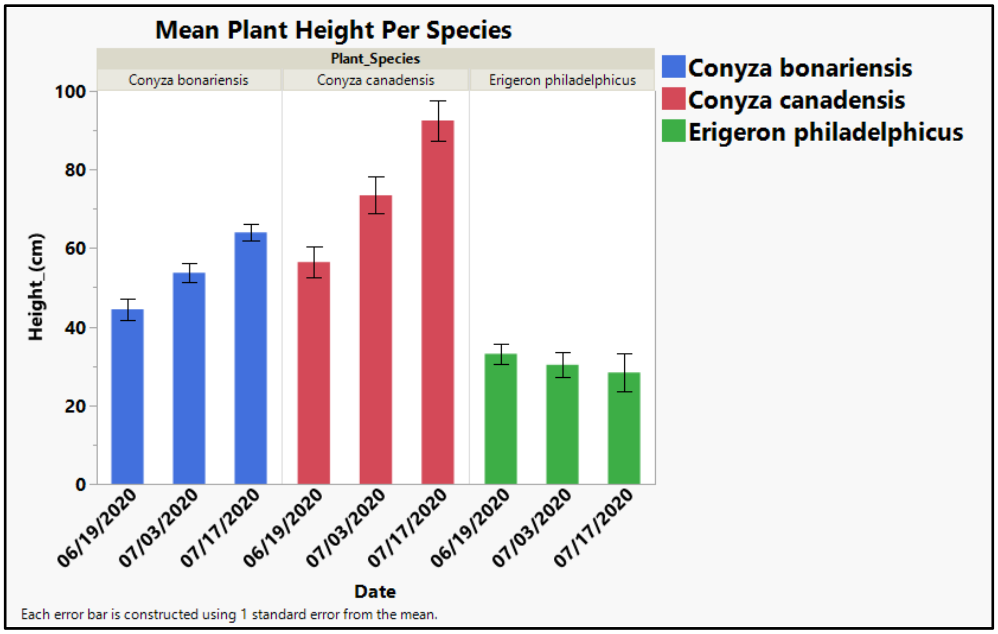

Figure 5**.** Mean plant height recorded for *C. bonariensis*, *C. canadensis*, and *E. philadelphicus* within the garden plot over a 39-day period, along with the standard error for mean plant height per recording date. There were 36 plants per species, and each plant was measured twice per week.

There was a clear difference in the three plant species observed in this study (Fig. 6). A significant difference in plant height was seen in both *C. canadensis* ($F_{1,69}$ = 19.20, P < 0 .0001) and *C. bonariensis* ($F_{1,98}$ = 31.37, P < 0.0001); and over time the height of both of these species steadily increased. However, there was no significant difference in the height of *E. philadelphicus* ($F_{1,35}$ = 0.96, Pvalue= .3329) over time. *C. bonariensis* height increased with date by 7.989e-5 (SE: 0.07099), *C. canadensis* height increased 1.487e-6 (SE: 0.05488) with date, and *E. philadelphicus* decreased – 2.024e-6 (SE: 0.07099) with date.

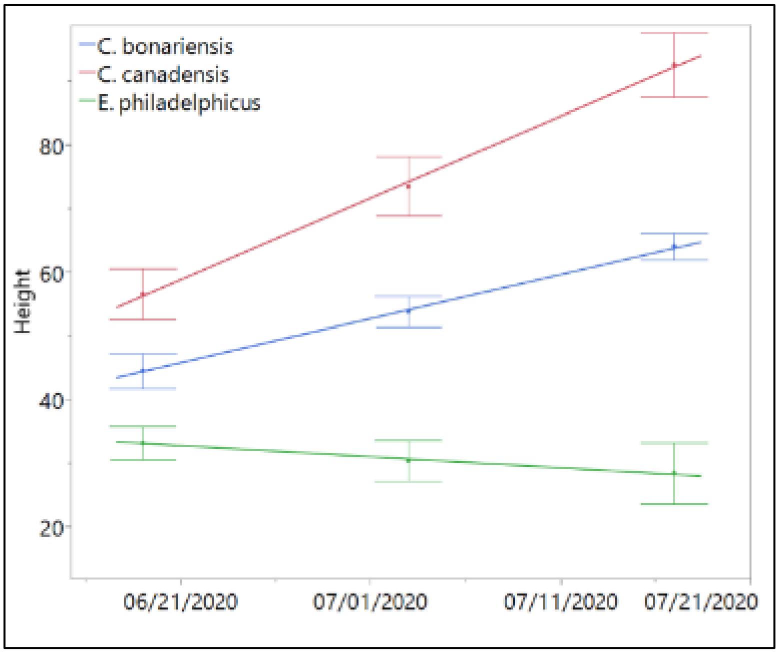

Figure 6. Mean height of *C. bonariensis*, *C. canadensis*, and *E. philadelphicus* during June and July 2020, when the experiment took place. An exploratory line of fit connects the means for each species.

Several insect species were observed attacking the plants in the garden plot, and for all three plant species, phloem feeders were the most dominant guild observed (Fig. 7). This feeling guild was comprised primarily of insects and the family Miridae, Aphididae, and Tingidae. Plant-fluid feeders were the second most dominant feeding guild to be observed on all three plant species including insects primarily in the family Agromyzidae.

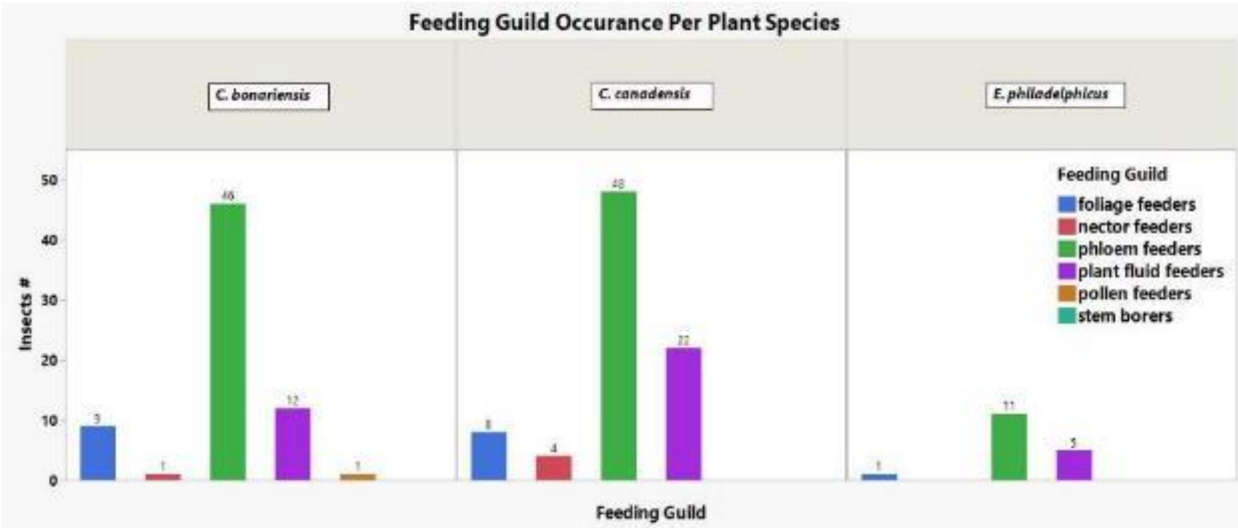

Figure 7**.** Insect feeding guilds associated with each plant species. There was a total of 6 feeding guilds observed; they included: foliage feeders, nectar, phloem feeders, plant-fluid feeders, pollen feeders, and stem borers.

A total of 168 insects were found associated with the different plant species within the plot. These insects were found to belong to 21 different families for both *Conyza* spp. (Fig. 8). For these plant species, insects in the family Miridae where the most dominant group and were found primarily feeding near the petiole of the flowers of all plants within the plot. Insects in the family Agromyzidae, which were found feeding within the leaves of all plant species as immatures, were the second most dominant group. For *E. philadelphicus*, both insects in the family Agromyzidae and Aphididae were the most dominant group of insects observed; this was followed by insects in the family Miridae. Insects in the family Aphididae were found throughout the entire plant, primarily on the stem.

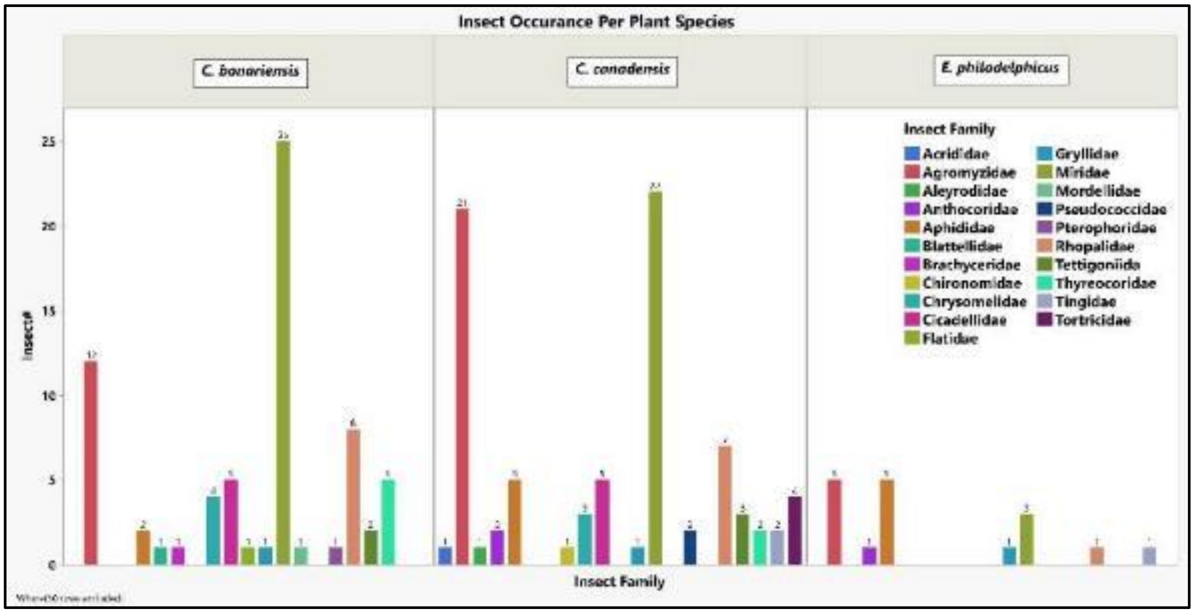

Figure 8**.** Abundance of insect families found feeding on *C. bonariensis*, *C. Canadensis*, and *E. philadelphicus* within the plot. All insects observed feeding on the plants or completed their lifecycles within a plant species were counted and identified to family level.

A Shannon diversity index was conducted in order to characterize community species diversity (Table 1). The closer the equitability value is to 1, the more diversity community of organisms is. The index was conducted for each plant species grown within the garden plot for both insect family and species number. When conducted with insect family numbers; the equitability value for each plant species is greater than 0.75. This indicated that the community of organisms within the garden is fairly diverse. When conducted with insect species numbers, the equitability value was still greater than 0.70.

Table 1**.** A table showing the variables found and used to calculate the Shannon Diversity index for all plant species within the garden plot. “Plant sp.” Is the plant species. “Family #” is the number of different insect families found on each plant species. “Species #” is the number of different insect species found on each plant species. “H” is the proportion of individuals in each species /family out of the total number of individuals of all the species (Sp. H) or families within the community. “H-max” is the max proportion of individuals in each species (Sp. H-Max) or family out of the total number of individuals of all the species or families within the community. “Eq” or equitability value, is value indicating how diverse a community of organisms is (“Sp. Eq.” is the value calculated for insect species).

Plant species	**Insect Family #**	**H**	**H-Max**	**Eq.**	**Insect Species #**	**Sp. H-Max**	**Sp. Eq.**
C. bonariensis	14	2.040	2.639	0.773	17	2.833	0.720
C. canadensis	16	2.220	2.773	0.801	20	2.996	0.741
E. philadelphicus	7	1.693	1.964	0.870	10	2.303	0.735

Several insect species were found associated within the garden plot. From the insects observed from the common garden experiment, a total of roughly 26 morphospecies were observed associated with both *Conyza* and *Erigeron* spp. Roughly 35 morphospecies have been found associated with *C. bonariensis* within 38 different families in this study. All insects that were found associated with *C. bonariensis* the common garden plot experiment were found to be generalist feeders of various plants species, including several in the family Asteraceae. Within the plot, no insects were found to be specialists of plants in the genera *Conyza* or *Erigeron*.

For *C. bonariensis*, each feeding guild is represented by its relative abundance: phloem feeders (primarily hemipterans in the families Miridae and Aphididae) compromised 40% of the observed feeding guilds *bonariensis* (Fig. 9-A). Phloem-fluid feeders (Primarily dipterans in the family Agromyzidae) compromised about 20% of the total relative abundance. Additionally, leaf miners (primarily larval dipterans in the family Agromyzidae) compromised about 20% of the total relative abundance. The relative abundance of the other feeding guilds was fairly low (foliage feeders, detritus feeders, nectar feeding adults, and fluid feeders).

For *C. canadensis*, each feeding guild is represented by its relative abundance: phloem feeders (primarily hemipterans in the families Miridae and Aphididae) compromised 51% of the observed feeding guilds (Fig. 9-B). Phloem-fluid feeders (Primarily dipterans in the family Agromyzidae) compromised 17% of the total relative abundance. Additionally, leaf miners (Primarily larval dipterans in the family Agromyzidae) compromised about 9% of the total relative abundance. The relative abundance of the other feeding guilds is fairly low (Foliage feeders, Detritus feeders, Nectar feeding adults, and Fluid feeders).

For *E. philadelphicus*, each feeding guild is represented by its relative abundance: phloem feeders (primarily hemipterans in the families Miridae and Aphididae) compromised up 56% of the observed feeding guilds (Figure 9-C). On this plant species, nectar feeding insects (adult lepidopterans in the family Pterophoridae) compromised 18% of the total relative abundance. Phloem-fluid feeders (Primarily dipterans in the family Agromyzidae) compromised about 10% of the total relative abundance. Additionally, leaf miners (Primarily larval dipterans in the family

Agromyzidae) compromised about 11% of the total relative abundance. The relative abundance of the other feeding guilds is fairly low (Foliage feeders, Detritus feeders, Flowerhead feeders, and Fluid feeders). It was seen that fewer insects with varying feeding strategies were observed on *E. philadelphicus*. However, in all plant species, the feeding habits with the most abundant number of insect occurrences were phloem feeders followed by phloem-fluid feeders.

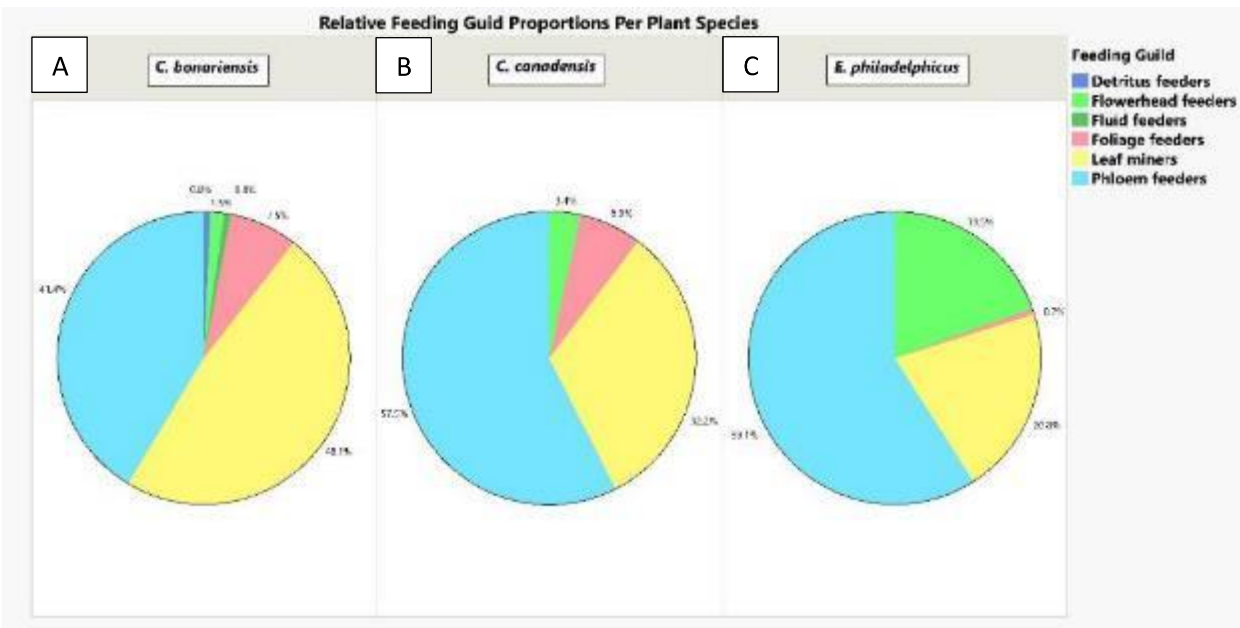

Figure 9. Relative proportion of observed feeding guilds for *C. bonariensis* (A), *C. canadensis*. (B), and *E. philadelphicus* (C) Grown within the common garden plot. All insects were assigned feeding guilds based on the feeding habits observed during their herbivorous stages.

.

The diversity of insect families in *C. bonariensis* and *C. canadensis* were similar compared to those present on *E. philadelphicus* (Fig. 10). The GLM of family level diversity from June to July indicates that there is a significant difference between *C. bonariensis* and *E. philadelphicus* ($Z_{1,323}$ = -2.199, P = 0.02789), but no significant difference between *C. bonariensis* and *C. canadensis* ($Z_{1,323}$ = -1.288, P = 0.19777). However, there was not a difference between *C. canadensis* and *E. philadelphicus* ($Z_{1,323}$ = 0.993, P = 0.3206). It suggests differences in family occurrence among plant species, date, and the interactions. Diversity of insect family changed by plant species and date. Results showed that there was a difference in the changes of insect diversity within *C. bonariensis* and *E. philadelphicus*.

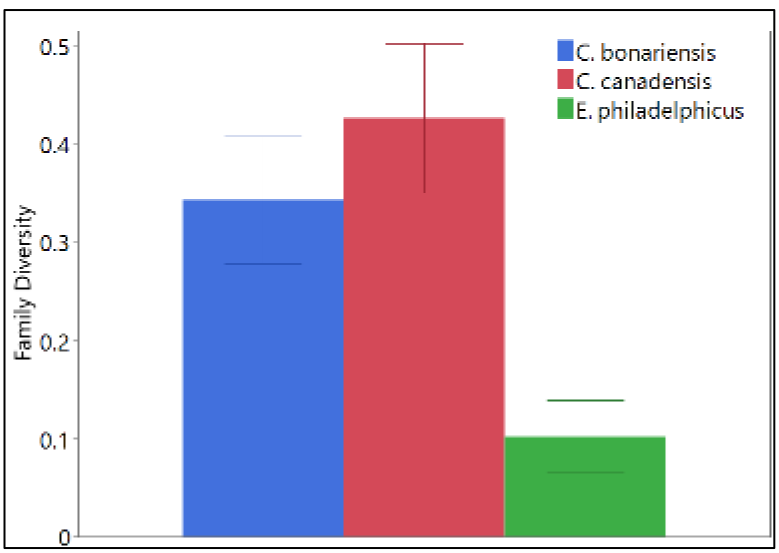

Figure 10**.** Bar graph depicting the mean family diversity of insects associated with *C. bonariensis*, *C. canadensis*, and *E. philadelphicus* within the plot. The mean number of different insect families which occurred on each plant species for all plant within the plot was calculated; giving the diversity of insect families per plant species.

When looking at the community of insect in all three plant species, the diversity and abundance of insects increased overtime (Table 2). This is somewhat expected; as the seasons change and temperatures begin to increase, the activity of insects may increase as well.

Table 2. Temperature (°C) relative humidity, and total insect diversity and abundance for the sampling dates in April and May 2020 for all plants within the plot. Climate data recorded twice per week, with mean insect diversity and abundance for all insects within the plot being calculated from insect observations using the R-code programming software. Included is also the SE (standard error) for each calculation.

Date	**Mean temp**	**SE**	**R.H.**	**SE**	**Mean Diversity**	**SE**	**Mean Abundance**	**SE**
4/20/2020	21	0	68.00%	0	0.08	0.06	0.17	0.12
4/24/2020	25	0	54.00%	0	0.08	0.06	0.22	0.15
4/27/2020	26	0	35.00%	0	0.08	0.05	0.17	0.10
5/1/2020	25	0	39.00%	0	0.11	0.05	0.19	0.10
5/4/2020	28	0	57.00%	0	0.17	0.08	0.28	0.16
5/8/2020	24	0	76.00%	0	0.17	0.06	0.36	0.19
5/11/2020	24	0	37.00%	0	0.22	0.11	0.61	0.32

(table cont’d.)

Date	Mean temp	SE	R.H.	SE	Mean Diversity	SE	Mean Abundance	SE
5/15/2020	27	0	74.00%	0	0.78	0.20	2.03	0.70
5/18/2020	26	0	57.00%	0	0.67	0.19	1.58	0.66
5/22/2020	30	0	62.00%	0	1.06	0.23	2.72	0.78
5/25/2020	28	0	72.00%	0	0.64	0.17	1.33	0.44
5/29/2020	29	0	49.00%	0	0.58	0.14	1.19	0.35

From April 2020- May 2020, the mean abundance of insects was found to be very similar between *C. bonariensis* and *E. philadelphicus*, but lower for *C. canadensis* (Table 3). Additionally, the mean diversity of insect families associated with each plant species was found to be similar each species within the plot as well.

Table 3. Mean insect abundance and diversity in all *C. bonariensis*, *C. canadensis*, and *E. philadelphicus* is depicted, along with the SE for each calculation. All insects observed feeding on plants were counted and individuals were identified to family to calculate the mean diversity.

Plant Species	Mean Abundance	SE	Mean Diversity	SE
Conyza_bonariensis	0.95	0.25	0.38	0.08
Conyza_canadensis	0.69	0.17	0.34	0.07
Erigeron_philadelphicus	1.08	0.23	0.43	0.06

Here a GLM was used to examine differences in abundance between plant species and dates (Fig. 11-A). For both *C. canadensis* and *C. bonariensis* ($Z_{1,431}$ = -2.468, P = 0.01360) and *C. canadensis* and *E. philadelphicus* ($Z_{1, 431}$ = 3.542, P = 0.000398) were significantly different, but *E. philadelphicus* and *C. bonariensis* ($Z_{1,431}$ = 1.110, P = 0.26715) and were not. There was no plant affect, but there was a date effect on insect diversity. To conclude, all plant species are statistically similar, but for the dates; the initial dates are the same, but the later dates differ. No differences in diversity was detected amongst plant species (Fig. 11-B). For *C. canadensis* and *C. bonariensis* ($Z_{1, 431}$ = -0.558, P= 0.576909) and *C. bonariensis* and *E. philadelphicus* ($Z_{1, 431}$ = 0.727, P= 0.466965), which indicates no significant differences in insect diversity amongst plant species. When compared to the diversity of insects found during the later experiment from June -July 2020, it was seen that the diversity of insect species associated with all three plant species was greater than the diversity of insect families associated with each plant species. As there could be multiple insect species found within individual insect families, such as with *Lygus lineolaris* and *Taylorilygus apicalis* in the family Miridae, a difference in insect species and family diversity would be expected for each plant species; with a larger insect diversity being seen for insect species than for insect families.

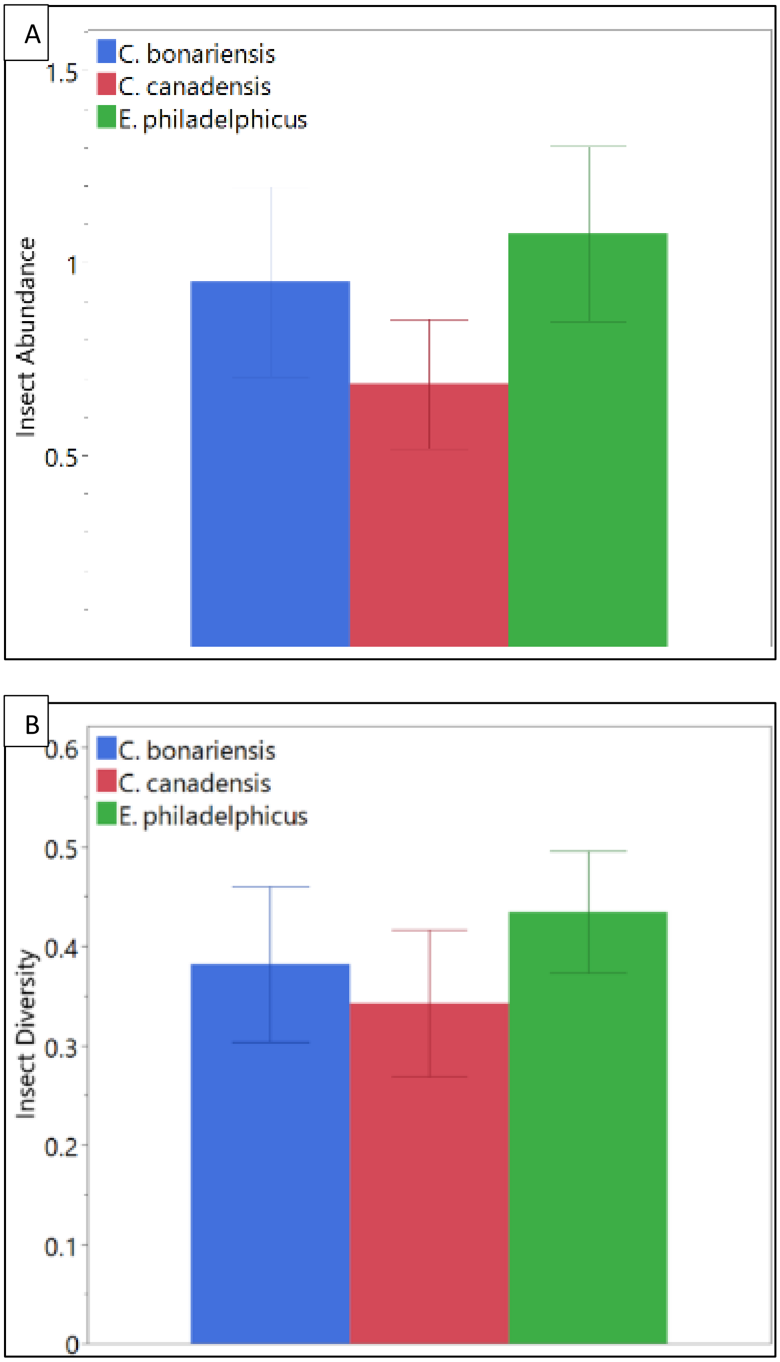

Figure 11. Mean insect abundance (A) (±SE) and insect family diversity (B) (±SE) of all insects found associated *C. bonariensis*, *C. Canadensis*, and *E. philadelphicus*. Both mean insect abundance and family diversity were calculated using the total occurrence of each insect family observed feeding on each plant species.

Conyza bonariensis exclusion experiment: Initially there was no damage observed on any plants; however, as time progressed many of the plants that were not treated with insecticides began to show signs of insect damage - leaf mines/ blotches and piercing/sucking, chewing, and defoliation damage, and many plants that were not treated with fungicides began to show signs of pathogen damage – leaf discolorations, spots, and blight - on the rosette leaves. After a freezing event, which occurred within southern Louisiana during February 2021, plants treated with both fungicide and insecticides began to show signs of pathogen damage on rosette leaves. Necrosis of rosette leaves was observed more often 2-3 weeks after the freezing event. Before the freezing event, most plants had not developed stems; but after this event, it was found that all plants had developed stems.

As rosette diameter was recorded for all plants up until the point where each individual plant had developed a stem, it was seen that all plants it had been treated with fungicide had a smaller rosette diameter (figure 12). Plants treated with insecticides seemed to have a larger leaf diameter than for all other treatments; however, the results for the rate of growth of rosettes was very similar in this treatment, to what was seen in the control. In all, plants treated with fungicide only had the lowest mean rosette diameter of all treatments. This may indicate that insect herbivory could play a major role in the reduction of plant development, or at the least the size of rosette leaves.

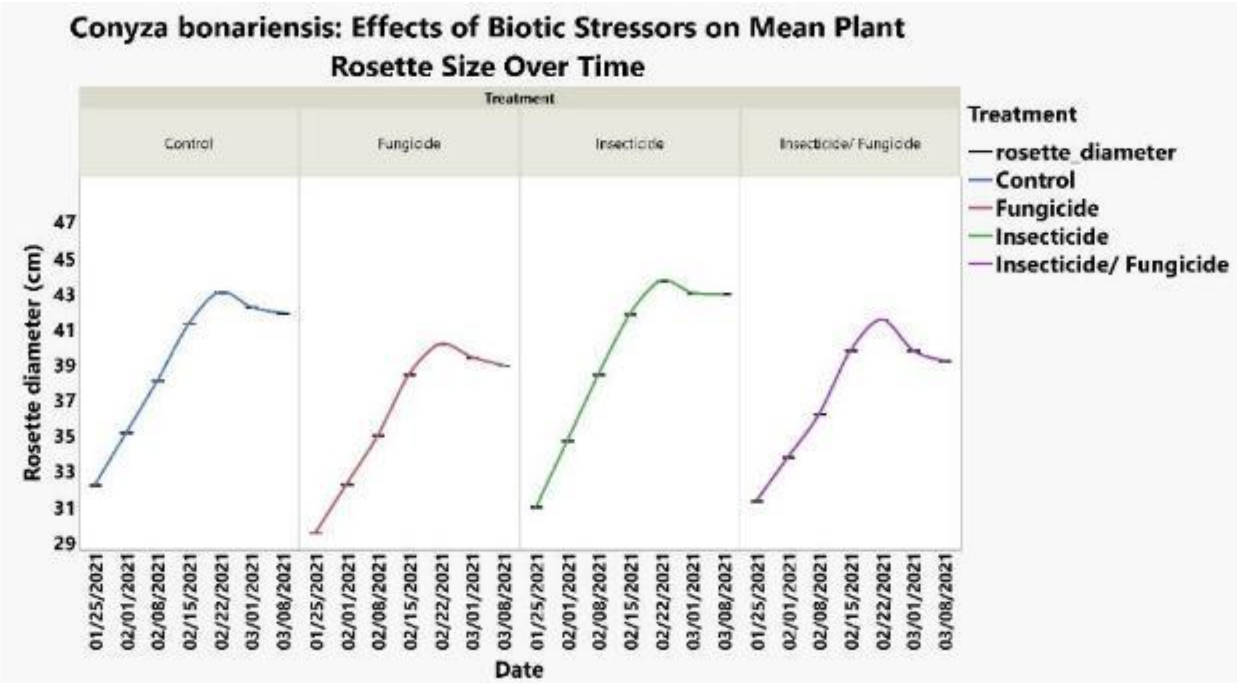

Figure 12. The effects of biotic stressors on plant rosette diameter of *C. bonariensis* in the presents of exclusion treatments: fungicide, insecticide, fungicide and insecticide, and water control. The rate of rosette growth and decline for all plants within the plot was recorded by from January 2021 to May 2021.

As plants began to develop stems, leaf necrosis and even insect feeding damage was observed in all plants; with the necrosis resulting in the loss of rosette leaves. As plants begin to develop axial leaves along their stems, leaf mines began to appear on many that were not treated with insecticides. Throughout different stages of development, the untreated plants were attacked by various insects. Some insects that were found feeding on plants before the development of flowers included primarily insects in the family Miridae and Aphididae. As flowers begin to develop on plants,

Lepidoptera in the family Pterophoridae were seeing feeding on flower heads as immatures. While rosette leaves were still present, blotch mines were found on these plants as well; but as these leaves begin to wither, these mines became less abundant. However, throughout the experiment, leaf mines of insects in family Agromyzidae were quite abundant.

Of the plant damage observed for each treatment, it was seen that in both the control and the insecticide treatments, more plants were damaged by a combination of insects and pathogens (Fig. 13). During this time, the majority of insect found associated with these plants where the agromyzid flies, which mined through the leaves of plants. All treatments had plants which were observed being attacked by insects only during recording. Insect Square found to be most abundant in the fungicide only treated plants; and both insecticide only and the combination of insecticide and fungicide treatment had similar occurrences of pathogen damage during recordings.

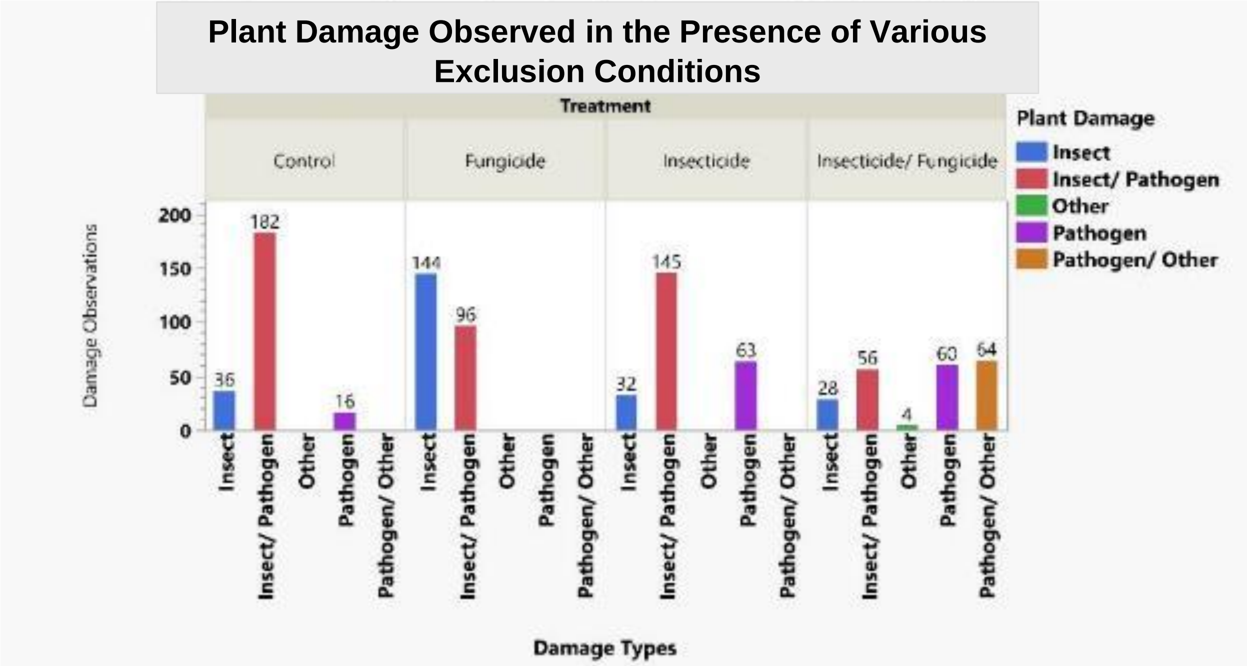

Figure 13. Plant damage observed in the presence of the various exclusion conditions (fungicide, insecticide, and insecticide/fungicide) including a water control. The types of damage observed included only insect, only pathogen, both insect and pathogen, both pathogen and other, and only other types of damage. The type of damage described as "other" included any type of damage that couldn't be attributed to either visible pathogen or insect feeding damage.

Though there was a freezing event which occur during the early portion of the experiment, which covered all plants with a layer of ice over five days; this seemed to have little to no lasting effects on the plants as all plants began to develop stems afterwards within two weeks. However, heavy rainfall caused parts of the garden plot to become saturated with water; despite there being drainage for the garden plot during the experiment; As the experiment concluded plants were exposed to heavy rainfall

over a two-week period, and at this point, all plants seemed to be affected by the soil saturation at this point as many began to darken and become brown to black in coloration. After the second week of heavy rainfall, all plants seem to be either completely dead or only have parts of the upper stems what flower heads still alive.

Throughout the monitoring of plant height over the course of the exclusion study, it was seen that despite the treatment, all plants had a similar trend of growth from the initial height after transplanting, two plants developing flowers and entering senescence (Fig. 14). The main plant height for each treatment did vary; however, as it was seen that plants treated with insecticides tended to be much taller or had a more rapid rate of growth than all other treatments. The other hand, plants treated with both insecticide and fungicide had a slower rate of growth and were ultimately smaller than for all other plant treatments. This may have been due to other factors besides insect and pathogen damage, as plants with this treatment were the only plants that seemed to have damages not attributed to insect feeding or pathogen infection. The mean plant height and rate of growth for both the control and fungicide treatments were very similar, showing a similar trend over the course of the experiment.

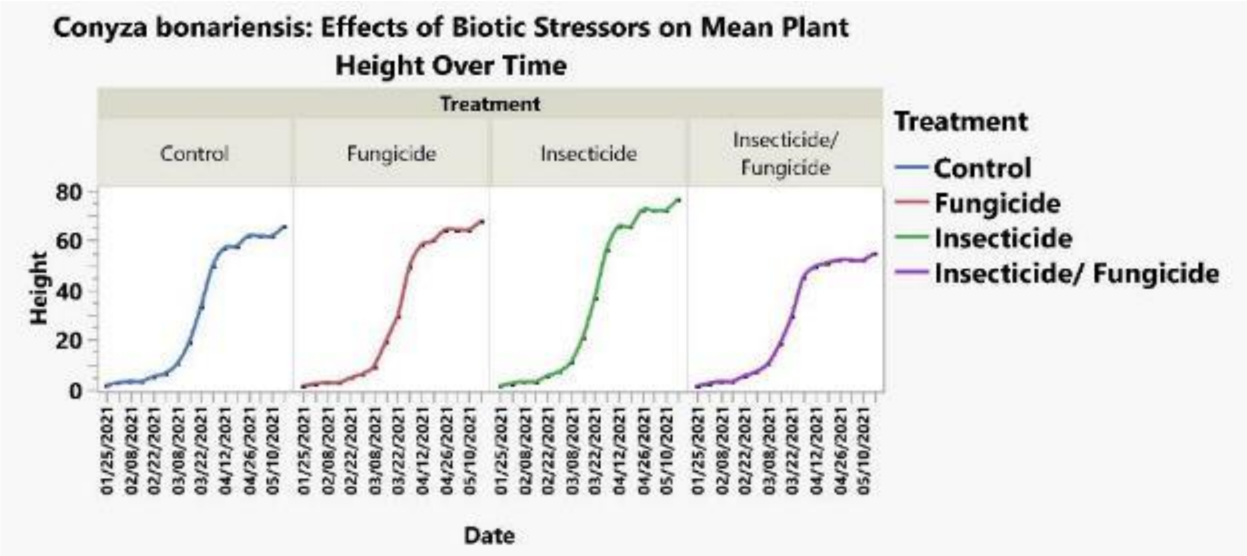

Figure 14. The effects of biotic stressors on mean plant height of C. bonariensis in the presents of exclusion treatments: fungicide, insecticide, fungicide and insecticide, and water control. The rate of growth for all plants within the plot was recorded from January 2021 to May 2021.

3.4. Discussion

In this study, all insects found associated with *C. bonariensis* were found to be non-host specific. All insects found were generalist feeders, feeding on a variety of plants; some of which also fed on plants in other families. The only host specific insect was *Neolasiopera erigerontis* and found associated with *C. canadensis*, which is native to North America (USDA 2014b). *Neolasiopera erigerontis* larvae were found inside the stem galls (Appendix1: Fig. 17). Though galls were found in *C. bonariensis*, they were associated with *Mordellistena pustulata*, as the larvae fed on the pith of the plant. In the United States, *C. bonariensis* is considered an introduced species (USDA 2014a);

unlike *C. canadensis*. This may explain the lack of specialist insects found associated with the species in Louisiana.

During this study, it was seen that both *C. bonariensis* and *C. canadensis* shared a lot of the same insects herbivores. Also, both insect diversity and abundance were higher for these species than they were for *E. philadelphicus*. This could be due to both *Conyza* spp. being more closely related to one another than the *Erigeron* sp. despite *C. canadensis* and *E. philadelphicus* being native to the United States (Nelson 2008). This could also explain the similar types of damage observed between the two *Conyza* spp. as opposed to what was observed in *E. philadelphicus*. It was also seen the insects associated with and the feeding damages observe for each plant species varied, even between the more closely related *Conyza* spp. As these two species vary in the types of defensive chemical compounds they utilize and physical defense of traits they poses, it would be expected that the diversity of insects associated with each species would vary (Araujo et. al. 2013; Ayaz et. al. 2017; Perveen et. al. 2016)

The diversity of insects and the number of insects associated with each species within the plot may also vary due to the size difference of plants. *Conyza bonariensis* can reach a height of 2 m, and *C. canadensis* can reach a height of 3 m. Additionally, these two plants produce multiple axial branches from their main stems (Shrestha 2008), increasing the amount of food available for potential herbivores. Whereas *E. philadelphicus* can only grow up to 0.9 m in height, and only has a main stem that may or may not branch into multiple flower heads (Immel 2000). *Erigeron philadelphicus* may not be able to support as many insects as either *Conyza* spp. due to its smaller size.

During the common garden experiment, it was seen that the three plant species, *C. bonariensis*, *C. canadensis*, and *E. philadelphicus*, all had distinct rates of growth. During the study no additional *Conyza* and *Erigeron* spp. were able to be grown. All other plant species either failed to sprout after being planted in the greenhouse or died before they could be transplanted to the garden plot. Some of these plants included: *C. sumatresis*, *E. annuus*, and *E. procumbans*. During the late spring to early summer when this study took place, both *Conyza* spp. had a steady increase in mean plant height, while there was no difference in the overall mean plant height of *E. philadelphicus* during this period. Within the plot, the *E. philadelphicus* plants began to flower earlier in the year between late March and April. During that time, both *Conyza* spp. were still in their rosette stages despite all plants initially sprouting within one week of each other in a greenhouse before being planted in the garden.

It has been observed in California that *C. canadensis* emerges in the fall from October to February and in the spring from March to May, the growth and bolting phase of the species begins in late April to mid-August, and the flowering and sitting face seeding phase begins from August to mid-October (Shrestha 2008). However it is also seen that when conditions are favorable, seeds can germinate throughout the year in this species (Buhler et. al. 1997). The fall and spring emergence of *C. bonariensis* is similar to C*. canadensis*. However, the bolting and growth phase of the species has a shorter duration lasting from late April to early July, and the flowering and seating phase last thing from July to mid-September (Shrestha 2008). As California has a hot and dry climate, the phenology of *C. bonariensis* and *C. canadensis* may differ in the cooler more humid climate of the southeastern United States. These cooler and more humid

conditions may facilitate earlier flowering, seeding, and emergence with variations in periods of growth or development. Phenology of *E. philadelphicus* seems to vary depending on the region (Novak 2015; USDA 2014c), but during my study it was seen that this species emerged in mid-November and was seen flowering between late February and July.

For the exclusion it was seen that during the early stages of development*, C. bonariensis* may be more susceptible to insect feeding damage then to other types of stressors; as it was seen that plants treated with fungicide tended to have smaller rosette diameters than plants treated with any other treatment; and despite not being treated to exclude pathogens or insects, plant rosette size for the control treatment mirrored those of the insecticide treatment; which had the greatest mean rosette diameters over time. It was also seen that plants treated with the combination of insecticide and fungicide, had a lower rate of growth and were overall smaller than all other plants look treated during this study. Plants treated with insecticides only, had a more rapid rate of growth and tend to be larger than all other plants within the study. It was also seen that plants treated with the water control seemed to grow better than those that had been spread with any other treatment. Visually, these plants looked healthier -dark green leaves and stems- despite of them being fed upon by insects and infected with pathogens. Only plants grown on the edge of the plot same to show any signs of stress throughout the study, but this what’s most likely due to the water saturation of the soil; as all plants along the edge were extremely stressed, showing either yellowing or necrosis of the leaves.

In addition, when looking at plant damages observed in the presence of the various treatments, it was seen that more combined insect and plant damage was observed between the control plants and those treated with insecticides. However, these two treatments along with the combination insecticide and fungicide treatment had the lowest occurrence of insect only feeding damage. The plants treated with both insecticide and fungicide were observed how many damage that was not attributed to either pathogen for insect feeding damage, which could've ultimately been a result of the chemicals themselves; has no other treatments were seen to have this type of damage.

In conclusion, this study demonstrated that plants may higher sensitivity to insect feeding damage. As it was seen in the study, young plants in the presence of higher insect herbivory tended to be smaller than those where attempts were made to exclude. Additionally, plants treated with insecticides tended to grow at a much faster rate and were seen to be larger than those treat it with any other treatment; indicating that plants grow better without these types of stressors. Though this study did not focus on any insect species or pathogen strain, it may be suggested to conduct further studies looking at how individual species of insects or strains of pathogens affect the development of *C. bonariensis* over time. In addition, it may be suggested to look further into other regions of the United States for populations of *C. bonariensis* that may have different insects associated with them, to potentially find host specific species that may act as a biological control agent for this weed.

Conclusions and Recommendations

Flaxleaf fleabane, *Conyza bonariensis* [L.] Cronquist is a major economic weed for various crops in several countries due to its invasiveness, effectiveness in competing with other plants for essential nutrients and water, and its subsequent development of herbicide resistance (Holm et. al. 1997; Thébaud et. al. 1995; Agostinetto et. al. 2018). *Conyza bonariensis* is native to South America but is widespread throughout Central America and the Southern United States. The introduction of *C. bonariensis* to Australia greatly impacted the no-till agricultural systems in regions where this weed established and became widespread. The impact of this weed on Australia's no -till agroecosystem is in part, due to the cost of managing this plant; as more and new herbicides are needed to keep these weeds under control. The purpose of this thesis was to survey areas within in the southern United States, characterize herbivorous insects associated with *C. bonariensis*, and find potential biological control agents that may be beneficial in the management of this weed in Australia.

The second chapter of this thesis aimed to identify and record herbivorous insects associated with both *Conyza* and *Erigeron* spp. commonly found throughout the southern United States, and to determine the feeding guilds and potential level of specificity for those associated insect species. During field surveys, several herbivorous insects were found associated with *Erigeron annuus* (L.) Pers., *E. philadelphicus* (Felt 1907.), *C. bonariensis*, *C. canadensis* (L.) Cronquist, and *C. sumatrensis* (Retz.); however, more insects species were found associated with the larger *Erigeron* sp., *E. philadelphicus* (L.), and all *Conyza* spp. Many of the insects found associated with these

plant species were found to be phloem feeding hemipterans - *Lygus lineolaris* (Palisot de Beauvois, 1818) (Hemiptera: Miridae), *Taylorilygus apicalis* (Fieber 1861) (Hemiptera: Miridae), and *Corythucha marmorata* (Uhler 1878) (Hemiptera: Tingidae) - which in high densities could cause severe damage and stress to their host plants. Though there were 29 morphospecies of herbivorous insects found associated with both *C. bonariensis* and *C. canadensis* during these surveys of natural enemies, none of the insects were found to be host specific species. All insects found associated with the *Erigeron* spp. were also found associated with both *Conyza* spp.; and though several insects found associated with *C. bonariensis* and *C. canadensis* were not found to be associated with any observed *Erigeron* spp., those insects were also found to be generalist feeders, associated with other plant species.

As a recommendation for further studies in searching for potential biocontrol agents of *C. bonariensis* within the southern United States, it would be suggested to expanding the survey range. The survey for this chapter was conducted within the neighboring states in the southern United States: Louisiana, Texas, and Mississippi. With the range of *C. bonariensis* extending west from California and east to Virginia (USDA 2014a), the community of insects associated with different populations of this species could vary drastically in other parts of the country. Additionally, I would suggest looking further into what types of feeding damage could result in the most injury *to C. bonariensis*, or even investigate what potential pathogens may be transmitted from vectors during plant feeding. As it was seen from this study, even when plants appeared to have severe damage by insect herbivores, such as with *Mordellistena pustulata* (Melsheimer, 1846) and *Hippopsis lemniscate* (Fabricius 1801), their feeding damage

did not necessarily result in an overall plant decline. On the other hand, the damage by the generalist feeder, *Corythucha marmarata*, resulted in leaf discoloration and even death of *Conyza* plants under high infestation.

An additional recommendation may be to conduct focused surveys on *C. canadensis*, which is native to the United States (USDA 2014b). During this study, two putative specialist insect species were found associated with *C. canadensis, Calycomyza minor* (Spencer 1973) and *Neolasioptera erigerontis* (Felt 1907). However, they were found to be difficult to either rear or maintain colonies. *Calycomyza minor* is a leaf mining fly in the family Agromyzidae, and is known to only be associated with *C. canadensis*, feeding within the leaves of this plant species (Spencer 1990). During this study many larvae were reared from leaf material, but after emerging as adults, would die within a few days. Many attempts were made to maintain colonies of adults, but finding a food source for these insects was fairly difficult. *Neolasioptera erigerontis* is a gall forming midge in the family Cecidomyiidae. Similarly to *C. minor*, this species is known to be host specific, associated only with *C. canadensis* (Gagne 1994). The larvae of *N. erigerontis* form galls and feed within the stem of *C. canadensis*. Larvae have been found within the stems of both live and dead plants, and adults have been reared from both stem types placed within growth chambers; however, after several attempts at placing adults within insect cages with live *C. canadensis* plants, no female ovipositioning or larval, gall formations were observed. It would be suggested to conduct further studies on the biology of these two species for proper colony maintenance of adults.

The third chapter of this thesis aimed to study the interactions of various herbivorous insects associated with *Conyza* and *Erigeron* spp. Here, a common garden plot was utilized to monitor the growth of *Conyza bonariensis*, *Conyza canadensis*, and *Erigeron philadelphicus* and to record the community of insects associated with these plants species over time. Insect feeding damages was also recorded during this time, and it was found that over a 39 day period, 12 different types of insect feeding damage were observed, along with pathogen damage. All types of damage - (leaf defoliation, leaf mines, leaf blotch mines, leaf puncture marks, leaf nutrient drain coloration, stem holes, stem puncture marks, stem nutrient drain coloration, flower petiole puncture marks, disk flower damage, flower head holes, seed feeding damage, and pathogen) - were observed on *C. bonariensis*, 12 types, excluding stem holes, were observed on *C. canadensis*. Though *C. canadensis* had one less observed type of feeding damage associated with it during the common garden experiment, this type of feeding damage was observed in the field, so it doesn't exclude the insect species associated this damage entirely.

For each plant species, there was a total of six possible feeding guilds that could be observed in the insects associated with them - foliage feeders, nectar feeders, phloem feeders, plant fluid feeders, pollen feeders, and stem borers. During the common garden experiment, the majority of insects found associated with all three plant species were found to be phloem feeders, primarily hemipteran in the family Miridae. Of the 168 insects found associated with the different plant species, all were found to belong to 21 different families on both *Conyza* spp. Though more types of feeding damage were found on *C. bonariensis,* a larger number of insect families were found on *C.*

canadensis. A Shannon diversity index was conducted to characterize community species diversity of insects found on both individual plants and all plants within the garden plot. The results showed that both the equitably values for the plot and for individual plant species was > 0.70, indicating that the community of organisms within the garden and on individual plants was fairly diverse. Of the 35 morphospecies found associated with *C. bonariensis* during this study, 26 were observed associated it with this plant species during the common garden experiment.

As both *C. bonariensis* and *C. canadensis* are known to flower and grow throughout the summer months (Shrestha et. al. 2008), it would be expected that these plant species would have steady growth during the time of the common garden experiment, which took place from June to July. These plants are also known to grow larger than *E. philadelphicus*, indicating that they may be able to support a greater diversity and density of insects (Immel 2000: Shrestha 2008). As a suggestion for any future studies looking at the diversity or community composition of insects associated with *Conyza* and *Erigeron* spp.; individual plant species should be grown at a given time, and the insects associated with them should be monitored for the entirety of the life cycles of the plant each plant species.

Being a host for various insects and pathogens, *C. bonariensis* may be attacked by different types of enemies during different stages of development. As the immature or overwintering stages of these plants are in a rosette form, they do not possess a stem or flowers (Strek et.al. 2020). The insects which utilize these parts of the plant for feeding or development may not be present during periods where plants are in this form. However, there could be insects which utilize this species during overwintering

periods. Future surveys should focus on the insect associated with the overwintering forms of the plant.

Heavy rainfall, as well as periods of freezing weather may affected some of the results of the exclusion study since the experiment was conducted in an outdoor setting. In the field, *C. bonariensis* was rarely found in areas where the soil could be heavily saturated with water, and though there was drainage for the plot that prevented flooding, the heavy rainfall over long periods caused parts of the garden plot to become saturated with water. When looking at the impact of stressors on plant development for and individual species, such as *C. bonariensis*, it would be suggested to take in account, any abiotic factors they may affect the results of an experiment that is being conducted outdoors. During this study, factors such as severe weather and soil water saturation may have affected the health of plants grow within the plot. An answer to this for any future studies may be to add some sort of covering to plots to prevent direct water saturation and ice from forming plants. Additionally, it may be suggested to look at the long-term effects of different or the same chemical applications on *C. bonariensis*. Being herbs or weedy plants, this species me be negatively impacted or sensitive to various chemical applications. As it was seen in this study, an additional stressor, not attributed to insect feeding damage or pathogen infection seemed to have had a major negative affect on plant health, growth, and development. As this stressor was only observed in the combination of both fungicide and herbicide treatments, it was suspected that the combination of the chemical insecticide and fungicide may have play a role in the deterioration of plants; however, the reaction of *C. bonariensis* to the

chemicals present in the products used during this study must be further investigated before coming to a conclusion.

As a recommendation for any future exclusion studies looking at their various biotic stressors affecting the development of *C. bonariensis*, it would be suggested to conduct studies using individual insect species or pathogen strains, as well as communities of these organisms in a more controlled setting such as in a greenhouse or a laboratory. Conducting similar studies in a more controlled environment may prevent the need chemical pesticides to prevent insects and pathogens from attacking plants, which would negate the chance of stress by treatments.

Appendix B. Neolasioptera Erigerontis

Neolasioptera erigerontis (Felt 1907) is a gall forming dipteran known to be host specific, forming galls in *Conyza canadensis* (Figure 15). This species is widespread, and form galls along the stem of the plant. These insects were also reported to form galls in the petiole of leaves, which can be found to contain larvae in the summer, which overwinter within the galls and emerge as adults in the following spring (Painter 1935).

Plant galls from *C. canadensis* were collected from the New Orleans sites found during the Summer 2019 intensive surveys. The number and types of galls (stem, bark, or branch) collected were recorded. Galls of each type were grouped by whether exit holes were present or not. Galls were placed in containers and all containers were placed in growth chambers set to 20 °C, with a 70% RH; and were monitored for insect emergence. The types of insects found emerging from galls were recorded, and samples were taken. All emerging gall midges were removed from each container, and the number collected, gall type, and date of collection were recorded. The removed midges were transferred to an insect cage that contained a young *C. canadensis* plant with a stem.

Plant galls that were collected from *C. canadensis* in November were observed for insect emergence (Figure 16). Galls were grouped by location collected, whether exit holes were present or absent, and the types of galls observed- branch, stem, or bark (just below the surface of the stem). These galls were monitored daily for signs of insect emergence, and it was seen that only galls of the axial branches did not have adult all

midges reared from them. Midges emerged from both plants with and without exit holes, and all emerged within two weeks of the first emergence.

In the field, no adult midges were found; but upon dissecting stem galls, most plants -dead and living- contained larvae of *N. erigerontis.* The larvae of these insects were often found within or near the pith of the plant. Though the target of this study focuses on *C. bonariensis*, *C. canadensis* it's still a problematic weed in Australia; and with there being a host-specific insect associated with the species, biological control may be an option of controlling it using a natural enemy.

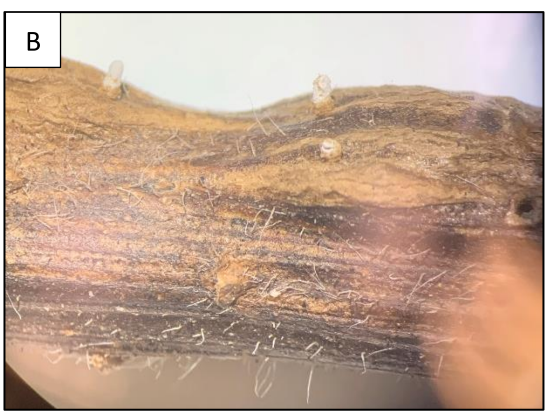

Figure 15. Parasitoids (Eulopidae) of *Neolasioptera erigerontis* which emerged during rearing attempts (A). Emergence holes and cases from the parasitoid wasp from bark stem galls of large *C. canadensis* plant (B).

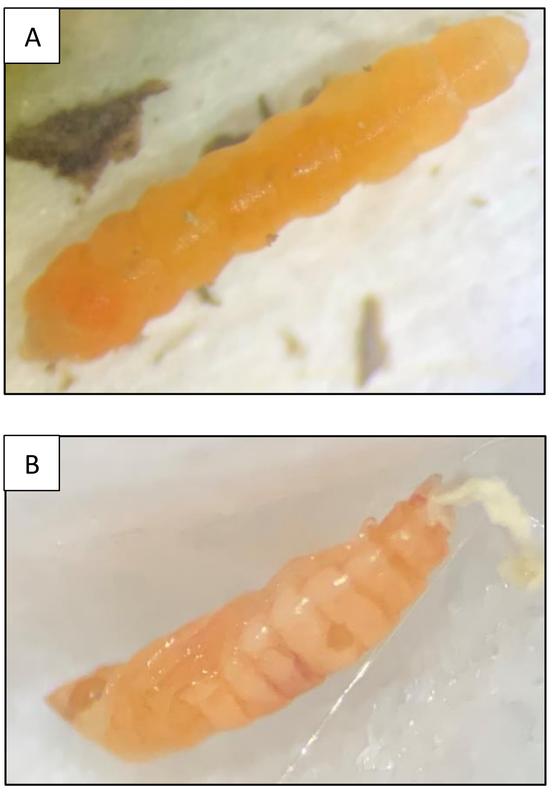

Figure 16. Dorsal view of *N. erigerontis* larvae extracted from stem gall (A). Developing pupae of *N. erigerontis* extracted from stem gall (B). Adult *N. erigerontis* individual collected after emerging from stem gall (C).

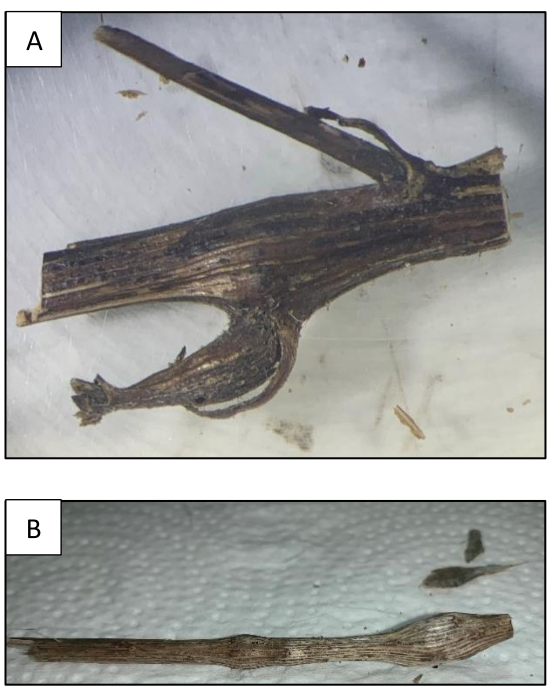

Figure 17. Intact (A and B) and dissected stem gall (C) of *N. erigerontis* from dead *C. canadensis* plant.

Appendix B. Insects of Conyza and Erigeron spp.

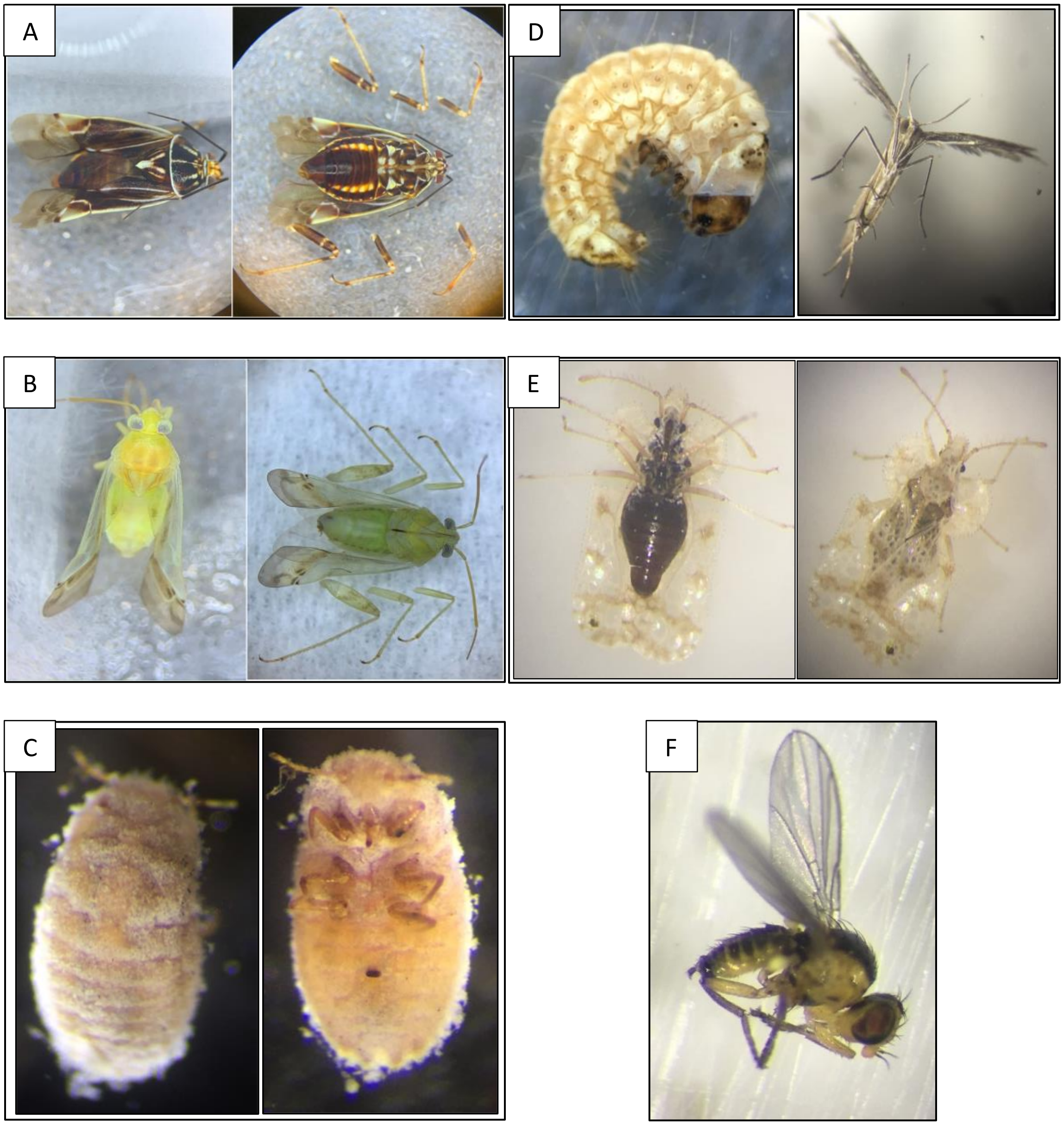

Figure 18: Generalist herbivorous insects found associated with both *Conyza* and *Erigeron* spp. during surveys and observed during common garden experiments in Baton Rouge, Louisiana. (A) Miridae: *Lygus lineolaris* (Palisot de Beauvois, 1818): *C.* J. Wiggins, 2020. (B) Miridae: *Taylorilygus apicalis* (Fieber, 1861): *C.* J. Wiggins, 2020. (C) Pseudococcidae: *Phenacoccus solani* (Ferris): *C.* J. Wiggins, 2019. (D)

Pterophoridae: *Lioptilodes albistriolatus* (Zeller 1877): C. J. Wiggins 2019. (E) Tingidae: *Corythucha marmarata* (Uhler,1878). (F) Agromyzidae: *Calycomyza humeralis* (von Roser 1840).

References

Acedo, M. B., De Ulzurrum, Patricia D., Garavano, M. E., Gianelli, V., and Ispizua, V. N. (2018). Morphological Characterization of *Conyza* blakei, *Conyza bonariensis* var. *bonariensis*, *Conyza sumatrensis* var. *sumatrensis* and *Conyza* lorentzii in the southeast of Buenos Aires (Argentina)". Boletin De La Sociedad Argentina De Botanica; 53(3): 359-373.

Agostinetto, D., Ruy Orsolin da Silva, D., and Vargas, L. (2018). Soybean yield loss and economic thresholds due to glyphosate resistant hairy fleabane interference. Arquivos do Instituto Biológico 84: 1-8.

Alpen, K., Gopurenko, D., Wu, H., Lepschi, B., and Weston, L. (2014). The development of a DNA barcode system for species identification of *Conyza* spp. (fleabane). Nineteenth Australasian Weeds Conference. Tasmanian Weed Society Inc., 4: 401-404.

Andreas, J. E., Milan, J., Randall, C.B., and Winston, R.L. (2016.) Biology and Biological Control of Rush Skeletonweed. U.S. Forest Service. Forest Health Technology Enterprise Team, Morgantown, West Virginia.

Araujo, L., Moujir, L., Rojas, J., Rojas, L., Carmona, J., and Rondón, M. (2013). Chemical Composition and Biological Activity of *Conyza Bonariensis* Essential Oil Collected in Mérida, Venezuela. Natural Product Communications, 8 (8); 1175 – 1178.

Armengol, G., Filella, I., Llusià, J., and Peñuelas, J. (2017). β-Ocimene, a Key Floral and Foliar Volatile Involved in Multiple Interactions between Plants and Other Organisms. Molecules, 22: 1148.

AVH (2021). Australian Virtual Herbarium. Available: https://avh.chah.org.au/. Accessed 21, Sept. 2021

Ayaz, F., Küçükboyacı, N., and Betül Demirci, B. (2017). Chemical composition and antimicrobial activity of the essential oil of *Conyza* canadensis (L.) Cronquist from Turkey. Journal of Essential Oil Research. Journal of Essential Oil Research, 29 (4): 336-343.

Beadle, D. and Leckie, S. (2018). Field Guide of Moths of Southeastern North America. Seabrook Leckie and David Beadle. Mariner Books; Illustrated edition, 640.

Ben-Dov, Y., Denno, B. D., Hardy, N. B., García, Morales M., Miller, D. R., and Miller, G. L. 2016. ScaleNet: A literature-based model of scale insect biology and systematics. Database. Doi: 10.1093/database/bav118. http://scalenet.info.

Bonacci, M., Curioletti, L., Caglirari, D., Stacke, R., and Guedes, J., (2016). Hairy fleabane as a source of major insect pests of soybean”, Planta Daninha, Viçosa-MG, 34(3) 403-409.

Bonacci, M., Formento, A., Morales, M., Orlando, J., Ibáñez, F., Sartori, M., Etcheverry, M., Nesci, A., and Barros, G. (2018) *Conyza bonariensis* as an alternative host for Colletotrichum species in Argentina. Journal of Applied Microbiology, 30(5):1656-1670.

Brodeur, J. (2012). Host specificity in biological control: insights from opportunistic pathogens. Evolutionary Applications, 5(5): 470–480.

Capinera, J. L., University of Florida (1999). Featured Creatures, Entomology and Nematology: Garden Fleahopper. http://entnemdept.ufl.edu/creatures/veg/leaf/fleahopper.htm, University of Florida, Institute of Food and Agricultural Sciences. (Accessed Feb. 2, 2020)

Coley, P., Dexter, K., Donoso, D., Endara, M., Ghabash, G., Kursar, T., Nicholls, J., Pennington, R., and Stone, G. (2017). Coevolutionary arms race versus host defense chase in a tropical herbivore–plant system. http://www.pnas.org/cgi/doi/10.1073/pnas.1707727114, Department of Biology, University of Utah, Salt Lake City, UT: Proceedings of the National Academy of Sciences of the United States of America (PNAS), 14 (36) E7499-E7505;.

Cook, T., Walker, S., Widderick, M. (2011). Flaxleaf Fleabane (*Conyza bonariensis*)-Strategic Solutions Using Best Management Practices. The Sebel Cairns, 1: 585-591.

Cook, T., Walker, S., Widderick, M. (2012). Northern IWM fact sheet: Flaxleaf fleabane: a weed best management guide. www.daf.qld.gov.au/__data/assets/pdf_file/0005/65903/Flaxleaf-fleabane.pdf. Department of Agriculture, Fisheries and Forestry.

Cronquist, A. (1980) Vascular Flora of Southeasturn United States: Asteraceae. The University of Carolina Press, 1: 165-166.

CSIRO (2015) Flaxleaf Fleabane Biological Control. https//research.csiro.au/flaxleaf-fleabane/our-research; Commonwealth Scientific and Industrial Research Organization. (Accessed: 13 May, 2019).

Dalazen, G., Formento, A. N., Daita, F., Sartori, M., Etcheverry, M., Nesci, A., and Borros, G., (2016). Hairy fleabane as a source of major insect pests of soybean. Planta Daninha, Viçosa-MG, 34 (3): 403-409.

Dhandapani, S., Kim, M., Chin, H., Leong, S., and Jang, C. (2020). Identification and Functional Characterization of Tissue-Specific Terpene Synthases in *Stevia rebaudiana.* International Journal of Molecular Sciences, 21: 8566.

Dolan, R.W., M.E. Moore (2021). Indiana Plant Atlas. [S.M. Landry and K.N. Campbell (original application development), USF Water Institute. University of South Florida]. Butler University Friesner Herbarium, Indianapolis, Indiana.

Dolan, R.W., M.E. Moore (2021). Indiana Plant Atlas. [S.M. Landry and K.N. Campbell (original application development), USF Water Institute. University of South Florida]. Butler University Friesner Herbarium, Indianapolis, Indiana.

Erasto, P. and Viljoen, M. (2008). Limonene - A Review: Biosynthetic, Ecological and Pharmacological Relevance. Natural Product Communications, 3 (7): 1193 – 1202.

Faccini, D., Metzler, M., Puricelli, E., Torres, P. (2015). “Differential Susceptibility of *Conyza bonariensis* Biotypes to Glyphosate and ALS-Inhibiting Herbicides in Argentina”. Agricultural Sciences, 6: 22-30.

Fizdale M., Normark B. B., and Schneider S. A. (2019). Key to Commonly Intercepted Aspidiotini (Hemiptera: Diaspididae). USDA ARS Systematic Entomology Laboratory and USDA APHIS Identification Technology Program (ITP). Fort Collins, CO. (Accessed Feb. 3, 2020)

Gagne, R. J., (1994). The gall midges of the Neotropical region”, Cornell University Press.

Gao, L., Zhang, X., Zhou, F., Chen, H., and Lin, Y. (2015). Expression of a Peppermint (E)-β-Farnesene Synthase Gene in Rice Has Significant Repelling Effect on Bird Cherry-Oat Aphid (*Rhopalosiphum padi*). Plant Molecular Biology Reporter 33(6).

GRDC (2013). Flaxleaf Fleabane Fact Sheet: Northern, Southern, and Western Regions Managing Flaxleaf Fleabane. https://grdc.com.au/__data/assets/pdf_file/0026/108980/grdc_fs_fleabane_high-res-pdf.pdf.pdf. Grain Research and Development Corporation.

Hao J. H., Qiang S., Liu Q. Q., Cao F. (2009). Reproductive traits associated with invasiveness in *Conyza sumatrensis*. Journal of Systematics and Evolution, 47: 245–254.

Harborne, J (1993). Introduction to ecological biochemistry, 4th edn. Academic Press, San Diego

Heap, I. (2015). International Survey of Herbicide Resistant Weeds. http://www.weedscience.org/summary/home.aspx. Accessed March 12, 2020

Holm L., Doll J., Holm E., Pancho J., Herberger J. (1997). World Weeds, Natural Histories and Distribution. New York, NY: John Wiley & Sons, Inc., ISBN: 978-0-471-04701-8; 1152

Immel, D. (2000). Plant Guide: Philadelphia Fleabane *Erigeron philadelphicus* L. USDA NRCS, National Plant Data Center.

Jermy, T. (1984). Evolution of Insect/Host Plant Relationships. The University of Chicago Press for The American Society of Naturalists:The American Naturalist, 124(5): 609-630.

Keane, R. and Crawley, M. (2002). Exotic plant invasions and the enemy release hypothesis. Trends in ecology & evolution, 17(4), pp.164-170.

Kil, J. and Kim, D. (2014) Geographical Distribution and Host plants of *Corythucha marmorata* (Uhler) (Hemiptera: Tingidae) in Korea. Korean Society of Applied Entomology, Korean Journal of Applied Entomology; 53(2): 185-191.

Kortbeek, R., gragt, M., and Bleeker, P. (2018).Endogenous plant metabolites against insects. European Journal of Plant Pathology, 154:67–90.

Kumar, V. and Mathela, C. (2017). Toxicity and repellent effect of essential oils and a major component against Lipaphis erysimi.. Journal of Crop Protection, 6 (1): 15-23.

Laidlaw, M. and Taylor, M. (2011). Potential for childhood lead poisoning in the inner cities of Australia due to exposure to lead in soil dust. Environmental Pollution, 159(1):1-9.

Lankau, R. A. (2007). Specialist and generalist herbivores exert opposing selection on a chemical defense New Phytologist, 175(1):176-84.

McKay, F. and Gandolfo, D. (2001). Description of the immature stages of *Trupanea bonariensis* Diptera Tephritidae with notes on its biology. Revista de la Sociedad Entomologica Argentina 60(1-4): 129-138.

Michael P.W. (1977). Some weedy species of Amaranthus (amaranths) and *Conyza*/*Erigeron* (fleabanes) 69aturalized in the Asian-Pacific region. In: Proceedings of the 6^{th} Asian PacificWeed Sci. Soc. Conference (Jakarta, Indonesia, 11–17). Asian-PacificWeed Science Society, Jakarta, 1: 87–95.

Middleton, B. (2019). Invasive plant Species. Encyclopedia of Ecology (Second Edition); Elsevier, 431-440.

Mitchell, P.L., (2004). Heteroptera as vectors of plant pathogens. Neotropical Entomology, 33 (5): 519–545.

Nesom, G. (2008). Classification of Subtribe Conyzinae (Asteraceae: Astereae). Lundellia 11: 8–38.

Ninkuu V., Zhang, L., Yan, J., Fu, Z., Yang, T., and Zeng, H. (2021). Biochemistry of Terpenes and Recent Advances in Plant Protection. International Journal of Molecular Science, 22: 5710.

O'Brien, C. W. (2009). New Notiodes semiaquatic weevil (Curculionidae) from sporocarps of *Marsilea mollis* (Marsileaceae) in southern Arizona, USA," Western North American Naturalist; 69(4): 421-425.

Painter, R., (1935). The Biology of Some Dipterous Gall-Makers from Texas", Kansas (Central States) Entomological Society Journal of the Kansas Entomological Society, 8(3): 81-97.

Paulin, M., Hirka, A., Eötvös, C., Gáspár, C., Mikó, Á., and Csóka, G. (2020). Known and predicted impacts of the invasive oak lace bug (Corythucha arcuata) in European oak ecosystems – a review. Folia Oecologica, 47 (2) doi: 10.2478/foecol-2020-0015.

Perveen, A., Khan, M., Mansuri, S., and Tabassum, T. (2016). Morphological studies on trichome of family AsteraceaeInternational Journal of Biology and Biotechnology, 13 (2): 177-182.

Pippa, M., Scott, J. K., Yeoh, P. B. (2016). Methods to select areas to survey for biological control agents: An example based on growth in relation to temperature and distribution of the Weed *Conyza bonariensis*. Biological Control, 97: 21-30.

R Core Team (2021). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org/, Version: 4.1.1.

Rafter, M. and Morin, L. (2017). Nomination of a Target Weed for Biological Control. CSIRO, Health and Biosecurity, Invasive Plants & Animal Committee.

Richard, A., Linhart, S., and Santos, A. (2002). Plant diversity, herbivory and resistance of a plant community to invasion in Mediterranean annual communities. Oecologia 130:96–104.

Shrestha, A., Hembree, K., and Wright, S. (2008). Biology and Management of Horseweed and Hairy Fleabane in California. https://doi.org/10.3733/ucanr.8314. The Regents of the University of California.

Silva, J. (2004). Mining the essential oils of the Anthemideae. African Journal of Biotechnology, 3 (12): 706-720.

Soares, D. J., Oliveira, W. S., Uzuele, E. L., Carvalho, S. J. P., and Ovejero, R. F. L., Christoffoleti, P (2017) Growth and development of *Conyza bonariensis* based on days or thermal units. Pesquisa Agropecuaria Brasileira, 52(1), 45-53.

Spencer, K. A. (1990). Host Specialization in the world of Agromyzidae (Diptera). Kluwer Academic Publishers, 444.

Strother, J. (1943). *Conyza bonariensis*. http://floranorthamerica.org/*Conyza_bonariensis*. Bulletin of the Torrey Botanical Club 70: 632

Thébaud, C. and Abbot, R.A. (1995). Characterization of Invasive *Conyza* Species (Asteraceae) in Europe: Quantitative Trait and Isozyme Analysis. American Journal of Botany 82(3): 360-368.

Treiber, M. (1979). Compodites as Host Plants and Crypts for Synchlora aerata (Geometridae). Journal of the Lepidopterists' Society; 33(4): 239-244.

Trezzi, M., Vidal, R., Patel, F., Miotto, E., Debastiani, F., Balbinot, A., and Mosquen, R. (2015). Impact of *Conyza bonariensis* density and establishment period on soyabean grain yield, yield components and economic threshold. Weed Research, 55: 34–41.

USDA (2014a). *Conyza bonariensis* (L.) Cronquist, Asthmaweed. https://plants.usda.gov/home/plantProfile?symbol=COBO. Natural Resources Conservation Service. Accessed: Oct. 13, 2021

USDA (2014b). *Conyza canadensis* (L.) Cronquist, Canadian horseweed. https://plants.sc.egov.usda.gov/home/plantProfile?symbol=coca5. Natural Resources Conservation Service. Accessed: Oct. 13, 2021

USDA (2014c). *Erigeron philadelphicus* (L.) Philadelphis fleabane. https://plants.usda.gov/home/plantProfile?symbol=erph. Natural Resources Conservation Service. Accessed: Oct. 13, 2021

Wang, A., Wu, H., Zhu, X., and Lin, J. (2018). Species Identification of *Conyza bonariensis* Assisted by Chloroplast Genome Sequencing. Frontiers in Genetics. 9:374.

Wang, Y., Ma, Y., Zhou, D. (2017). Higher plasticity in feeding preference of a generalist than a specialist: experiments with two closely related Helicoverpa species. Scientific Report 7: 17876.

Wu, H (2007). The biology of Australian weeds 49. *Conyza bonariensis* (L) Cronquist. Plant Protection Quarterly, 22:122–131.

Vita

Carlos Wiggins grew up in Shreveport, Louisiana and received his bachelor's degree in Field and Organismal Biology at Louisiana State University; Shreveport, Louisiana. While working towards his Bachelor's degree at Louisiana State University, Shreveport; his fascination with insects and the systems that they are a part of grew. As a result, he decided to join the Department of Entomology at Louisiana State University, Baton Rouge, to pursue his master's degree with Dr. Rodrigo Diaz. He plans to receive his Masters Degree in Entomology in December 2021.