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Disturbance, Defense and Fitness in Silverleaf Nightshade (*Solanum elaeagnifolium*) in Their Native Range in South Texas

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DISTURBANCE, DEFENSE AND FITNESS IN SILVERLEAF NIGHTSHADE
(*SOLANUM ELAEAGNIFOLIUM*) IN THEIR NATIVE RANGE
IN SOUTH TEXAS

A Thesis

by

JESUS CHAVANA

Submitted to the Graduate College
The University of Texas Rio Grande Valley
In partial fulfillment of the requirements for the degree of
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DISTURBANCE, DEFENSE AND FITNESS IN SILVERLEAF NIGHTSHADE

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December 2020

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ABSTRACT

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Chapter 1: In this introductory chapter, we reviewed past and recent studies to understand weed ecology and species interactions. We specifically looked at how anthropogenic disturbance and insect mediated interactions affect natural and agricultural ecosystems. Ultimately, understanding these interactions will help create better management strategies.

Chapter 2: In this data chapter, we examined how mowing affects reproductive fitness, defense, and possible transgenerational traits using *Solanum elaeagnifolium*. Our results show that mowed plants germinated more and faster. Mowed plants were also better defended against generalist chewing herbivores when compared to generalist piercing/sucking herbivores. However, Texas potato beetle (*Leptinotarsa texana*), a co-evolved specialist on *S. elaeagnifolium*, did not show any differential feeding effects. We also found that specific root length, an indicator of nutrient acquisition, was significantly higher in first-generation seedlings from mowed plants. Taken together, we show that mowing is a selective pressure that enhances some fitness and defense traits and can contribute to producing superweeds.

Chapter 3: In this conclusions chapter, we discuss how the results from chapter 2 should lead into future experiments examining the mechanistic underpinnings of *S. elaeagnifolium* – herbivore interactions, and their effects on defense and fitness traits.

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TABLE OF CONTENTS

	Page
ABSTRACT.....	iii
ACKNOWLEDGEMENTS.....	v
TABLE OF CONTENTS.....	vi
LIST OF TABLES.....	viii
LIST OF FIGURES.....	ix
CHAPTER I. INTRODUCTION.....	1
Study system: Silver leaf nightshade (<i>Solanum elaeagnifolium</i> ; Solanaceae).....	2
SLN as a model for insect-plant interactions.....	3
Weed Herbivore Interactions.....	4
CHAPTER II. MAKING SUPER WEEDS? CONTINUOUS MOWING IMPROVES FITNESS AND DEFENSE TRAITS WITH TRANSGENERATIONAL EFFECTS IN THE NOXIOUS WEED <i>SOLANUM ELAEGNIFOLIUM</i>	7
Abstract.....	7
Introduction.....	9
Materials and Methods.....	12
Statistical analysis.....	20

Results.....	21
Discussion.....	38
CHAPTER III. CONCLUSIONS AND FUTURE DIRECTIONS	44
REFERENCES	46
APPENDIX.....	56
BIOGRAPHICAL SKETCH	59

LIST OF TABLES

	Page
Table 1: Details of response variables, treatment means, statistical tests and significance from WinRhizo experiment. Variables that are statistically significant are in bold P values at $P < 0.05$	37
Table A1 Location coordinates of mowed and unmowed sub-populations of SLN used for this study.....	58

LIST OF FIGURES

	Page
Figure 1: Results of field fitness traits show: (1A) Number of fruits collected from mowed and unmowed locations ($P < 0.001$), (1B) number of seed per fruit (Y-axis) ($P = 0.809$), (1C) Seeds mass (Y-axis) (treatment $P < 0.001$), (1D) total number of seed (Y-axis) ($P < 0.001$) mowed and GA (orange) and unmowed and control (blue) plants. Means are shown by scale bars and asterisk denotes significantly different results at $p < 0.05$, while ns denotes non-significant results.	22
Figure 2: Results of seedling germination in green house show: (2A) Seeds germinated per week on mowed and unmowed plants ($P < 0.002$), (2B) Seeds germinated per week with GA treatment and control ($P = 0.974$), (2C) Seeds germinated over time (early vs late season) (treatment $P < 0.006$; GA $P = 0.977$), mowed and GA (orange) and unmowed and control (blue) plants. Means are shown by scale bars and asterisk denotes significantly different results at $p < 0.05$, while ns denotes non-significant results.....	24

Figure 3: Results of seedlings health and vigor show: (3A) number of leaves on seedlings on mowed and unmowed plants ($P < 0.001$), (3B) seedling height (cm) on both treatments ($P < 0.001$) mowed (orange) and unmowed (blue) plants. Means are shown by scale bars and asterisk denotes significantly different results at $p < 0.05$, while ns denotes non-significant results.....26

Figure 4: Results of seedling herbivory in field show: (4A) herbivore presences on mowed and unmowed plants ($P < 0.001$; binary logistic regression; Wald's test), (4B) damage by insects on both treatments (scale 0-4) ($P = 0.037$; ordinal logistic regression; Wald's test) mowed (orange bar) and unmowed (blue bar) plants. Means are shown by scale bars and asterisk denotes significantly different results at $p < 0.05$, while ns denotes non-significant results.....28

Figure 5: Results of herbivory in lab results from 3 herbivores show: (5A) number of *A. craccivora* (Y-axis) ($P < 0.001$; Poisson distribution fit model regression), (5B) mass gain of *M. sexta* caterpillars (Y-axis) ($P = 0.022$; Kruskal-Wallis test) and (5C) mass gain of *L. texana* (Y-axis) on mowed (orange bar) and unmowed (blue bar) plants ($P = 0.715$; Kruskal-Wallis test). Means are shown by scale bars and asterisk denotes significantly different results at $p < 0.05$, while ns denotes non-significant results.....30

Figure 6: Results of trichome density: (6A) number of trichomes on leaves for mowed and unmowed treatments (Y-axis) ((Two sample T-test; $t = -2.53$; P-value = 0.02), (6B) compares mean number trichomes on abaxial and adaxial side of the leaf for both mowed (Y-axis) (Two-way Anova; $F = 1.27$; P-value= 0.26) and unmowed (Y-axis) (Two-way Anova; $F = 3.50$; P-value= 0.06) treatments respectively, (6C) mean number of stellate trichomes on leaves for mowed and unmowed treatments (Y-axis) (Two sample T-test; $t = -2.47$; P-value >0.02), (6D) mean number of glandular trichomes on leaves for mowed and unmowed treatments (Y-axis) (Two sample T-test; $t = -0.10$; P-value= 0.918), (6E) number of spikes on stellate trichomes for mowed and unmowed treatments (Y-axis) (Two sample T-test; $t = -6.26$; P-value <0.00). Means are shown by scale bars and asterisk denotes significantly different results at $p < 0.05$, while ns denotes non-significant results.....32

Figure 6A: Non-glandular trichomes on adaxial side of SLN.....33

Figure 6B: Non-glandular trichomes on abaxial side of SLN.....34

Figure 7: Results of six major root traits show: (7A) Whole root area ($P < 0.108$; Kruskal-Wallis tests), (7B) Root surface area ($P = 0.017$; Kruskal-Wallis test), (7C) Fine roots pooled ($P < 0.0002$; Kruskal-Wallis tests), (7D) Root length ($P = 0.004$; Kruskal-Wallis tests), (7E) Shoot length ($P = 0.225$; Kruskal-Wallis tests), (7F) Specific root length ($P < 0.049$; two tailed T test) on mowed (orange bar) and unmowed (blue bar) plants. Means are shown by scale bars and asterisk denotes significantly different results at $p < 0.05$, while ns denotes non-significant results.....36

Figure A1: SOLANUM ELAEAGNIFOLIUM flowering on the side of the road.....57

Figure B1: Results of fruit diameter from plants collected from 4 mowed and unmowed sub-
populations(P=0.269).....58

CHAPTER I

INTRODUCTION

Weed as a term is very broad and can have many different definitions based on the audience and context. (Holzner et al. 1982). To a farmer or homeowner, a weed could be defined as ‘A plant species or a group of species interfere with their activities, health or pleasures’ (Holzner et al. 1982, Fryer 1979). However, the general consensus is weed can be any plant out of its place. Regardless of the definitions, it is clear that if not managed, weeds have the potential to cause a lot of damage, especially in agroecosystems where they significantly reduce crop yield (Cousens and Mortimer 1995; Pimentel et al., 2000; Vila et al., 2004; Soltani et al., 2017). A recent estimate shows that the potential yield loss in corn form in US is \$26.7 billion (Soltani et al., 2016).

In addition to disrupt and negatively impact agriculture, a majority of weeds also tend to be highly invasive. Invasion occurs when an alien plant is introduced into a non-native habitat. Weeds are able to colonize in new environments primarily due to their enhanced traits in reproductive fitness, self-compatibility, seed dormancy, persistent seed bank, and better defense traits, to name a few (Kleunen et al., 2016; Kariyat et al., 2013; 2019). In addition, invasi

species also have better resource allocation towards leaf area and shoots, and consequently had higher growth, size and fitness which make them better suited than non-invasive species in similar habitats (Kleunen et al. 2010). Invasive species also tend to compete better with other plant species, and defend better against herbivores and pathogens in its native habitat. Taking all these factors into consideration many theories on invasion have been proposed and well-studied by many ecologists. One of these, the enemy release theory states that when exotic plants are introduced into a new habitat the lack of predation (herbivores) above and below ground can provide a significant advantage over native plant species in the same area (Keane & Crawley et al. 2002; Agrawal et al. 2005). Invasive species can disrupt complex ecological interactions between native plants and other organisms, and can compete for nutrients, light, and water, and pose a serious threat to local biodiversity and ecosystem functioning (Pimentel et al., 2001; Tscheulin et al., 2009). Although recent studies have suggested a strong correlation between invasiveness and certain physiological and ecological traits (Pysek and Richardson, 2007)- we still lack an understanding of the key characteristics, especially the role of human disturbance on invasion success.

Study system: Silver leaf nightshade (*Solanum elaeagnifolium*; Solanaceae)

Silverleaf nightshade or SLN (*Solanum elaeagnifolium*), is a competitive and noxious weed native to Southern US and Mexico, which is also invasive worldwide (Petanidou et al., 2018). It has been considered as invasive in all countries of the Mediterranean Basin, Australia, Chile, Australia, India, New Zealand, Pakistan, Puerto Rico, South Africa, Taiwan and Zimbabwe (Bouhache and Tanji 1985; Boyd et al. 1984; Mekki 2007). The species has been suggested to be introduced to various countries worldwide, either by contaminated crop seeds (e.g., Morocco; Tanji et al., 1984) or as a contaminant in fodder (e.g., South Africa., Wasserman

et al., 1988). SLN causes adverse economic and ecological impacts by suppressing commercial and native vegetation, and inhabits agricultural land, pastures, and crop fields. In addition to that, it also serves as host to pathogens and herbivorous insects that cause severe economic losses to cotton, grain sorghum, and potatoes (Thinakaran et al. 2015) to name a few. For example., a study by The University of Oklahoma in Knox County, Texas found that one silverleaf nightshade per square foot of cotton reduces cotton yield by 45%, which estimates a revenue loss of \$8 per acre (Alexander et al. 1990). Each parent plant can on average produce more than 30 fruits, with 50-100 seeds, creating a seed bank that can last for years (Moore et al. 1975; Khanna & Singh. 1987). The plant can also reproduce asexually i.e., through underground rhizomes and can spread and sprout easily, making it almost impossible to get rid of. The root system can grow up to one meter deep, and can extract nutrients from deeper soil levels, thereby outcompeting neighboring plant species. SLN also has a variety of physical and chemical traits that play a role in defense against herbivores. Both leaves and stems are covered by spines; leaves, stem and flower tissues are also covered with stellate trichomes that gives a silvery white appearance, and hence the common name “Silver nightshade” (Bowers et al., 2013). Collectively, these characteristics makes SLN a noxious and invasive weed (Heap et al. 1997; Cuthbertson et al. 1976).

SLN as a model for insect-plant interactions

Our lab had been using SLN as a model to understand insect-plant interactions, plant defenses and herbivore counter defenses. We have estimated and quantified the herbivores of SLN (Davies et al., 2020), characteristics of sub-populations in their native range in south Texas regarding their growth-defense tradeoffs (Kariyat and Chavana 2018), trichome morphology and the role of leaf trichomes in herbivore growth and development (Kariyat et al., 2018; Kariyat et

al., 2019; Kaur and Kariyat, 2020a,b). Previous work on this species has also documented growth and fitness traits in native and invasive populations (Petanidou et al., 2018). These studies were instrumental in understanding the species interactions mediated by SLN and paved the way for the questions answered through this thesis. Our goal is to understand plant phenotypes vary within and among populations of *S. elaeagnifolium*, due to biotic and abiotic factors. As biotic factors, we focus on three common herbivores, their incidence, and damage on SLN. These include tobacco hornworm (*Manduca sexta*), a generalist Solanaceae caterpillar, cowpea aphid (*Aphis craccivora*) a generalist aphid and Texas Potato Beetle (*Leptinotarsa texana*), a co-evolved specialist on SLN, and we used mowing, as our abiotic factor of interest. Ultimately, our objective is to understand how disturbance (mowing) affects reproductive fitness, defenses, and possible cascading transgenerational effects using a combination of field, growth chamber, common garden, and lab experiments.

Weed Herbivore Interactions

The impact of weeds on arthropods in various ecosystems has been well studied and thoroughly reviewed (Norris and Kogan, 2005 and references within). Most of these studies have examined this interaction through the eye of integrated pest management, and weeds are also considered as beneficial plants that would improve biodiversity in agroecosystems (Gabriel et al., 2005), thereby improving the density of predatory and parasitoid arthropods (Norris and Kogan., 2005; Tixier et al., 2013). This is especially important in monoculture intensive agriculture systems where species diversity is low, leading to severe herbivore and disease incidence, and faster resistance build-up. A key measure to address this concern is understanding and employing

multitrophic interactions by building a healthy density of predators and parasitoids that enhance other pest management practices.

However, multitrophic interactions are highly complex and require a deeper understanding of food web ecology, and producer-consumer interactions. For example, weeds are the primary producers in an ecosystem, and can offer food for herbivores, but also benefit the next trophic level (predators and parasitoids) by sustaining a healthy population of their prey, representing a bottom up effect (Norris and Kogan, 2005). They can also provide services to beneficial insects in two ways, one by serving as an alternate host to insect herbivores on which they feed and the other is serving as source of pollen and nectar. But it could be opposite as well and can lower the activity of predators or parasitoids (Naranjo and Stimac 1987, Powell et al., 1985). For instance, Florida beggarweed (*Desmodium spectabilis*) and crotalaria (*Crotalaria spectabilis*) weeds in soybean lower the activity of *Geocoris punctipes* against their prey (Naranjo and Stimac 1987). On the other hand, weed integrated crop models have been suggested to manage the insect resistance in crops by sowing weeds. In Bt-corn, to control the resistance build-up in the major crop, crops can be grown in 80:20 ratio with weeds. This increases the choice of insects to feed on other plants and helps to escape resistance in the major crop (Losey et al., 2001; Onstad et al., 2003).

Weeds may also indirectly affect the feeding behavior of insect herbivores (Coop and Croft 1993). For example, *Digitaria ciliaris* produce allelochemicals which reduce the oviposition and consequently feeding of *Heliothini* spp on pearl millet (*Pennisetum glaucum*). (Mitchell et al., 1993). Weeds can also serve as oviposition sources for beneficial insects. For example, hophornbeam copperleaf (*Acalypha ostriaeifolia*) weed was selected as source of oviposition by *Coleomegilla maculate* (Cottrell et al., 1998,1999). However, they can also be

used as only oviposition but not feeding source by insect herbivores (Saad and Bishop 1969, Niemczyk and Flessel 1970, Waldrep et al., 1969). Henbit (*Lamium amplexicaule*; hollow stemmed weed) can be used as alternative source of oviposition by alfalfa weevil (*Hypera postica*), quackgrass (*Elytrigia repens*) by stalk borer (*Papaipema nebris*; Levine 1985) and foxtail (*Setaria* spp.) by western corn rootworm (*Diabrotica virgifera*; Kirk et al., 1968).

On the other hand, weeds can serve as a refuge crop or alternative host to herbivores (mostly generalist herbivores) while the major crop is not in season or unavailable due to their phenology (Green et al., 2003). For instance, weeds can be used as alternative source of overwintering by *Rhagoletis mendax* (Geddes et al., 1992). Weeds can also act as a reservoir for pathogen incidence and establishment (Capinera 2005), also affected by intraspecific genetic variation (Kariyat et al., 2012 PSB). For example, weeds can be alternate host to whitefly, aphids and thrips, which can transmit viruses and sugar beet (*Beta vulgaris*) and tomato (*Solanum lycopersicum*) curly top virus can be transmitted by beet leafhoppers surviving on weeds.

Taken together, while weeds are a major concern in natural and agricultural ecosystems, weed management should also focus on identifying weed traits and species interactions mediated by weeds to sustainably manage them. This thesis examines how disturbance through mowing affects weed traits and species interactions, and whether these effects if any cascade to offspring generation

CHAPTER II

MAKING SUPERWEEDS? CONTINUOUS MOWING IMPROVES FITNESS AND DEFENSE TRAITS WITH TRANSGENERATIONAL EFFECTS IN THE NOXIOUS WEED *SOLANUM ELAEAGNIFOLIUM*.

Abstract

The role of disturbance in accelerating weed growth is well understood. While most studies have focused on soil mediated disturbance, mowing can also impact weed traits. Using silverleaf nightshade (*Solanum elaeagnifolium*), a noxious and invasive weed, through a series of field, lab and greenhouse experiments, we asked whether continuous mowing affects growth and plant defense traits, and whether they cascade into offspring. We found that mowed plants produced significantly fewer fruits, and lower seed fitness, but had higher seed mass, and a higher germination rate and faster germination. When three herbivores were allowed to feed, tobacco hornworm (*Manduca sexta*), caterpillars, gained more mass on seedlings from unmowed plants, while cowpea aphid (*Aphis craccivora*), a generalist, established better on mowed seedlings, but leaf trichome density was higher on unmowed seedlings, suggesting possible negative crosstalk in defense traits. However, Texas potato beetle (*Leptinotarsa texana*), a co-evolved specialist on *S. elaeagnifolium* did not show any differential feeding effects. We also found that specific root length, an indicator of nutrient acquisition, was significantly higher in first-generation seedlings from mowed plants. Taken together, we show that mowing is a selective pressure that enhances some fitness and defense traits and can contribute to producing superweeds.

This chapter is under review for Scientific Reports as

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Introduction

Weeds are generally defined as undesired plant species that can invade ecosystems and can cause harm both biotic and abiotic ecosystem components (Holzner 1982, Randall 1996). The factors contributing to the ability of weedy plant species to establish and colonize have been well understood (Atkinsosn 1997; Goslee et al., 2001; Dawson et al.,2009). A general consensus is that weed species tend to have enhanced traits that allow them to succeed either in their native or introduced habitats, when compared to their non-weedy counterparts (Baker 1974; Perrins et al., 1992; Mack 1996). This could be their ability to either outcompete heterospecifics and/or have better growth, fitness, and defense traits, to name a few (Sutherland 2004). For example, allelopathy in weedy sunflower (*Helianthus spp.*) inhibits mustard (*Brassica spp.*) seed germination and lantana (*Lantana camara*) inhibits wheat (*Triticum aestivum*), soybean (*Glycine max*), and corn (*Zea mays*) growth (Leather, 1983; Mersie and Singh, 1986). On the other hand, weeds such as *Rhododendron ponticum* and *Rhododendron maximum* colonize forests by adaptative switching between sexual and asexual reproduction, thereby reducing tree growth and regeneration, causing immense forest damage. They are also more tolerant to cold and shade, and have plasticity in morphological and physiological adaptations to varying environmental conditions (Derya et al., 2006). More recently, there has been tremendous interest to identify and quantify other contributing factors to weed success including their ability to cope with climate change, and more importantly, human disturbance (Clements and Ditommaso 2011; Sebask et

al.,2014; Hodgins 2014). However, most of these studies are limited to traits observed in a single growing season, ignoring any possible transgenerational effects.

The effects of human disturbance on weed success has also been well researched. Collectively, these studies suggest that land and soil disturbance due to human activities tend to enhance weed success both in natural and agricultural environments (Hobbs and Huenneke 1992; Lozon and Maclsaac 1997; Ditomaso, 2000). Many plants that thrive in hot and dry environments tend to become weedy with fast growth and drought resistance, primarily facilitated and enhanced by human disturbance (Larson et al., 2001). These can range from clearing, draining, and human activities that promote erosion, collectively damaging non-weedy vegetation with slower growth and environmental resistance (Baker, 1974). Recently, in the semi-arid open forest with *Prosopis caldenia* (Caldenal) demonstrated that anthropogenic disturbance (e.g., fire, grazing) played a significant role in the establishment of widely distributed ruderal weed species (Chiuffo et al., 2018). In rhizomatous weeds such as Carolina horsenettle (*Solanum carolinense*; Solanaceae), a single mother plant can produce ~21 new sprouts in the following season, a grave concern to farmers when rhizomes are broken apart in agricultural lands as part of tillage (Kariyat et al., 2011). While obvious human-driven disturbances like clearing and draining have been studied extensively among management practices, mowing has been overlooked in this context even though mowing dramatically reduces photosynthetic area and removes growing regions of plants, forcing them to reallocate resources to regain fitness traits (Bo Li et al., 2004; Jia et al., 2008; Ramula, 2020).

Weeds in urban, agricultural, and other forms of managed systems undergo multiple mowing events during their growing season and must constantly reprogram growth, defense and fitness traits to ensure at least some fitness is maintained, else risk extinction in local populations

(Liu et al., 2002; Biazzo et al., 2019). For example, Yong et al. (2015) demonstrated in the invasive weed *Erigeron annuus*, mowing reduced seed mass but led to variation in pappus length, and achene size, and speculated that these differences lead to better spread and higher survival rate (Yong et al., 2015). Moreover, mowing is also considered as mechanical wounding, leading to enhanced defenses, both locally and systemically with short and long-term effects (Mithöfer et al., 2005; Engelberth et al., 2019). This interplay of anthropogenic disturbance and weed ecology, and its role in transgenerational growth and defense traits needs to be better understood, especially since many weed species are perennial and can propagate asexually over multiple years and growing seasons (Kariyat et al., 2011).

Clearly, anthropogenic disturbance has a huge impact on weed success, and we are yet to understand in detail the factors that contribute to this. Among the many weedy traits that provide an edge over non-weedy plants, the ability to self-fertilize is considered critical (Baker 1955; Etten et al., 2017). In line with Baker's theory (Tabassum and Leishman 2019), it is observed that self-fertility is common in weeds, and the ability to self-pollinate and set viable seeds ensures fitness in founding populations, when cross pollen from conspecifics may be low, due to small population size and reduced number of unrelated individuals (Pannell and Barrett, 1998; Pannell et al., 2015). While most studies have addressed these questions using fitness measures as variables of interest (Kariyat et al., 2011; Mena-Ali et al., 2008), we still lack a complete understanding of whether other biotic and abiotic factors can contribute and complicate the interactions at multiple trophic levels. For example, in their home ranges, weeds are constantly subjected to high herbivory pressure from co-evolved herbivores, a phenomenon lacking in their invasive habitats- commonly known as enemy-free space. For example, the tropical fire ant, *Solenopsis geminata* (Fabricius), and the Asian house rat, *Rattus tanezumi* Temminck, are widely

known to consume weed seeds of *Digitaria ciliaris*, *Echinochloa colona* and *Eleusine indica* and control weed populations in rice fields (Chauhan, 2010) in their native ranges. A large body of work has demonstrated that in the absence of these natural predators, weeds in an enemy-free space are more likely to thrive and become a larger problem as they are highly invasive (Muniappan and Viraktamath, 1993; Ziller, 2000; Javid and Riaz, 2012; Alves et al., 2017). In addition, there is the possibility for local herbivores that differ in specialization and feeding guild to also impact these defense traits and weed fitness, by checking weed populations at an ecosystem threshold. For example, we previously found that in *S. carolinense*, intraspecific variation due to experimental inbreeding affected the recruitment of herbivores and natural enemies in the field, selectively improving fitness of outbred progeny when compared to inbreds through better defenses, in addition to better growth and reproductive fitness (Kariyat et al., 2012) with transgenerational effects (Nihranz et al., 2019). Expanding this line of research into anthropogenic disturbance is critical to determine the evolutionary ecology, an area traditionally under-explored, but has gained momentum recently (Kariyat et al., 2012, Nihranz et al., 2019). Being the chief contributor of fitness, seeds are loaded by mother plants with nutrients, which directly affect offspring success (Nihranz et al., 2020). Parental stress can lead to depleted resource allocation to seeds since lack of resources due to photosynthetic tissue loss by herbivory, or reallocation and tradeoffs at defense-fitness traits (Wilkens et al., 1996; Zanyab et al., 2018; Neilson et al., 2013).

To examine reproductive fitness, defenses, and possible cascading transgenerational effects due to mowing, we used a combination of field, growth chamber, common garden, and lab experiments with multiple genets from 4 mowed and unmowed sub-populations of Silverleaf nightshade (SLN) to ask the following questions: (1) Does mowing affect growth and fitness

traits, (2) Does mowing affect herbivore incidence and field damage, (3) Does mowing lead to transgenerational effects on seed germination, seedling growth and fitness, (4) Are there any transgenerational effects on plant defenses against generalist and specialist herbivores that vary in feeding habit, and (5) Are these transgenerational effects if any, also prevalent in root traits, since the species also reproduce through rhizomatous roots?

We hypothesized that due to consistent loss of photosynthetic area and growing time, mowed plants will exhibit lower growth and fitness traits, but have enhanced defenses since mowing is mechanical wounding, and can lead to defense signaling. We also hypothesized that offspring, from mowed mother plants will have compromised growth and fitness traits due to lower resource allocation but will also have higher constitutive defenses due to damage in the parental generation.

To answer these questions, we used three herbivores: tobacco hornworm (*Manduca sexta*), a generalist Solanaceae caterpillar, cowpea aphid (*Aphis craccivora*), a generalist aphid and Texas potato beetle (*Leptinotarsa texana*), a co-evolved specialist on SLN for our herbivory experiments. And, to examine root traits, we used WinRhizo Pro 2019 root scanner to measure the key root traits involved in weed success.

Materials and Methods

1. Study system:

SNL is a noxious, drought-resistant, perennial weed that is believed to have originated in the southwestern border of the United States and Mexico (Boyd et al., 1984) but is invasive worldwide (EPPO datasheet, 2019). The species thrives well in all environmental conditions

including poor soil and nutrient availability (Travlos, 2012). It can easily spread to other locations by rivers and streams, through livestock manure and anthropogenic activity such as plowing and mowing (Mekki, 2007). The species also exhibits allelopathy, physical and chemical defenses, and high reproductive fitness by seeds and asexual reproduction through rhizomes, collectively making it highly competitive and extremely invasive (Cuthbertson et al., 1976; Heap et al., 1997). Additionally, the species exhibits gametophytic self-incompatibility, but is also plastic for the trait, producing selfed seeds when outcrossed pollen is limited (Petanidou et al., 2012).

2. Study populations and plant materials:

For all the experiments detailed in this study, we used plants and seeds derived from 8 locations in the McAllen-Edinburg area of Rio Grande Valley, Texas, USA where SLN is native. We had been monitoring these locations for over three years (Kariyat and Chavana, 2018) and have confirmed the disturbance status of these populations; 4 out of eight populations were disturbed by continuous mowing by the city, and the rest were left undisturbed, but within 20-30 meters from each other. The GPS coordinates and population size of these locations have been detailed in supplementary table 1.

3. Fruit collection

All the fruits produced by ~100 different genets with at least ten plants (genets) from each location were collected over one week in December 2019. The mowing treatments (4 rounds of mowing) ended in late summer (September) and the plants could set and mature fruits. Care was taken to ensure that the genets were at least 5 meters apart to minimize any clonality effects in sampling since SNL can vegetatively reproduce through rhizomatous roots. The

collected fruits were pooled per genet and bagged and stored at room temperature in the lab for seed extraction.

3.1 Seeds/ fruit and total seeds:

Seeds were extracted from the fruit by cutting each fruit in half and gently squeezing the fruit to push out all the seeds into a fine mesh strainer (250 microns). Seed were washed to remove the pulp of the fruit from the seed. Once washed, seeds were set out to dry on a paper towel at 70°C and 50% relative humidity for 24 hours.

3.2 Fruit diameter and seed mass measurements.

To measure fruit diameter and seed mass, five random fruits from the pooled fruits of a single genet (individual plant in each sub-population/ treatment were chosen. Fruit diameter was measured using a digital caliper (ABSOLUTE Super Caliper SERIES 500, IL, USA). Afterward, the fruits were carefully cut in halves without causing any seed damage. The fruit's pulp was removed, and the seeds were separated using a fine mesh strainer and then were dried on a napkin for at least 12 hours. Once dried, the seeds were counted and stored in falcon tubes at 70°C and 50% relative humidity.

To create a seed bank from each location for germination experiment and mass measurement, 200 seeds were randomly chosen from the pooled seeds of all the fruits from each genet per location. One hundred of these seeds from both treatments (mowed and unmowed) from each of the eight locations were then weighed using an analytical balance (Accuris Dx W3101A-220, Mid Sci, MO, USA) to get 100 seed mass.

3.3 Seed germination and establishment

To examine seed germination and seedling establishment, 200 seeds from each of the pooled seed banks (from all eight locations) were used (1600 seeds in total; 800 per treatment). Before sowing, half of the seeds (400 each from each treatment) were treated with 20 milliliters of gibberellic acid in deionized water (mowed/unmowed) (GA₃, 1000ppm; Sigma-Aldrich, MO, USA) for 24 hours to examine whether treatment X seed germination is impacted by the rooting hormone (Centibas and Koyuncu 2013). After the seed treatment, 50 seeds each were sown each in a plastic tray (7.5 in x 12.5 in x 2 in) using a sterilized potting mixture (Sunshine professional growing mix: Sun Gro Horticulture Canada Ltd., Agawam, MA, USA). All the trays were placed in popup cages (24 in x 24 in x 36 in, Biogentex Laboratories, Inc., TX, USA) inside the greenhouse conditions at 27⁰C and RH 70%. Trays were monitored daily for germination for 70 days until no further germination was observed for 5 days. Germination was measured in two ways; number of seeds germinated over total seeds planted (germination rate), and number of seeds germinated per week (speed of germination). After seedlings produced 2-4 true leaves, they were transplanted to square pots (4 in x 4 in x 6in) inside the popup cages.

3.4 Growth traits

The seedlings were monitored for height and leaf count every two weeks after transplanting. The height was measured using a ruler (cm) and the number of fully developed leaves were counted. In addition, total shoot length was also measured before harvesting for root traits.

3.5 Root traits

Morphological characteristics of roots were measured from a total of 30 plants composed of both treatments: 15 mowed and 15 unmowed. These plants were randomly chosen from the transplanted seedlings, all at the same age post-transplanting (4 weeks after transplanting). The plants were cut at soil level to separate the shoots from the roots and then gently removed from their respective pots and placed on a 3-part strainer, where soil was gently washed off the roots. Image acquisition of roots was completed by placing washed roots submerged in water on a transparent tray and scanned with an EPSON Flatbed Scanner (EPSON Expression 11000XL 1.8 V3.49 3.49), part of the WinRHIZO package (Regent Instruments Inc., Quebec, Canada). WinRHIZO has been used to efficiently and precisely determine complex root parameters that are normally prone to human error (Pornaro et al., 2017.) WinRHIZO was used to digitize and quantify various root traits such as total root length (cm), area (cm²), the number of tips, forks, crossing (fine roots) and root volume (cm³), among others. Specific root length (m/g) was determined by dividing the total root length by total root dry biomass.

Detailed explanation of the variables measured are in Table 1.

3.6 Dry Biomass

After root trait quantification, roots and shoots were dried at room temperature in brown paper bags for two days. After drying, the bags were placed in a drying oven (Quincy lab. INC, Fisher Scientific, USA) at 75°C for 48 hours, and dry mass was measured using an analytical balance (Accuris Dx W3101A-220, Mid Sci, Valley Park, MO, USA).

4. Herbivory

4. 1. Field herbivory

To determine whether mowed and unmowed plants experience similar herbivory levels in field, we did a field survey on ~10 plants per sub-population and estimated herbivory levels on a 0-4 scale; 0= 0%, 1= 25%, 2= 50%, 3= 75% and 4= 100% of the leaves damaged (Kariyat et al. 2012). Since mowing continuously reduced leaf area, we restricted our estimation to the youngest 5-6 fully developed leaves to be consistent across treatments. In addition, we also estimated herbivory presence on a yes or no (0 or 1) binary scale as an additional line of data for herbivory in field. The same methodology was repeated for seedlings from the next generation, except the transplanted seedlings in pots were transported to field and placed in SNL populations as a pair (one mowed and one unmowed; 15 pairs) 1 m apart for 7 days, followed by damage and herbivore assessment as before.

4.2 Herbivory in lab

To determine whether mowing impacted plant response against specific herbivores, we followed up the field experiment with lab assays with three different herbivores; Tobacco hornworm (*Manduca sexta*), a generalist on Solanaceae caterpillar, cowpea aphid (*Aphis craccivora*) a generalist aphid, and Texas Potato Beetle (*Leptinotarsa texana*), a specialist on SNL. These three herbivores are commonly found in the native SNL populations, and have been documented to successfully complete their life cycle on SNL.

a. *Manduca sexta* larval mass gain

M. sexta caterpillars were collected from the lab colony reared on a wheat germ based artificial diet (Frontier Scientific Services, Newark, DE, USA; Kariyat et al. 2019). Two days

old *M. sexta* eggs were placed on a 1cm³ cubes diet inside a petri dish until they hatched. After hatching, first instar caterpillars were pre-weighed and placed on fully developed leaves of 4 weeks post transplanted seedlings of SLN. In this experiment, sixty SLN plants (thirty mowed and thirty unmowed) were used. The plants were not randomly selected, but chosen based on similarity in size, height, and the number of leaves to reduce any confounding traits on herbivore mass gain. A coffee filter paper was wrapped around the potted plants around the midpoint of the stem, such that each plant was divided into two halves, with each half receiving one caterpillar each. Two first instar *M. sexta* caterpillars were placed on each plant, one caterpillar above the coffee filter and one caterpillar below the filter on a fully developed leaf and could feed continuously for 4 days. The caterpillars were starved for 4-6 hours before the experiment to clear their gut. After 4 days, the caterpillars were removed, and post mass data was collected. Using the following equation mass gain was calculated: $\text{mass gain} = (\text{final mass} - \text{initial mass}) / \text{initial mass}$ (Tayal et al., 2020; Singh and Kariyat, 2020).

b. *Aphis craccivora* population growth

A. craccivora used for the experiment was from a lab colony reared on multiple Solanaceae species. In this experiment, a total of twenty-four plants; twelve mowed and twelve unmowed were used. For the population assay, three third instar aphid nymphs were transferred from the host plants to a young leaf of treatment plant using a paintbrush and were allowed to grow and reproduce. The plants were separated and caged individually to minimize any accidental spread. Aphids were monitored and counted every five days and were counted twice. For both counts, adults and nymphs were counted separately.

c. *Leptinotarsa texana* larval mass gain

Like *A. craccivora*, we used a lab colony of *L. texana* reared on Solanaceae species from individuals collected from SNL from the native populations in the Summer of 2019. We used newly molted second instar grubs for the experiment, pre-weighed and placed on fully developed leaves of 15 mowed and 15 unmowed treatment plants. After four days, beetles were removed from the plant using a small paintbrush and weighed on a balance (Accuris Dx W3101A-220, Mid Sci, Valley Park, MO, USA) for the second mass. Like *M. sexta*, mass gain was then calculated.

4.3. Trichome density

To examine whether trichome density in offspring was affected by mowing treatment imposed on maternal plants, we chose one leaf from 10 randomly chosen offspring seedlings from mowed and unmowed parents. These leaves were cut near the node of the plant to avoid any damage to the leaf, then using a hole punch two small disks (6 to 8 mm diameter) were cut for each leaf. Leaf disks were taped with carbon tape and then was placed on a 15 mm aluminum stage. To examine the trichomes in detail, we used a desktop scanning electron microscope (SNE-4500M Plus Tabletop SEM; Nanoimages LLC, Pleasanton, California, USA). Images of abaxial and adaxial sides of leaves were taken at 60x magnification with 5KV using SE detector. For each sample, we did the following measurements: trichomes on abaxial and adaxial surface, number of glandular vs. non-glandular trichomes, and then for each sample, 10 random non glandular stellate trichomes were chosen and the number of individual spikes on them were counted. For counting, the scanning electron micrograph was saved as a .jpg file and each trichome was identified, labeled and counted (Kariyat et al., 2013).

Statistical analysis

For total fruits, seed mass, seeds/fruit and total seeds data, we used a General Linear Model (GLM) with Poisson distribution, with sub-population and treatment as factors. Tukey comparisons were carried out to determine pairwise differences among the factors. Fruit diameter was analyzed using the non-parametric Kruskal-Wallis test to determine if mowed populations varied from unmowed, since data failed to meet normality assumptions after transformation. Plant height from mowed and unmowed sub-populations were collected twice and analyzed separately using Kruskal-Wallis tests after transformation attempts failed to attain normal distribution. Damage assessment on 0-4 scale data was analyzed using Poisson regression. Seed germination data was also analyzed with a General Linear Model with mowing (or unmowed control), GA (no GA control) treatment and week (week 0-9) as factors, followed by Tukey posthoc tests to tease apart pairwise comparisons. For analyses of root traits, we used a combination of two-tailed T-tests and non-parametric Kruskal-Wallis tests based on the distribution of the data. Variables for which the data did not meet normality assumptions even after transformation were analyzed by Kruskal-Wallis tests. Specific root length was analyzed using two-tailed t-tests. Transformed data were back transformed for reporting as means and for making plots. In both analyses, mowed/unmowed treatment was used as the predictor. The detailed statistics are displayed in the table, and a few of the most relevant root traits of interest (Paez-Garcia et al. 2015) are displayed as plots (see table 1). For field herbivore presence (yes or no) and herbivory scale (0-4) data analyses, we used binary logistic regression and ordinal logistic regression respectively with treatment (mowed /unmowed) as the predictor. P values were reported based on Wald's test. To confirm that any preexisting variation in plant traits did not factor into field herbivory assessment, we also ran a t-test on plant height (supplementary

data). *M. sexta* mass gain and *L. texana* mass gain were analyzed using the Kruskal-Wallis test due to non-normal distribution. *A. craccivora* population growth was analyzed by examining total aphids found (adults and nymphs) using a Poisson distribution fit model regression due to non-normal count data. Both treatment and replicate were used as predictors and P values were reported from Wald test. For trichomes we ran multiple analyses; total trichomes were analyzed using a 2-sample t-test, and a Two-way Anova was used for estimating whether the trichomes varied due to mowing treatment of leaf surface. Treatment (mowed/unmowed) and side (abaxial / adaxial) were used as factors. Similar to total trichomes, stellate and non-glandular trichome number, and the number of spikes on stellate trichomes were also analyzed using 2 sample t-tests. All analyses were carried out using Minitab (Minitab Inc, State College, PA, USA) and plots were made using GraphPad Prism (LA Jolla, California, USA) software.

Results

1. Fitness traits (parents)

Analyses of total fruits production showed that unmowed genets produced significantly more fruits (GLM; $F=48.72$; $P<0.001$; Figure 1A), and total seeds (Mean seeds X total fruits; GLM; $F=41.90$; $P<0.001$; Figure 1B). However, we also found no difference for fruit diameter (Kruskal-Wallis Test; $P=0.269$; see supplementary figure 1), and for mean number of seeds per fruits (GLM; $F=0.06$; $P=0.809$; Figure 1C). Surprisingly, when we measured 100 seed mass, we found that seeds from mowed genets were significantly heavier than unmowed genets, suggesting that these embryos may be better fit (GLM; $F=3.35$; $P<0.001$; Figure 1D), a question we addressed with the germination assays.

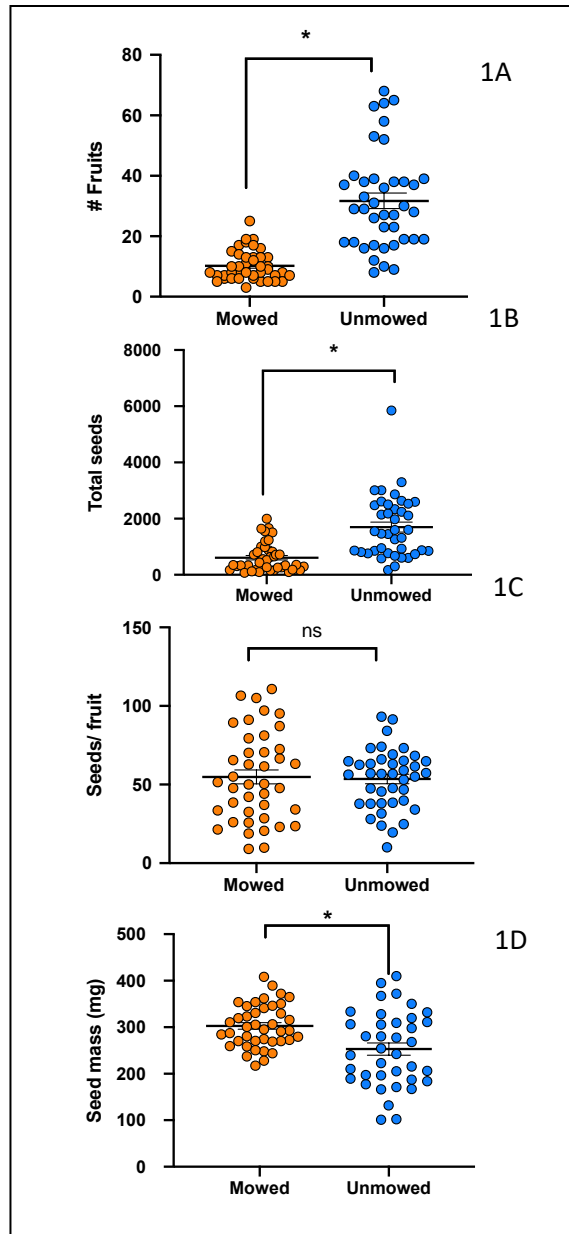


Figure 1: Results of field fitness traits show: (1A) Number of fruits collected from mowed and unmowed locations ($P < 0.001$), (1B) number of seed per fruit (Y-axis) ($P = 0.809$), (1C) Seeds mass (Y-axis) (treatment $P < 0.001$), (1D) total number of seed (Y-axis) ($P < 0.001$) mowed and

GA (orange) and unmowed and control (blue) plants. Means are shown by scale bars and asterisk denotes significantly different results at $P < 0.05$, while ns denotes non-significant results.

2. *Seed Germination*

Following our fitness traits experiments, seed germination showed that seeds from mowed maternal plants had significantly higher germination rate than unmowed (GLM; $F=9.85$; $P < 0.002$; Figure 2A). However, the phytohormone GA3 had no significant effect on germination rate for mowed and unmowed populations (GLM; $F=0.00$; $P= 0.974$; Figure 2B). Like other weed species, we also found significantly more seeds germinated during the early season than in the late season (GLM; $F=95.33$; $P < 0.001$) with most of the germination taking place in the first five weeks (Figure 2C).

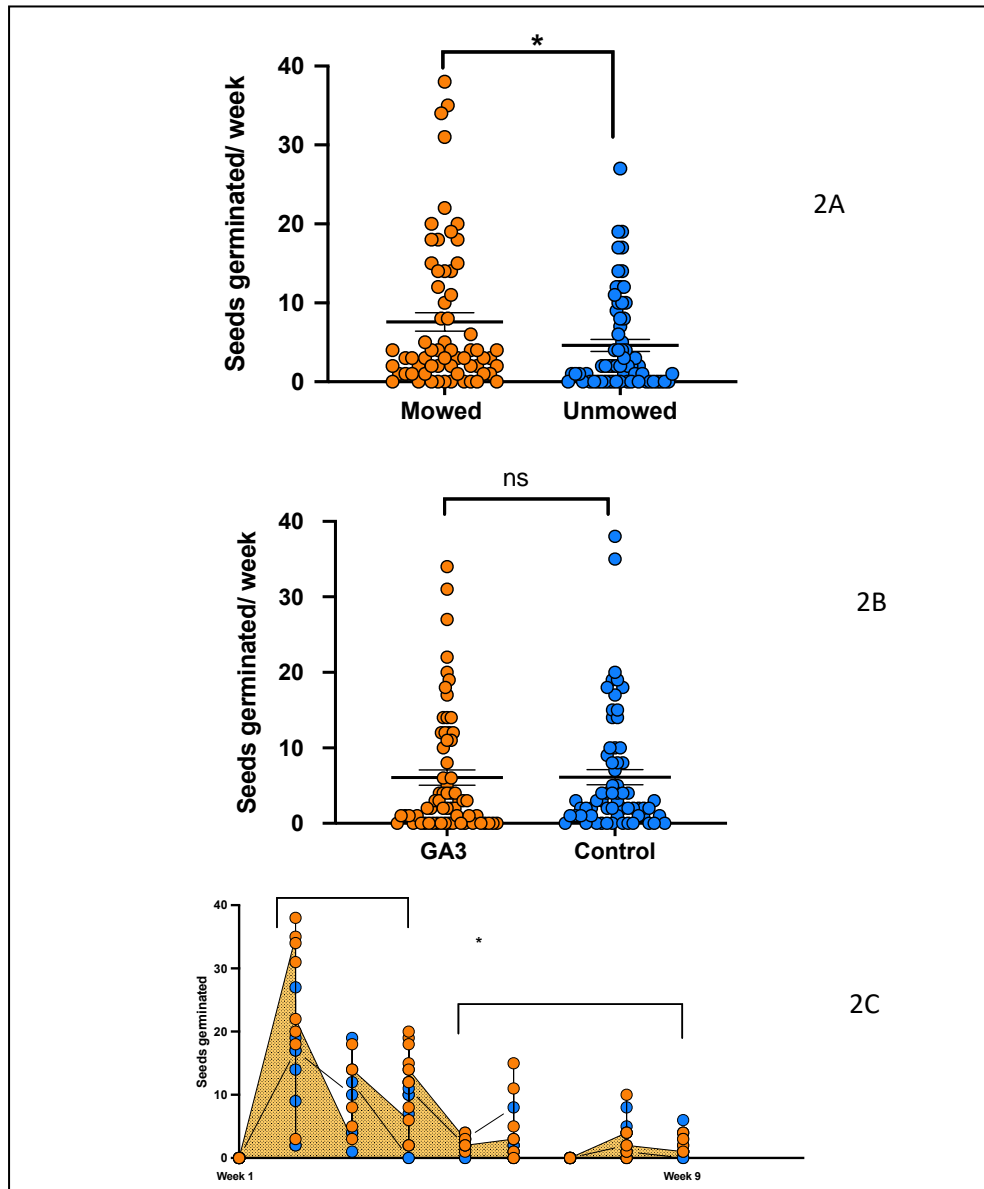


Figure 2: Results of seedling germination in green house show: (2A) Seeds germinated per week on mowed and unmowed plants ($P < 0.002$), (2B) Seeds germinated per week with GA treatment and control ($P = 0.974$), (2C) Seeds germinated over time (early vs late season) (treatment $P < 0.006$; GA $P = 0.977$), mowed and GA (orange) and unmowed and control (blue) plants. Means are shown by scale bars and asterisk denotes significantly different results at $P < 0.05$, while ns denotes non-significant results.

3. Growth (offspring)

Transplanted seedlings from the germination experiment were also monitored for health and vigor. We found that seedlings from unmowed parents had significantly more leaves per seedling (Poisson distribution fit model regression; Chi-square=15.16; $P < 0.001$) and were also significantly taller than mowed plants (Kruskal-Wallis test; $t=-3.81$; $P < 0.001$), suggesting better growth and developmental traits in unmowed seedlings (Figures 3A & B).

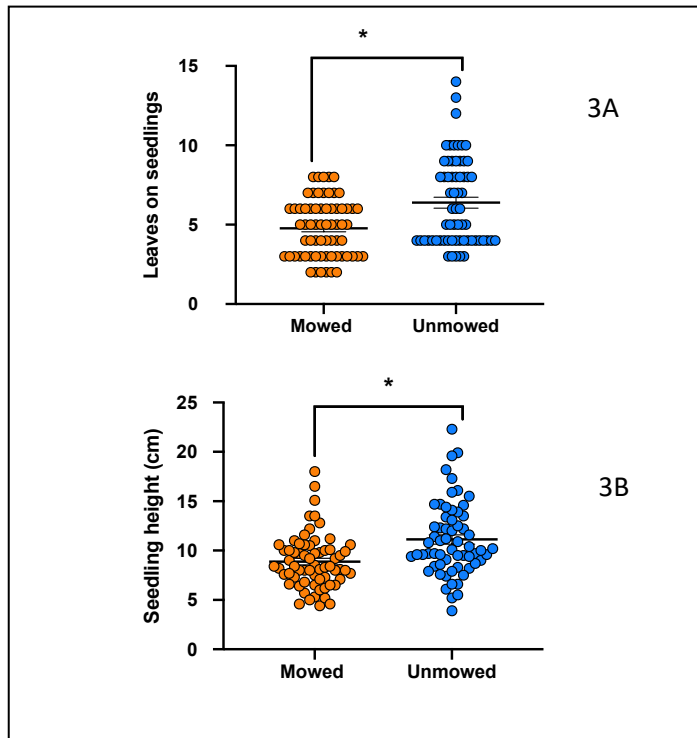


Figure 3: Results of seedlings health and vigor show: (3A) number of leaves on seedlings on mowed and unmowed plants ($P < 0.001$), (3B) seedling height (cm) on both treatments ($P < 0.001$) mowed (orange) and unmowed (blue) plants. Means are shown by scale bars and asterisk denotes significantly different results at $P < 0.05$, while ns denotes non-significant results.

4. *Field damage (parental generation)*

Analysis of herbivory data from field showed that there were significantly more herbivores on unmowed plants in comparison to mowed plants (Binary logistic regression; Chi-square=16.92; $P > 0.001$; Figure 4A). Consequently, damage done by herbivores was also significantly more on unmowed plants (Ordinal logistic regression; $P = 0.037$; Figure 4B).

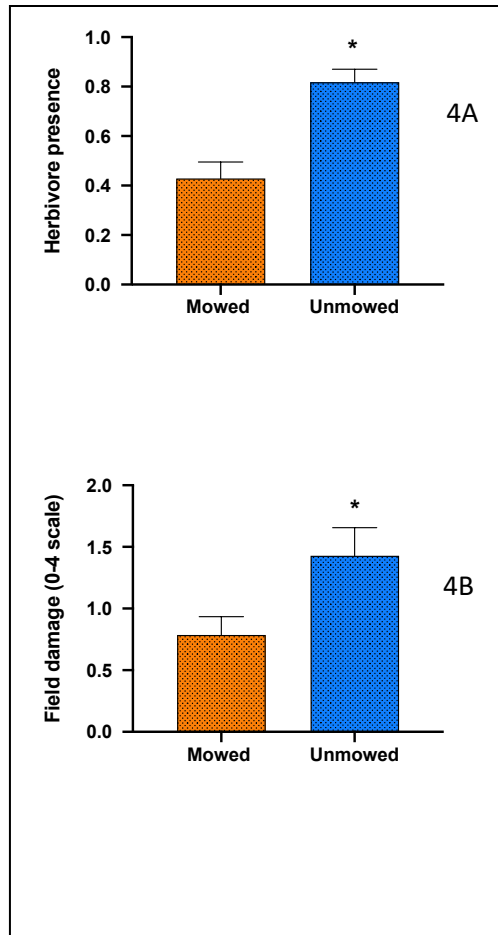


Figure 4: Results of seedling herbivory in field show: (4A) herbivore presences on mowed and unmowed plants ($P < 0.001$; binary logistic regression; Wald's test), (4B) damage by insects on both treatments (scale 0-4) ($P = 0.037$; ordinal logistic regression; Wald's test) mowed (orange bar) and unmowed (blue bar) plants. Means are shown by scale bars and asterisk denotes significantly different results at $P < 0.05$, while ns denotes non-significant results.

5. *Herbivory in lab (offspring)*

Field damage results were further confirmed by herbivory experiments conducted in lab. We found the number of *A. craccivora* on mowed plants to be significantly higher than on unmowed plants (Poisson distribution fit model regression; $P < 0.001$; Figure 5A). However, our mass gain experiments of *M. sexta* show significantly lower mass gain on mowed plants than on unmowed plants (Kruskal-Wallis test; $H=5.22$; $P = 0.022$) (Figure 5B). On the other hand, we found no significant difference in mass gain of *L. texana* on both mowed and unmowed plants (Kruskal-Wallis test; $H=0.13$; $P= 0.715$) (Figure 5C). Therefore, we speculate higher induction of jasmonic acid (JA) signaling pathway in mowed treatments, which is induced in plants upon attack by chewing insect pests. These results are akin to JA-SA (salicylic acid pathway induced in plants upon attack by sucking insect pests) pathway negative crosstalk, where induction of one pathway downregulates the other.

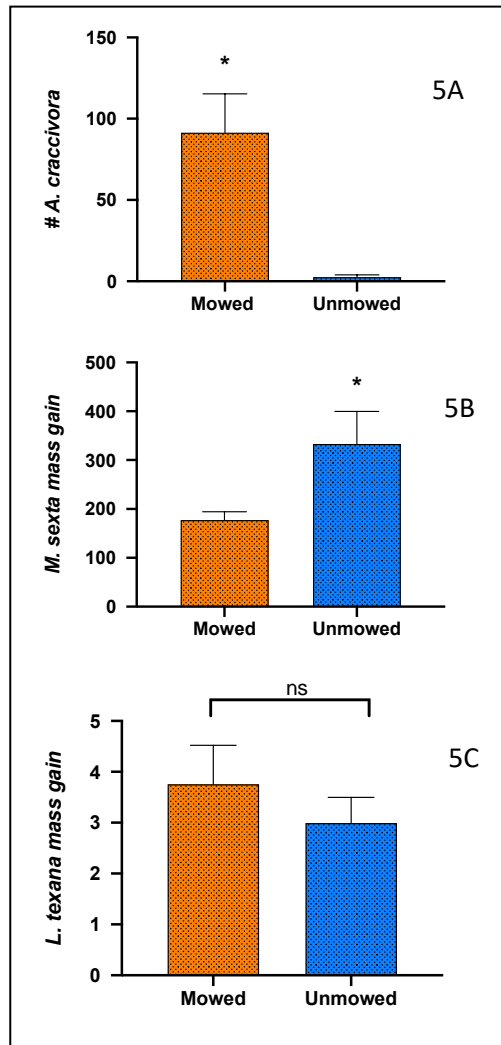


Figure 5: Results of herbivory in lab results from 3 herbivores show: (5A) number of *A. craccivora* (Y-axis) ($P < 0.001$; Poisson distribution fit model regression), (5B) mass gain of *M. sexta* caterpillars (Y-axis) ($P = 0.022$; Kruskal-Wallis test) and (5C) mass gain of *L. texana* (Y-axis) on mowed (orange bar) and unmowed (blue bar) plants ($P = 0.715$; Kruskal-Wallis test). Means are shown by scale bars and asterisk denotes significantly different results at $P < 0.05$, while ns denotes non-significant results.

6. *Trichomes (offspring)*

Contrary to our expectations, our results show that unmowed plants have significantly more trichomes than mowed treatments (Two sample T-test; $t = -2.53$; $P = 0.02$) (Figure 6A). We followed this by examining the abaxial and adaxial sides of the leaves for both treatments. However, there was no significant differences in mean number of trichomes per side (Two-way Anova; $F = 1.27$; $P\text{-value} = 0.26$) (Figure 6B) in either of the treatments (Two-way Anova; $F = 3.50$; $P = 0.06$) (Figure 6B). SLN has both glandular and non-glandular (stellate) trichomes, dominated by non-glandular stellate trichomes. Similar to trichome density we also found that unmowed treatment had significantly more stellate trichomes (Two sample T-test; $t = -2.47$; $P\text{-value} > 0.02$) (Figure 6C), while no difference was found between treatments for glandular trichomes (Two sample T-test; $t = -0.10$; $P = 0.918$) (Figure 6D). Using enhanced measurement features of the tabletop SEM, we also examined detailed morphology of non-glandular (stellate) trichomes and found that the seedlings from unmowed treatment also had significantly more spikes on their trichomes (Two sample T-test; $t = -6.26$; $P < 0.00$) (Figure 6E).

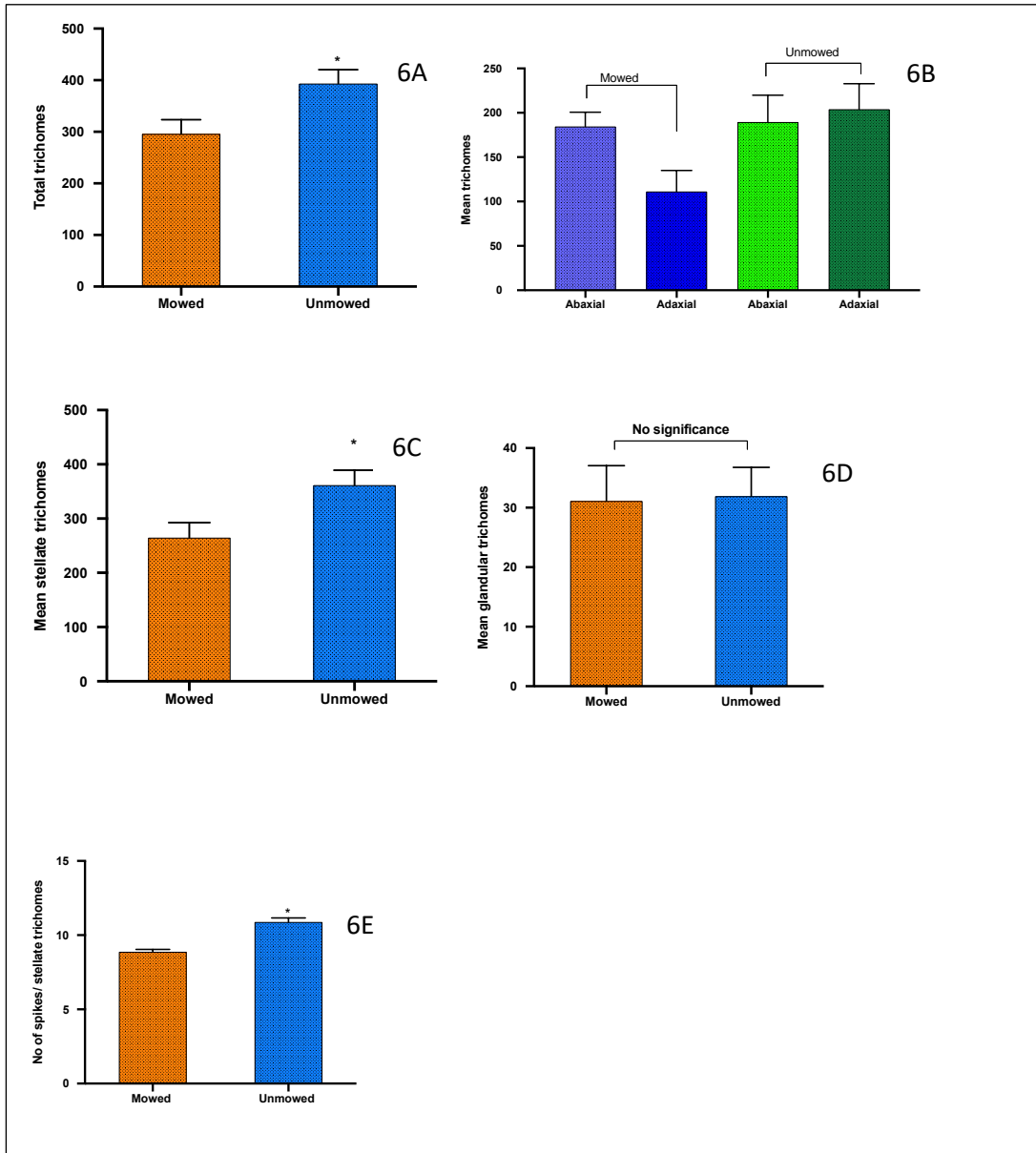


Figure 6: Results of trichome density: (6A) number of trichomes on leaves for mowed and unmowed treatments (Y-axis) ((Two sample T-test; $t = -2.53$; $P = 0.02$), (6B) compares mean number trichomes on abaxial and adaxial side of the leaf for both mowed (Y-axis) (Two-way Anova; $F = 1.27$; $P\text{-value} = 0.26$) and unmowed (Y-axis) (Two-way Anova; $F = 3.50$; $P = 0.06$) treatments respectively, (6C) mean number of stellate trichomes on leaves for mowed and unmowed treatments (Y-axis) (Two sample T-test; $t = -2.47$; $P > 0.02$), (6D) mean number of glandular trichomes on leaves for mowed and unmowed treatments (Y-axis) (Two sample T-test; $t = -0.10$; $P = 0.918$), (6E) number of spikes on stellate trichomes for mowed and unmowed treatments (Y-axis) (Two sample T-test; $t = -6.26$; $P < 0.00$). Means are shown by scale bars and asterisk denotes significantly different results at $P < 0.05$, while ns denotes non-significant results.

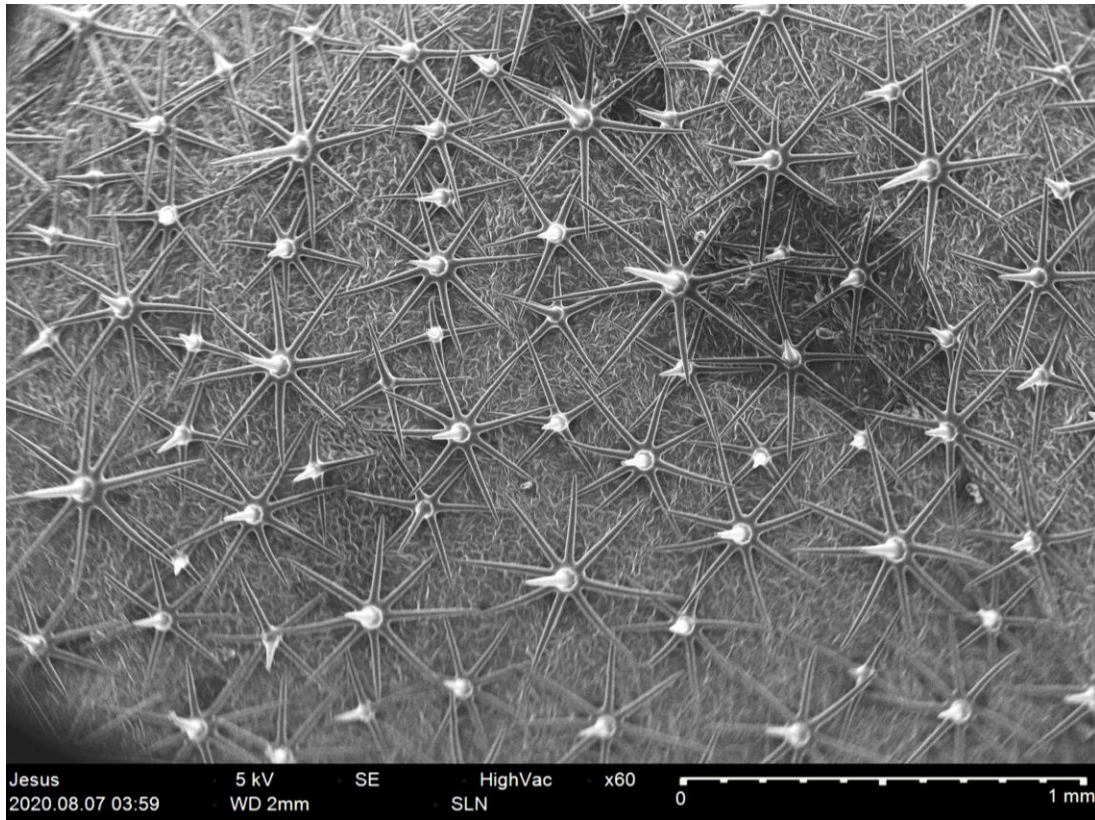


Figure 6A: Electron micrograph of abaxial (underside) trichomes on *S. elaeagnifolium* collected using a Scope M desktop scanning electron microscope at 60X magnification.

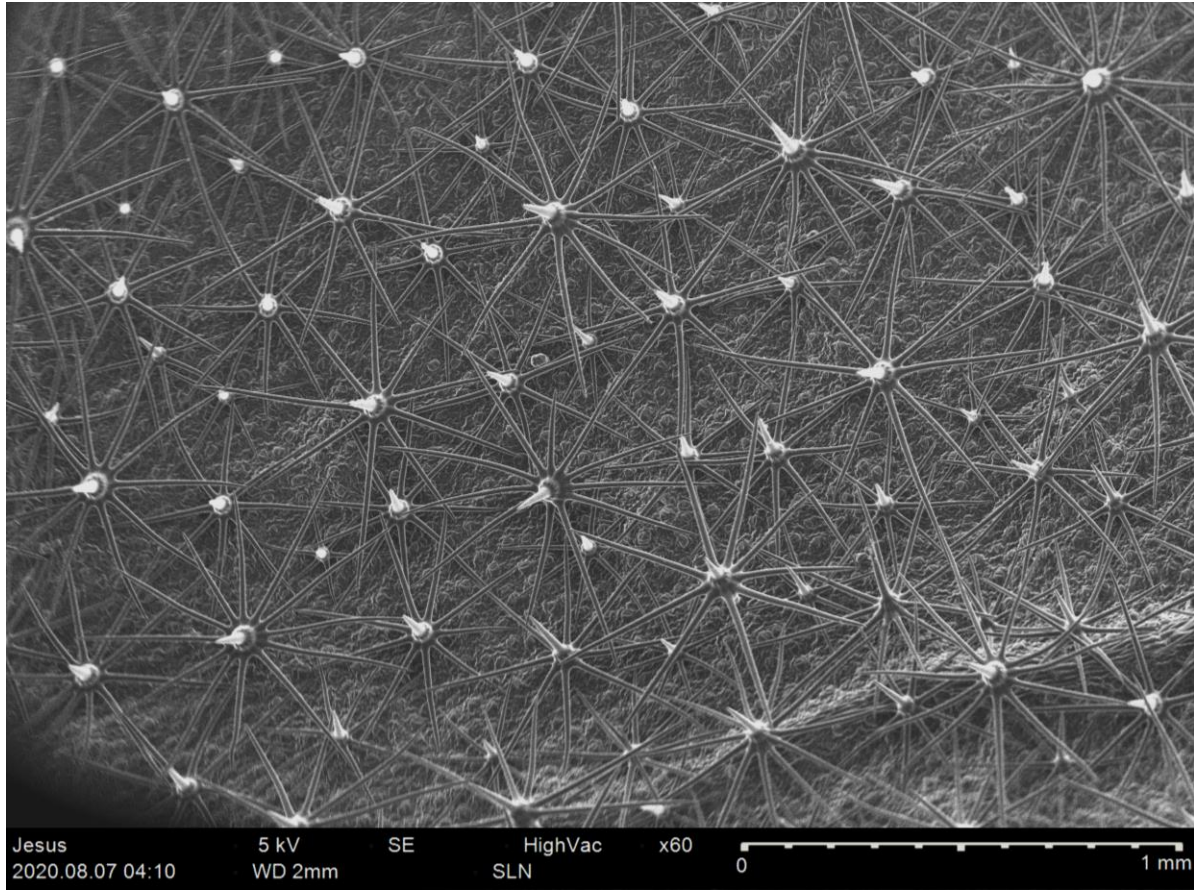


Figure 6B: Electron micrograph of adaxial (aboveside) trichomes on *S. elaeagnifolium* collected using a Scope M desktop scanning electron microscope at 60X magnification.

7. Root traits

In addition to above ground traits, we also examined below-ground root traits and their differences between seedlings from mowed and unmowed parents. There were no significant differences between the two groups in root (Kruskal-Wallis tests; $t = -0.130$; $P < 0.108$) (Figure 7A, Table 1) and shoot (Kruskal-Wallis tests; $t = 1.24$; $P = 0.225$) (Figure 7B, Table 1) length. However, we found that the major root traits such as whole root area (Kruskal-Wallis tests; $t = 2.54$ $P = 0.017$) (Figure 7C, Table 1), root surface area (Kruskal-Wallis tests; $P = 0.017$) (Figure 7D, Table 1), and fine roots (Kruskal-Wallis tests; $t = -3.42$ $P < 0.0002$) (Figure 7E, Table 1), were significantly higher in roots from seedlings of unmowed parents than their mowed counterparts. Interestingly, specific root length (SRL), a key root trait in resource acquisition, was significantly higher on mowed when compared to the unmowed seedlings (Two-tailed T-test; $t = 2.02$; $P < 0.049$) (figure 7F, Table 1).

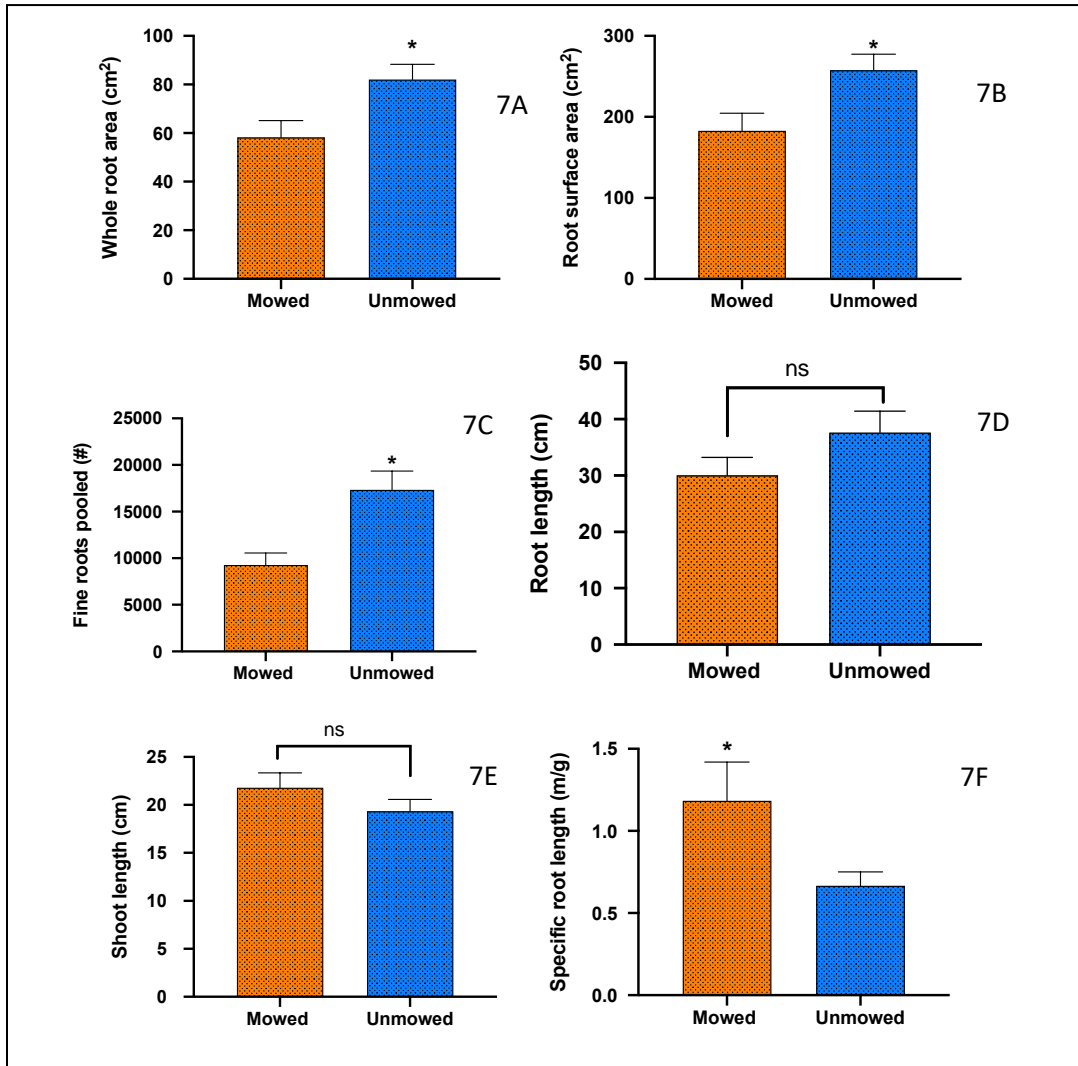


Figure 7: Results of six major root traits show: (7A) Whole root area ($P < 0.108$; Kruskal-Wallis tests), (7B) Root surface area ($P = 0.017$; Kruskal-Wallis test), (7C) Fine roots pooled ($P < 0.0002$; Kruskal-Wallis tests), (7D) Root length ($P = 0.004$; Kruskal-Wallis tests), (7E) Shoot length ($P = 0.225$; Kruskal-Wallis tests), (7F) Specific root length ($P < 0.049$; two tailed T test) on mowed (orange bar) and unmowed (blue bar) plants. Means are shown by scale bars and asterisk denotes significantly different results at $p < 0.05$, while ns denotes non-significant results.

<i>Response Variable</i>	<i>Mean and SE (Mowed)</i>	<i>Mean and SE (Unmowed)</i>	<i>T Value</i>	<i>P-Value</i>
Shoot length	21.8+/-1.6	19.3 +/-1.2	1.24	0.225
Root width	23.46+/-0.93	25.0+/-0.73	-1.30	0.204
Root height	16.12+/-0.63	17.23+/-0.35	-1.54	0.138
Root length	1142+/-110	1623+/-104	-0.91	0.004
Total surface area	182.9+/-22	257.7+/-20	-2.54	0.017
Root volume	2.45+/-0.39	3.38+/-0.40	-1.66	0.108
Root tips	7928+/-1150	15447+/-1904	-3.38	0.003
Root forks	13483+/-1700	19381+/-1569	-2.55	0.017
Root crossings	1148+/-131	1563+/-144	-2.14	0.042
Pooled fine roots	9268+/-1256	17349+/-2000	-3.42	0.0002

Table 1. Details of response variables, treatment means, statistical tests and significance from WinRhizo experiment. Variables that are statistically significant are in bold P values at P<0.05.

Discussion

In this study, we examined how disturbance (mowing) affects reproductive fitness, defenses, and their cascading transgenerational effects on SLN over two growing seasons. Collectively, we found that although mowing reduces reproductive fitness by removing photosynthetic area, mowing also leads to better defended plants in both parental and offspring generations. More importantly, we also found that these differences are not similar for all the traits we measured. Enhanced fitness and defense traits in the offspring; from mowed parents indirectly suggest that disturbance can lead to better seedlings with a possibility of making species like SLN a super weed, adding another layer of complexity in understanding its invasion and management (EPPO datasheet, 2019). While examining fitness traits, we found that unmowed genets were taller and produced significantly more fruits and total seeds (total fruits X seeds/fruit) than their mowed counterparts (Figure 1 B and D), confirming that these genets can flourish in anthropogenically undisturbed environments (Pinke et al. 2010). In contrast, while unmowed genets produced more fruits, the seed mass of mowed individuals was significantly higher (Figure 2C). It has been well understood that heavier seeds tend to germinate more and rapidly, therefore seed mass is considered a strong indicator of fitness (Treymane and Richards 2008). This is particularly important for a weed species such as SLN that colonize agricultural land, pastures and areas that are prone to constant disturbance. Our data clearly demonstrates that higher seed mass can have a benefit by improving progeny fitness, as documented elsewhere (Ramesh et al. 2017).

As a consequence of heavier seeds on mowed plants, we found that they also germinated more and more rapidly when compared to unmowed. We also introduced the phytohormone GA₃ to test if the hormone treatment can differentially influence germination rate (Rha and Jamil

2007), but surprisingly found that GA₃ has no effect on germination rates on mowed or unmowed treatments. We also estimated the germination cycle of SLN in its native range. We show that SLN seeds have around 50% germination rate and tend to germinate the most within the first 5 weeks of seeding, as opposed to later in the season (Figure 2C). This coincides with a study on germination timings of weed species, where *Ambrosia artemisiifolia* L., *Ambrosia trifida* (rag weed), and *Polygonum pensylvanicum* L., (Knotweed) all had large flushes of germination in the first 5 weeks from their planting, followed by very little to no germination after (Stoller and Wax 1973). Gioria and Pysek, 2016 also found a strong tendency for invasive plants to germinate earlier and faster than their native counterparts. For a weed species that tend to undergo constant disturbance and possible extinction of founding populations, rapid and relatively high germination rate makes it a grave concern for conservation, invasion and management policies (Gioria et al. 2017; Mahmood et al; 2016). A key follow up question would be to test if invasive populations differ in these traits under similar treatments, an area we are currently exploring (Petanidou et al. 2012).

Interestingly though, enhanced germination rates and faster germination didn't translate into better growth traits in mowed offspring. We found that seedlings from unmowed parents were taller and had more leaves, suggesting that mowing-induced trait enhancement is possibly limited to germination, rather than cascading throughout the growing season (Mc Naughton, 1984; McNaughton, 1987). Additionally, weeds like SNL have higher root investment, since rhizomatous roots are a major reproductive strategy, ensuring fitness. Similar to growth and fitness traits, we found that unmowed plants had higher values of various metrics of root biomass, including root area and number of fine roots, but a key metric of the cost-benefit ratio of roots, specific root length, was significantly higher in offspring of mowed plants (Table 1).

Specific root length has been associated with an enhanced ability to acquire nutrients and is known to be independent of other plant trait economics spectra (Laliberté et al., 2015; Karamer-Walter et al., 2016). This is a significant result as it clearly shows that while total available resources are limited, mowed offspring construct fine roots more efficiently, and thus may be able to partially offset these negative effects, giving them enhanced ability to acquire water and nutrients at a given size. Additionally, this ability can be very important in limited resource environment, and when taken together, these tradeoffs between below and aboveground traits warrant more detailed examination. Their potential implications for invasion success, and consequential effects on plant diversity under different land management regimes (Losapio et al., 2020) can shed light into how invasive weed species can be successful under resource limitation (Onen et al., 2016).

A large body of previous research has investigated the defense related mechanisms in plants against insect herbivory in weedy species, (Wittstock et al., 2002; Mooney et al., 2009; Baldwin, 2010; Kariyat et al., 2018) and how these effects are modulated by resource availability, evolutionary history, and breeding status (Coley et al., 1985; Fine et al., 2004; Van Zandt 2007; Kariyat et al., 2011). In both years, a significant amount of our experimentation was carried out on herbivory and plant defenses. We hypothesized that mowing, a mode of mechanical wounding will enhance defenses and thereby affect herbivores (Kariyat et al., 2013) that feed on SLN. In the parental generation, we found that mowed plants suffered lower damage in the field. The major herbivore of SLN in our sub-populations was Texas potato beetle (*Leptinotarsa texana*) grubs and adults, other herbivores present were tobacco hornworm (*Manduca sexta*), cowpea aphid (*Aphis craccivora*), and eggplant tortoise beetle (*Gratiana pallidula*). Even more interesting was that reduced herbivory was consistent in offspring when

they were exposed to herbivores in an area close to our sub-populations. Clearly, mowing (damage) in parental generation cascaded to offspring and had possibly higher constitutive defenses that reduced both herbivore incidence and herbivory levels (Nihranz et al., 2019). It has been shown that higher alkaloid production in tall fescue (*Festuca arundinacea*) and perennial ryegrass (*Lolium sp.*) as a result of mowing results in less damage done by insect pests, supporting the idea these disturbances help plants to withstand and maybe even defend against herbivores (Salminen and Grewal, 2002; Freeman and Beattie, 2008).

We found that our lab herbivory results were species specific; *M. sexta* (chewing herbivore; feeds on most Solanaceae) gained less mass on mowed plants indicating less damage to these plants than unmowed plants (Figure 5B). However, *L. texana* (chewing herbivore, co-evolved and feeds exclusively on SLN (Davis et al., 2020) mass gain was similar in both mowed and unmowed plants (Figure 5C). On the other hand, *A. craccivora* (sucking herbivore, generalist aphid) population fared significantly better on mowed plants (Figure 5A). These results are consistent with the JA and SA pathway negative crosstalk. Plenty of studies have reported negative crosstalk of JA suppressing SA action (Traw et al., 2003; Bostock, 2005). Traw et al. (2003) also found suppression of SA due to JA in Wassilewskija wild type which increased their susceptibility to *Pseudomonas syringae* (Traw et al., 2003). We speculate that increased constitutive defenses under the JA pathway enhanced defenses against herbivores in general (as observed in field), and more specifically against *M. sexta* in lab. Consequently, JA mediated SA suppression possibly lead to mowed SLN being susceptible to sucking herbivore *A. craccivora*. However, the most important herbivore that damages SLN - *L. texana*, a potential biocontrol agent (Lefoe et al., 2020) was unaffected by mowing, clearly suggesting that regardless of any enhanced defenses due to mowing, the co-evolved specialist herbivore was able

to continuously feed and develop, as documented in other systems (Chung et al; 2011, Korpita et al; 2014). For example, Yang et al, (2013) looked at *Triadica sebifera* in its native (Asia) and invasive habitat (USA).

Finally, the variation in herbivore response to mowing in offspring in lab and field caused us to ask whether plant defenses correlate with these herbivore growth traits. Our comprehensive examination of the trichome morphology of SLN allowed us to address this directly. Using a series of manipulative experiments, we have previously documented pre and post ingestive roles of trichomes as a plant defense in *Solanum spp- M. sexta* system (Kariyat et al. 2017, 2018,2019). Surprisingly, our results showed that offspring from mowed parents had lower trichome density (stellate, the major trichome type), and that they also had lower number of individual spikes on them. Trichomes have been well documented to be an effective defense against herbivores, by either restricting their access (Karabourniotis et al. 2019; Kariyat et al. 2018), movement (Tian et al. 2012, Kang et al. 2010) and in many cases being toxic to them (Kariyat et al. 2017, 2019). We speculate that although trichomes are thought to be primarily regulated by JA pathway, other phytohormones including GA, Cytokinins, SA and Ethylene also plays key roles in both initiation and branching (An et al., 2011). Our data clearly shows that the interplay of JA, SA mediated defenses, herbivore feeding and trichomes are far more complicated. It would be interesting to identify and quantify secondary metabolites (alkaloids, and plant volatiles), signaling compounds (phytohormones) and their gene expression to tease apart these effects, and to examine potential tradeoffs between chemical and structural defenses and herbivory in this species. And, as an additional line of research, we are currently exploring how trichomes differentially affect the three herbivores used in this study.

Taken together, our data from both parental and offspring generations affected by mowing pressure strongly supports the idea that environmental anthropogenic disturbances significantly affect growth and fitness traits and leads to cascading effects from parent to offspring. The present experiments were unable to resolve the mechanistic components and their possible crosstalk dictating these effects. Moving forward, the role of epigenetics (Lamke et al., 2017; Weinhold., 2018; Miryeganeh et al., 2019) in offspring trait expression should be explored further and will be the subject of future work in SNL

CHAPTER III

CONCLUSIONS AND FUTURE DIRECTIONS

The objective of this thesis was to understand the factors that contribute to the success of Silverleaf nightshade (SLN) as a noxious weed in its native range in south Texas. We surveyed multiple populations across the Rio Grande Valley, and examined the correlations between plant health and herbivory, and soil characteristics. We found that across the valley, from 50 sub-populations surveyed, that soil type is an important factor affecting SLN success. Soil type also affected species interactions, specifically, clay soil was particularly interesting because plants grew taller and had low number of spines and consequently had highest herbivory damage. A caveat of this study was we assumed the soil properties stayed constant based on NRCS soil map for the valley. An interesting follow-up question would be to collect and analyze soil samples from these sites (on progress) and estimate soil properties and weed growth, including the presence and volume of mycorrhizae.

I had been tracking SLN populations in the valley since 2018, and have consistently observed that human disturbance, primarily through mowing affected SLN growth and development traits. It is fair to estimate that most populations of SLN are indeed disturbed, including the 50 sub-populations we surveyed. However, we were able to identify 4 unmowed and undisturbed sub-populations in the vicinity of mowed populations in the McAllen-Edinburg area. This allowed us to ask some interesting questions including growth, development, fitness,

and more importantly, the effects of mowing on plant defenses. Over two seasons of growth and through a series of field, greenhouse and lab experiments, we show that continuous mowing had a significant impact on growth, fruit and seed set, germination and progeny fitness. Even more interesting was that these differences also translated into variation in plant defenses, differentially affecting different herbivores. Mowing was found to enhance quite a few of the above said traits and can possibly lead to making superweeds, a grave management concern for the valley and beyond. This study has also given us more questions than answers; for example., what are the mechanistic underpinnings of these trait variations, especially defense response to herbivory? An RNA sequencing effort based on this is currently underway which we expect give us more detailed explanation at the molecular end. In addition, we also found that mowing had no impact on Texas potato beetle (*Leptinotarsa texana*), the specialist herbivore on SLN. In the past decade, we have understood that microbial communities (e.g., oral secretion) can beat host plant defenses, an area we are currently exploring.

Taken together, this thesis used field biology, and chemical ecology to understand fitness and herbivore defenses in SLN, a worldwide invasive species in its native range. The results from this thesis sheds light into reproduction and insect-plant interactions in this species, and the current experiments (plant volatiles, phytohormones, RNA sequencing), and the follow up questions will help us decipher the underlying mechanisms that mediate these traits. More importantly, these results will also assist us in devising appropriate management practices so we can minimize the impact of this weed on our agriculture and soci

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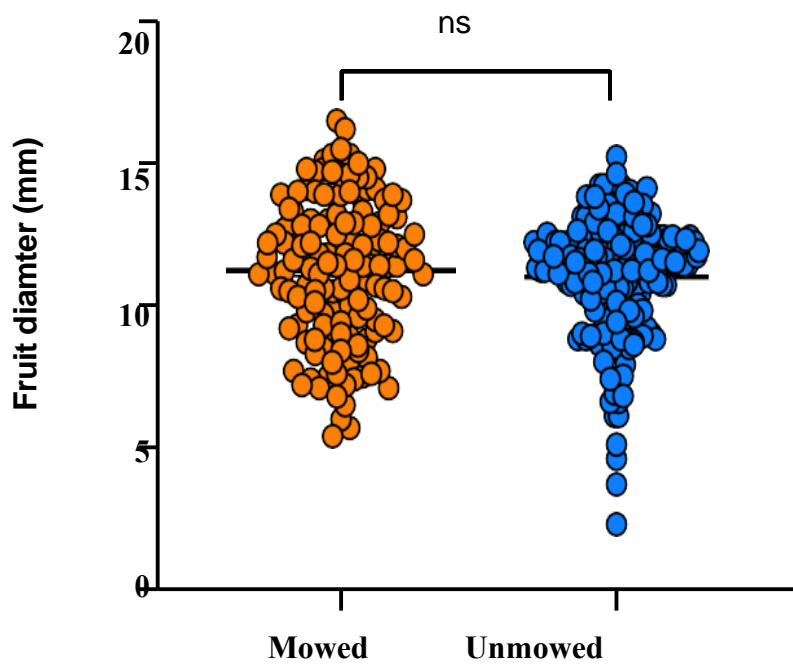
APPENDIX



Figure A1. *Solanum elaeagnifolium* flowering in its native range in McAllen, Texas.

Table B1. Location coordinates of mowed and unmowed sub-populations of SLN used for this study

<i>Location</i>	<i>Latitude</i>	<i>Longitude</i>	<i>Treatment</i>
Edinburg	26.313476	-98.145862	Mowed
Edinburg	26.306722	-98.145631	Mowed
McAllen	26.27475	-98.223654	Unmowed
McAllen	26.168613	-98.313712	Unmowed



Supplementary Figure B1: Results of fruit diameter from plants collected from 4 mowed and unmowed sub-populations(P=0.269)

BIOGRAPHICALSKETCH

Jesus Chavana studied in Arlington, Texas for his Elementary, Middle, and High schooling until 2012. He started at South Texas College in 2013 to obtain his associate degree in biology and was transferred to The University of Texas Rio Grande Valley in 2016 where he completed his Bachelor of Science with a Major in Biology, and continued to complete his Master of Science in Biology (2020). He has found a new passion of entomology and enjoys collecting insects. He is also passionate about learning new skills that will help him obtain a career in the future.

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