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Plant Mediated Effects on Tritrophic Interactions in the Solanaceae-Hornworm System

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PLANT MEDIATED EFFECTS ON TRITROPHIC INTERACTIONS IN THE SOLANACEAE-HORNWORM SYSTEM

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In loving memory of my Mother, MaryAnn Garvey, who pushed me to follow my dreams.

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ABSTRACT

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Top-down pressure from parasites is thought to be a key driver in herbivore diet breath, but studies investigating the evolution of food plant shifts as a defense against natural enemies in the environment are still lacking in the literature. I examined how plants alter insect-enemy interactions for a specialist herbivore utilizing solanaceous food plants, *Manduca sexta* (the tobacco hornworm) and the parasitoid wasp, *Cotesia congregata*, as a model.

In this study, I documented parasite infections in a field population of *M. sexta*, and then investigated from an eco-immunological perspective how plant toxins influence susceptibility to parasites in order to explain food plant choice. My research demonstrates that *M. sexta* exhibits a negative preference-performance relationship with plants in the Solanaceae. This is likely to gain protection from parasitoid attack via direct and indirect effects from plants on herbivore physiology. I show that herbivores are unpalatable and toxic to natural enemies when they consume more noxious host plants, but also provide a subsequent explanation for the adaptive value and maintenance of this interaction; specific plant secondary metabolites alter herbivore immune activity, where for *M. sexta* nicotine demonstrates immunotherapeutic properties by enhancing this insect's phenoloxidase activity.

I also examined phenotypic plasticity in caterpillar immune responses to nonlethal cues from natural enemies. Upon studying non-consumptive effects of natural enemies on *M. sexta* in the presence of *C. congregata* and the spined soldier bug, *Podisus maculiventris*, my work suggests that *M. sexta* generally accelerates their development in the presence of natural enemies at the cost of some immune defenses, implying a resource allocation tradeoff to physiological development and immunity.

Placed within a community level context, *M. sexta* can mitigate the consumptive and nonconsumptive effects parasitoids have on this herbivore's physiology by utilizing a food resource in parasite burdened habitats that increases direct resistance to parasites and also improves immune activity, even at the cost of development.

Following this, I investigated the consequences of crop domestication on plant-insect-parasitoid interaction via changes in plant traits for direct defense against herbivores and altered plant volatile signaling of natural enemies. I demonstrated that domesticated chili peppers showed no loss of plant direct defenses to *M. sexta* compared to wild peppers and that crop peppers had increased attraction and efficiency of parasitoids. This highlighted the context-dependent nature domestication has on trophic interactions and emphasizes the need for dedicated investigation in each unique crop system.

CHAPTER 1. INTRODUCTION

1.1 Overview

Nearly every plant described is susceptible to an insect herbivore; most plants to an array of them. Even plants seemingly too noxious to harbor and sustain growth of any animals, host insects. These insect herbivores are typically specialists, restricted to utilizing only a few closely related plant species. It is the maintenance of these plant-insect interactions over ecological time, in addition to how anthropomorphic selection has altered them, that I am interested in and will be the focus of this thesis. First, I will briefly discuss the evolutionary narrative of how the Lepidoptera came to be ravenous folivores. Next, I will discuss how plant traits and predation by natural enemies impose limitations on lepidopteran herbivore diet breath, and that only by examining their interplay in a tritrophic framework (plant-insect-enemy interactions) can a holistic understanding of the evolution of herbivore diet breadth be attained. Using the hornworm *Manduca sexta*, the host plants this caterpillar feeds on in the family Solanaceae, and the arthropod natural enemies that attack this insect as a model, I will argue in subsequent chapters that natural enemies help maintain plant specialization of herbivorous insects through plant-mediated effects on herbivore physiology that increase resistance to natural enemies. I will then use a subset of this system composed of the pepper plant (*Capsicum annuum*), *M. sexta,* and the parasitoid wasp *Cotesia congregata* to explore the consequences of human alteration through crop domestication on insect-enemy interactions.

1.2 Evolution of Herbivore Diet Breadth

The evolution of complete metamorphosis, coupled with the radiation of angiosperms, and the subsequent rapid exploitation of these plants by insects is believed to have led to the enormous species richness of the class Insecta, mainly in the orders Lepidoptera, Hymenoptera, Diptera, and Coleoptera (Grimaldi & Engel 2005; McKenna et al. 2007; Labandeira 2011). Recent estimates place the Lepidoptera as the second or third most speciose insect order, similar in magnitude to the Hymenoptera, and dwarfed only by the vast biodiversity of the Coleoptera (Grimaldi & Engel 2005). However, while both Hymenoptera and Coleoptera exhibit a broad range of resource utilization and feeding modalities, the Lepidoptera are overwhelmingly phytophagous. Of the described life histories, more than 90% of the known species feed and develop on plant foliar tissue (Pierce 1995). Rarely do alternative feeding strategies, such as parasitism or carnivory appear. Further, unlike today where herbivory by lepidopteran insects involves utilization of almost all plant tissues, the earliest herbivore feeding strategy in this order was mostly a result of leaf mining (Labandeira 2002; Mitter et al. 2017). The lepidopteran phylogeny implies this trend in host-plant use coupled with an increase in body size which started with internal feeding and leaf mining, transitioning to concealed external feeding, and followed finally by fully exposed folivory (Mitter et al. 2017).

1.2.1 Bottom-Up Pressure from Plants on Insect Herbivores

From what inferences can be derived upon examination of the fossil record, during the late Silurian–Middle Devonian periods detritivory and palynivory (consuming pollen) were the dominant feeding styles of the lepidoptera with herbivory being a less common strategy (Labandeira 2002). Although plant matter was abundant, accessing this resource was more difficult. This might be because compared to decaying organic matter and pollen, non-reproductive plant tissues are nutritionally poor, with high carbon to nitrogen ratios and low protein content, requiring ingestion of large amounts of plant biomass for herbivores to develop. Utilization of these abundant resources also required the evolution of specialized herbivore physiology and morphology, such as the development of unique mandibles and digestive systems (Labandeira et al. 1997).

1.2.1.1 Mechanical Plant Defenses

To utilize foliar tissue, insects must be able to break or pierce cell walls and the cuticle of plants. This selective pressure gave rise to specialized mandible morphology to surmount these mechanical plant defenses, as exemplified by comparing mandible structure between the lepidopteran families Saturniidae and Sphingidae (Bernays & Janzen 1988). Saturniids, which consume old tough leaves, have short simple mandibles conducive to cutting plant tissue, while sphingid mandibles tended to be long and toothed which make them better adapted to crushing the younger soft leaves these caterpillars eat. In addition, generalists caterpillars have similar mandibles, while specialist herbivores always have unique mandibles (Bernays & Janzen 1988). Leaf toughness, due to increased age or the presence of silica, also wears down insect mouthparts serving as a mechanical plant defense (Raupp 1985; Massey & Hartley 2009).

The other main mechanical plant defense herbivores must overcome are trichomes. These are hair-like plant structures that project out from leaves and stems, which serve the same ecological function as thorns to deter herbivory (Dalin et al. 2008). This plant defense can be systemic or induced, with herbivore damage causing an increase in the density of trichomes on the leaf surface. Trichome density affects herbivore foraging on plants, with caterpillars preferring to consume leaves with fewer trichomes (Eaton & Karban 2014). Some herbivores have subverted this plant defense though by first mowing the trichomes off the surface of the leaf before ingesting the plant tissue (Hulley 1988).

Given the high percentage of water and low nutrient content in foliar plant tissue, osmoregulation by insects is also key, so specialized alimentary tracks are necessary to feed on plants. This included the development of non-scleratized alimentary tracks, which allow the passage of less nutrient dense food, but also allowed deleterious secondary metabolites into the body cavity of lepidopteran herbivores.

1.2.1.2 Chemical Plant Defenses

Deleterious effects from phytochemicals also constrain the potential suite of plants available to herbivores. Plants contain general secondary metabolites and anti-nutritive

compounds such as tannins, phenolics, cinnamic acid, protease inhibitors, and polyphenol oxidase, with many plants also having developed neurologically toxic compounds such as alkaloids, glucosinolates, iridoid glycosides, and cardenolides (Fraenkel 1959; Bennett & Wallsgrove 1994; Wink 2003). These chemicals can be located in the reproductive, foliar, and root tissues of plants, in addition to being contained in glandular trichomes at their tip. Defensive plant compounds can be constitutive or induced after herbivore attack (Bennett & Wallsgrove 1994; Gatehouse 2002), with many studies documenting resource location and ecological costs that adversely affect plant fitness (i.e., lower seed production and foliar growth) upon deploying these chemical defenses (Baldwin 1998; Redman et al. 2001; Strauss et al. 2002; Alves et al. 2007). However, the diverse cocktail of toxic and anti-nutritive secondary metabolites that plants produce prevent rapid alimentation and slows herbivore development because herbivores must slow ingestion to prevent mortality (Steppuhn et al. 2004; Bhonwong et al. 2009), allowing the plant to survive herbivore attack.

Some lepidopteran herbivores have circumvented chemical plant defenses by specializing on only a few plant species or genera. These insects have evolved unique counter adaptions such as detoxification mechanisms and physiology to prevent mortality. Notably, specialist caterpillars in the genus *Pieris* that feed on plants in the Brassicaceae prevent the formation of toxic isothiocyanate products due to plant myrosinase hydrolysis of glucosinolates thru a nitrile-specifier protein, which promote the formation of less toxic nitriles instead (reviewed in Winde & Wittstock 2011). In another example, the solanaceous specialist *M. sexta* has a unique neural sheath compared to other insects making it less sensitive to the neurotoxic effects of nicotine on nicotinic acetylcholine receptors in the nervous system (Trimmer & Weeks 1989; Wink & Theile 2002). This plant-herbivory coevolutionary adaptation resulted in an arms race with caterpillars proliferating their detoxification mechanisms while plants increase their defensive secondary metabolites. This is most famously described by Ehrlich & Raven (1964), with current evolutionary models also supporting that this evolutionary arms race between insect herbivores and plants strongly contributes to the maintenance and expansion of phytochemical diversity (Speed et al. 2015).

1.2.2 Herbivore Specialization on Plant Genera and Families

Specializing on one plant genus or family is adaptive because it allows insects to better handle harmful plant compounds through investment in unique detoxification mechanisms that normally impair the development of generalist herbivores. This increased fitness on a few plants has come at the cost of not being able to feast on a wide array of possible plant species, which can be detrimental if the preferred plant species are rare or patchily distributed in the environment. This might impede shifts onto new host plant species due to the detoxification of novel xenobiotics, but this initial impediment and limitation of specialization could be outweighed by faster neural processing to more efficiently locate host plants (Bernays 1998a). Plant toxic secondary compounds are generally deterrents to generalist insect herbivores, but can be stimulants for specialists. For example, glucosinolates, while toxic to herbivores, also serve as feeding and oviposition stimulants for specialist *Pieris* sp. (Hopkins et al. 2009). This is also true of the buckeye butterfly, *Junonia coenia*, which uses plants containing iridoid glycosides as oviposition cues.

Overall, in the most comprehensive examination of global insect herbivore diet breath, encompassing over 7,500 insect species, Forister et al. (2015) found that 69% of lepidopteran herbivores specialize on a single host family; this includes 60% in sites at higher latitudes but increasing to 83% at locations closer to the equator. Plant species richness increases as latitude decreases and is believed to impact the degree of insect specialization in a community by allowing niche partitioning. Yet, even when accounting for this effect over the latitudinal gradient, the authors were only able to explain a fraction of the direct effect of latitude on herbivore specialization. This implies that while plant biodiversity, and by extension chemical diversity, is important for the evolution of specialization, it by no means fully accounts for it. This is because top down pressure from natural enemies and parasites also substantially contributes to variation in insect herbivore diet breath.

1.2.3 Top-Down Pressure from Natural Enemies on Insect Herbivores

“For an insect herbivore, the key problem is to eat without being eaten.” (Bernays, 1998b). The scientific literature is littered with examples of insects that purposely consume less

nutritious food plants over more nutritive ones (reviewed in Berenbaum & Feeny, 2008). While bottom up effects from plants constrain herbivore diet breadth and preferences, top down pressure from natural enemies can expand dietary utilization. Anti-predator and pathogen responses can shift insect dietary preferences, usually onto less nutritionally balanced foods to increase survival against natural enemies. The hypothesis that natural enemies influence herbivore diet breadth, which challenges resource-based competition in structuring ecological communities, was developed by Jeffries and Lawton (1984) and termed the Enemy-Free Space (EFS) hypothesis. EFS in its broadest sense describes "ways of living that reduce or eliminate a species' vulnerability to one or more species of natural enemies". The best example demonstrating EFS as a mechanism maintaining insect host plant shifts comes from Murphy (2004) who examined the Alaskan swallowtail butterfly, *Papilio machaon aliaska*, in natural habitats on its ancestral host plant, *Cnidium cnidiifolium*, and two recently colonized host plants, *Artemisia arctica* and *Petasites frigidus* in the presence of or protected from natural enemies. The author found that when predators were present caterpillar survival was higher on the recently colonized plants, but when predators were excluded both caterpillar survival and growth were greater on the ancestral host plant. Few examples, however, have documented EFS in nature. This may be due to the fact that natural enemies also exhibit distinct plant preferences because of variation in plant traits such as leaf trichomes, herbivore-induced plant volatiles (HIPVs), or host/prey quality due to sequestration of secondary metabolites.

1.2.4 Tritrophic Interactions

Only by examining insect herbivores together with the community of host plants they feed on and the natural enemies that attack them can a holistic understanding of the evolution of herbivore host plant diet breath be attained. This was the argument put forth by Price et al. (1980) in his classic review of the subject, which has served as a guide for generations of scientists researching insect ecology.

First, herbivore damage to plants induces the release of volatile organic compounds (VOCs) with natural enemies displaying differential preferences for these HIPVs. These VOCs provide long distance olfactory information aiding in host detection by natural enemies, making them an indirect plant defense via volatile signaling (Vet & Dicke 1992; Paré & Tumlinson 1999;

Thaler 1999; Van Poecke et al. 2001; Kessler & Baldwin 2001; Turlings & Wäckers 2004; Heil 2008; Turlings & Erb 2018). It has been shown that parasitoid wasps are capable of finding their specific host insect solely from the unique induced volatile bouquet emitted by damaged plants on which their host feeds (De Moraes et al. 1998), with the manual induction of these plant volatile signals also increasing parasitism of herbivores in the field (Thaler 1999). This has led many researchers to investigate the potential of artificially increasing/inducing plant VOCs in agricultural production systems for biological control of insect pests (reviewed in Turlings & Erb 2018).

Once a predator is on the plant harboring herbivorous prey, other plant defensive traits can interfere with predator efficiency directly and indirectly. The traits which have garnered the most attention are plant trichomes and sequestration of plant chemicals by insect herbivores. Chemical exudates from glandular trichomes can be directly fatal to natural enemies, such as egressing parasitoids or foraging predators (Kauffman & Kennedy 1989a; Kauffman & Kennedy 1989b), and also alter predator foraging strategies, with trichome density impeding natural enemies, slowing down foraging and acting as a deterrent (Mira & Bernays, 2002).

Plant chemicals can also be sequestered by herbivores, being stored in their body and act as a coopted direct defense against predation and parasitism. Many lepidopteran larvae in the families Nymphalidae, such as *Danaus plexippus* and *Junonia coenia*, and Papilionidae exhibit this behavior and thus are unpalatable to natural enemies (Bowers 1980; Price et al. 1980; Demain & Fang 2000; Nishida 2002). Caterpillars that feed on more toxic host plants are better defended against natural enemies, but can have impaired immune function due to resource allocation costs between detoxifying plant chemicals and immune activity (Smilanich et al. 2009; McMillan et al. 2017). Even caterpillars that do not sequester plant chemicals can be less preferred by natural enemies, thereby influencing herbivore abundance in the environment. For example *M. sexta*, a non-sequestering lepidopteran, when fed artificial diets containing nicotine had lower rates of parasitism and parasitoid survival by *C. congregata* (Gilmore, 1938b; Thurston & Fox, 1972; Thorpe & Barbosa, 1986; Barbosa et al. 1991). *M. sexta* are also able to exhale nicotine as an anti-predator defense, which in the field lowered predation by wolf spiders (Kumar et al. 2014).

1.2.5 Domestication Syndrome

Anthropomorphic selection in agriculturally important crops has destabilized many of these plant mechanisms of anti-herbivore defense, thus potentially altering tritrophic interactions. While plant breeding has improved traits such as fruit flavor and yield, some cultivated plants have lost or reduced foliar toxins (reviewed in Chen et al. 2015; Whitehead et al. 2017), making them more susceptible to insect pests. In addition, some domesticated plants have undergone changes in the amount and composition of HIPVs, especially in maize, impairing their ability to attract natural enemies (Rasmann et al. 2005; Köllner et al. 2008; Degenhardt et al. 2009; Gols et al. 2009; Gols et al. 2011; Tamiru et al. 2011; Tamiru et al. 2015; Tamiru et al. 2017). These unintended consequences due to anthropomorphic selection causing a reduction in plant resistance to herbivores are highly crop dependent (Chen et al. 2015; Whitehead et al. 2017). Drawing general trends even between related plant species can sometimes be problematic; thus, dedicated investigation in each crop system is required.

1.3 Study System

The tobacco hornworm, *M. sexta*, ranges from Canada to South America, and is distributed across the entire continental United States. This insect emerges from diapause in early summer, and has upwards of four generations per year at lower latitudes (Madden & Chamberlin 1945). *M. sexta* is a specialist herbivore on plants in the Solanaceae, or nightshades, feeding broadly on over 30 plant species (Madden & Chamberlin 1945; Yamamoto & Fraenkel 1960). Adults in natural populations have a high affinity for tomatoes and tobacco, making *M. sexta* a common pest in home gardens and agricultural fields (Ashmead 1887; Madden & Chamberlin 1945).

The nightshades include many agriculturally important species besides tomatoes (*Solanum lycopersicum*) and tobacco (*Nicotiana tabacum*) such as peppers (*Capsicum annuum*), potatoes (*Solanum tuberosum*), and eggplant (*Solanum melongena*) along with numerous weeds like horsenettle (*Solanum carolinense*), jimson-weed (*Datura stramonium*) and bittersweet nightshade (*Solanum dulcamara*). Besides containing general secondary metabolites (e.g., cinnamic acid, protease inhibitors, and polyphenol oxidase), members of this plant family vary in quantity and types of specific secondary metabolites, mainly the alkaloids (Eich 2008 and references therein), which are used for protection against pathogens and herbivores (Fraenkel 1959; Bennett & Wallsgrove 1994; Wink 2003). Of the more than 12,000 alkaloids known, the most significant for herbivore defense in the Solanaceae appear to be the ornithine-derived alkaloids, specifically tropanes and nicotinoids (Shonle & Bergelson 2000; Steppuhn et al. 2004; Eich 2008), along with terpenoids, mainly the steroidal alkaloids/glycoalkaloids (Weissenberg et al. 1998; Tingey 1984; Eich 2008).

Three common arthropod natural enemies of *M. sexta* readily found in agroecosystems are the spined stilt bug, *Jalysus wickhami* (Hemiptera: Berytidae), the spined soldier bug, *Podisus maculiventris* (Hemiptera: Pentatomidae), and the parasitoid wasp, *C. congregata* (Hymenoptera: Braconidae). Stilt bugs rapidly colonize trichome-rich solanaceous plants where they have been observed consuming hornworm eggs and aphids (Katanyukul & Thurston 1973; Jackson & Kester 1996; Kester & Jackson 1996), but the ecology and plant preferences of this

group, along with their impact on hornworm population dynamics are under studied. Historically, this insect was reared as a biological control agent for management of hornworms in tobacco fields (K. Kester, *personal communication*). Soldier bugs likewise are another omnivorous piercing-sucking hemipteran commercially available for augmentative biological control of a variety of soft bodied insect pests (McPherson 1980). Thus, given their promise in managed cropping systems this bug's chemical ecology and life history are more resolved compared to that of stilt bugs. The male produced pheromone of *P. maculiventris* contains a mixture of five compounds: (*E*)-2-hexenal, α-terpineol, linalool, terpinen-4-ol, and benzyl alcohol (Aldrich et al. 1984). This insect predator also dislikes foraging on plants containing glandular trichomes, such as many solanaceous species (M. Garvey, *personal observation*). The final natural enemy, and perhaps the main nemesis of *M. sexta*, is *C. congregata*, a gregarious parasitoid wasp that is capable of ovipositing >100 eggs into a caterpillar, and has been reported to specialize on 12 sphingid species (Gilmore 1938a, b; Fulton 1940; Lawson 1959). The incidence of *C. congregata* infection in tobacco hornworms is low early in the season but increases later in the summer, and it has been estimated that *C. congregata* may infect upwards of 30% of the population (Lawson 1959). This wasp, however, appears poorly synchronized with the emergence of *M. sexta*, emerging earlier in the season than this host, but may parasitize alternative host species during this time (Gilmore 1938a, b).

In order for *C. congregata* to complete its development within the body of *M. sexta* this wasp has evolved novel mechanisms to subvert and suppress the host immune response (Strand & Pech 1995; Schmidt et al. 2000; Schmidt et al. 2001; Beckage 2008; Kraaijeveld & Godfray 2009; Beckage & Drezen 2011). All wasps in the Braconidae are unique in that they have symbiotic viruses, called polydnaviruses (PDV), incorporated into their genomes (Fleming & Summers 1991; Fleming 1992; Whitfield 1997), which are injected into host caterpillars along with wasp eggs and venom proteins during oviposition (de Buron & Beckage 1992; Beckage 1998; Beckage 2008). These viruses cause immune suppression which allows for the initial survival of the wasp larvae in the caterpillar's body (Edson et al. 1981; Lavine & Beckage 1996; Beckage 1998; Amaya et al. 2005; Beckage 2008; Strand 2012).

The molecular, biochemical, and cellular aspects of the immune system of *M. sexta* during the juvenile stage are well described (Kanost et al. 2004; Zou et al. 2008; Jiang et al. 2010), and of these the most important to defense against parasitoids appears to be melanogenesis and the encapsulation response (Carton et al. 2008; Strand 2008). However, PDVs disrupt the host immune response by: 1) hemocyte apoptosis; 2) deregulation of encapsulation by preventing actin filament formation, resulting in the inability of hemocytes to join and properly spread over foreign objects; and 3) suppression of the serine protease cascade leading to the activation of prophenoloxidase to phenoloxidase, the enzyme that catalyzes the formation of melanin (Edson et al. 1981; Lavine & Beckage 1996; Beckage 1998; Jiang et al. 1998; Amaya et al. 2005; Thoetkiattikul et al. 2005; Beckage 2008; Strand 2012).

Although *C. congregata* has coevolved closely with *M. sexta*, the wasp is not always successful in infecting this host. Caterpillar diet affects parasitism success, with lower parasitoid emergence occurring in hornworms feeding on tobacco compared to tomato plants (Thorpe & Barbosa 1986; Kennedy et al. 1994). Gilmore (1938b) first noted this in field populations of *M. sexta* feeding on dark-fired tobacco, where *C. congregata* appeared absent, and speculated that the high levels of nicotine in the plants prevented parasitism. Later work by Thurston and Fox (1972), Thorpe and Barbosa (1986), and Barbosa et al. (1991) confirmed this empirically, showing that increasing levels of dietary nicotine lowered wasp survival and emergence.

1.4 Scientific Aims

1.4.1 Research Objectives

The **first** step of my dissertation work will be to test whether host plant quality influences moth ovipositional preference and parasite resistance (chapter 2). **Second**, I will quantify corresponding differences in immune investment of caterpillars on a broad range of food plants to determine if there are plant-mediated effects on herbivore physiology that alter herbivore-enemy interactions, and then will explore the possibility of plasticity in the immune response of larvae induced by the direct threat of natural enemy pressure (chapter 3). **Third**, I will explore how anthropomorphic selection through plant domestication has changed insect-enemy interactions (chapter 4).

1.4.2 Intellectual Merit of Work

To the best of my knowledge, such a global analysis of insect immune activity and susceptibility on numerous host plants relative to natural enemy pressure has never been conducted, making this experiment a novel endeavor. Further, by combining ecology with immunology, I will improve our understanding of how phenotypic plasticity in immune defenses contributes to host-parasite interactions. Lastly, I will address whether domestication in *C. annuum*, a globally important agricultural crop, has had deleterious consequences on insect pest performance and natural enemy recruitment such as described for the tomato *S. lycopersicum*.

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CHAPTER 2. TRITROPHIC INTERACTIONS MEDIATE A NEGATIVE PREFERENCE-PERFORMANCE RELATIONSHIP IN HORNWORMS

2.1 Introduction

Mother always knows best. This paradigm has permeated studies on plant-insect interactions for the better part of a half century. Formally known as the naïve adaptationist hypothesis, or the preference-performance hypothesis, it postulates that adult female insects should prefer to oviposit on host-plants that promote survival and growth of their young (Berenbaum & Feeny 2008; Gripenberg et al. 2010). Indeed, a meta-analysis of published data showed a positive correlation between plant preference and physiological performance of offspring, particularly for oligophagous herbivores in the Lepidoptera (Gripenberg et al. 2010). Under field conditions, however, researchers sometimes struggle to correlate larval performance with female oviposition choice (Rausher 1979; Underwood 1994; Berenbaum & Feeny 2008). Ecological factors such as top-down pressure from natural enemies can obscure the relationship, leading to alternative patterns of host use. This has led to more recent studies employing a tritrophic framework for understanding the role of enemy-free space in plant selection (Singer & Stireman 2003; Murphy 2004; Singer & Stireman 2005; Lefèvre et al. 2010; Murphy et al. 2014). The approach explicitly accounts for the likelihood that predators and parasitoids also show distinct plant preferences from interspecific variation in traits such as leaf surface architecture (e.g., trichomes), herbivore-induced volatiles, or host/prey quality due to sequestration of secondary metabolites (Price et al. 1980; Kaplan et al. 2016).

Here, I investigated the preference-performance relationship and tritrophic interactions involving *Manduca sexta* (Lepidoptera: Sphingidae), their natural enemies, and plants in the Solanaceae. Despite being among the most highly studied model insects in the world, the natural history and preferences of *M. sexta* across their preferred plant family, surprisingly, are poorly documented. Much of the existing knowledge comes from scattered records from applied research on their management as tobacco pests from the early- to mid-1900s or comparison of

two plants—tomato vs. tobacco (e.g., Kester & Barbosa 1994). Although the ecology of *M. sexta* and their enemies is also well-known from interactions with *Nicotiana* and *Datura* sp. in western U.S. deserts (e.g., Kessler & Baldwin 2001; Schuman et al. 2012, 2013; Wilson & Woods 2015), these are low diversity systems focusing primarily on a single plant species, often *N. attenuata*.

Commonly known as the tobacco hornworm or Carolina sphinx, *M. sexta* is a new world species that ranges widely from Canada to South America and is distributed across the entire continental US. This insect is believed to have originated in the Caribbean or Central America and subsequently colonized North and South America, forming two distinct lineages (Kawahara et al. 2013). Hornworms are considered oligophagous, broadly specializing on plants in the Solanaceae and capable of feeding on >30 species (Madden & Chamberlin 1945; Yamamoto & Fraenkel 1960); however, individuals have also been recently documented on devil's claw (*Proboscidea* sp., Martyniaceae), representing a possible host range expansion driven by escape from parasitism (Mechaber & Hildebrand 2000; Mira & Bernays 2002; Diamond & Kingsolver 2010). Because the nightshades include many agriculturally important crops (e.g., tomato, tobacco, pepper, potato, eggplant) and larvae are voracious folivores, hornworms are a common pest in home gardens and agricultural fields (Ashmead 1887; Madden & Chamberlin 1945).

Hornworms are heavily attacked by a wide diversity of predators and parasites that, collectively, reduce survival by >95% between the egg and pupal stages (Lawson 1959; Katanyukul & Thurston 1979). The relative importance of these natural enemies vary across development; for example, birds (Stewart 1969; Thurston & Prachuabmoh 1971) and *Polistes* wasps (Rabb & Lawson 1957) can be important predators of late-instar larvae, whereas tachinid flies parasitize pupae (DeLoach & Rabb 1971, 1972). In this research, I focus on two of the most commonly observed natural enemies of early-to-mid instar hornworm larvae: the spined stilt bug, *Jalysus wickhami* (Hemiptera: Berytidae) and the parasitoid wasp, *Cotesia congregata* (Hymenoptera: Braconidae). Stilt bugs rapidly colonize trichome-rich solanaceous plants where they have been observed consuming hornworm eggs and aphids (Katanyukul & Thurston 1973; Jackson & Kester 1996; Kester & Jackson 1996), but the ecology and plant preferences of this group, along with their impact on hornworm population dynamics, are relatively unknown. Historically, this insect was reared as a biological control agent for management of hornworms

in tobacco fields (K. Kester, *personal communication*). The wasp *C. congregata* is a gregarious parasitoid that prefers ovipositing in 2nd and 3rd larval instars of their host and has been reared from as many as 12 sphingid species (Gilmore 1938a, 1938b; Fulton 1940; Lawson 1959); although recent genetic analyses indicate that these may be separate host races segregated by plant species (Kester et al. 2015).

Plant-mediated differences in hornworm parasitism by *C. congregata* was previously noted in several studies. Originally, Gilmore (1938b) speculated that the high levels of nicotine in tobacco protected them from parasitism after noting the absence of *C. congregata* in field-collected *M. sexta* feeding on dark-fired tobacco. Subsequent experiments confirmed the lower parasitoid emergence from hornworms feeding on tobacco compared to tomato (Thorpe & Barbosa 1986; Kennedy et al. 1994), and that targeted titration of dietary nicotine into artificial diets lowers wasp survival and emergence from parasitized larvae (Thurston & Fox 1972; Thorpe & Barbosa 1986; Barbosa et al. 1991). Other studies similarly found reduced egg and larval parasitism on tobacco compared with alternative host-plants such as jimsonweed, but due to glandular trichromes that interfere with parasitoid foraging (Rabb & Bradley 1968; Katanyukul & Thurston 1973, 1979); and recent work shows that the disruptive effect of tobacco glandular trichomes on *C. congregata* is at least partly responsible for its diet breadth expansion to devil's claw (Mira & Bernays 2002; Diamond & Kingsolver 2010). Indeed, *C. congregata* are susceptible to being caught in the leaf glandular trichomes of *Nicotiana* sp. (M. Garvey, *personal observation*).

To determine if adult food plant preference correlated with larval performance, given variation in natural enemy pressure, I evaluated patterns of host plant use of *M. sexta* in a common garden containing plants across the Solanaceae. Then, through targeted laboratory manipulations, I tested the degree to which natural enemy pressure explained these observed field patterns. I predicted that caterpillar resistance to natural enemies would increase when reared on more toxic host plants, but individuals would have reduced growth compared to caterpillars reared on less toxic plants. Based on previous studies (Thorpe & Barbosa 1986; Thurston & Fox 1972), I expected that parasitism of hornworms feeding on *Nicotiana* will be lower compared to other plants across the family due to the presence of nicotine, and higher

nicotine content among plants in the *Nicotiana* will also result in a reduction in wasp performance (Barbosa et al. 1991). Finally, my experiments were designed to tease apart wasp attraction to herbivore-induced plant volatiles vs. plant-mediated host toxicity as two potential mechanisms explaining variation in parasitism across host plants.

2.2 Materials and Methods

2.2.1 Laboratory Insect Colonies

2.2.1.1 *Manduca sexta*

An outbred colony of *M. sexta* was established to avoid problems associated with inbred laboratory colonies, first by using individuals originally provided by Dr. Robert Raguso (Cornell University; origin and past rearing information provided in Ojeda-Avila et al. 2003); and then I founded a separate colony from field caught individuals in 2013 collected from tomatoes in the local area (Lafayette, IN) and kept in captivity for six generations before hybridizing with the original strain (wild ♀ x laboratory ♂). The laboratory rearing regime is similar to other hornworm colonies. Briefly, eggs were collected from plants and allowed to hatch communally onto a commercially available wheat germ based diet (Southland Products Inc., Lake Village, AR). Once neonates were 1-3 days old they were isolated individually in 44 mL plastic cups. Upon molting to the fourth instar, caterpillars were transferred to larger 96 mL cups. Caterpillars were provided artificial diet *ad libitum* throughout their larval stage. Larvae were reared in a scientific incubator (Percival Scientific, Perry, IA) on a 16:8 LD cycle at 25˚C and 25% RH. Once caterpillars became pharate and began their wandering stage, they were transferred first to a pupation block and then to a climate and light controlled incubator (VWR, Radnor, PA) on a 0:24 LD cycle at 25˚C and 25% RH. After adults eclosed, the moths were transferred to a 61 cm^3 collapsible field cage (BioQuip Products, Rancho Dominguez, CA) kept in an insect rearing room on a 16:8 LD cycle at 25˚C. These conditions facilitated mating. Moths were provided a 20%:10% sucrose:honey solution (w/v) and a solanaceous plant for oviposition.

2.2.1.2 *Cotesia congregata*

The laboratory wasp colony was initially derived in 2012 from individuals provided by Dr. Karen Kester (Virginia Commonwealth University; origin information contained in Bredlau et al. 2013) and supplemented every field season from the local population by collecting parasitized *M. sexta* larvae with egressed wasp cocoons on their cuticle. To maintain the parasitoid stock, wasps were presented with third to fourth instar caterpillars and watched until they were stung. Parasitized *M. sexta* were then reared as described above until wasp larvae

egressed from the caterpillar's body (~two to three weeks). Following cocoon spinning by the wasps, cocoons were gently harvested from the hornworm body and placed in a 30.5 cm^3 mesh rearing cage (BioQuip Products, Rancho Dominguez, CA). Adult wasps were provided with cotton macerated in DI water and a 20%:10% sucrose:honey solution (w/v), and maintained in a scientific growth chamber (Percival Scientific, Perry, IA) on a 16:8 LD cycle at 25˚C and 65% RH.

2.2.2 Field Experiment

2.2.2.1 Plants and Experimental Design

A common garden was established near the Purdue University campus at the Meigs Horticultural Farm (9101 S 100 E, Lafayette, IN) over the summers of 2014 and 2015 to examine *M. sexta* preference among plant species in the Solanaceae using a randomized complete block design, with 3 blocks per year. Eighteen plant species with three replicates were transplanted into each block (see Table 2.1 for a list of plant species and locality information). Plants within blocks were spaced between each other using the growing recommendations for the largest cultivated plant, tobacco, at 0.91 meters, and 1.82 meters between blocks. These food plants were chosen because of their natural occurrence in the geographic area, agricultural importance, and/or documented preference by *M. sexta* in the literature (Madden & Chamberlin 1945; Yamamoto & Fraenkel 1960). All seeds, except *Nicotiana* sp., were treated with a 20% bleach (NaOCl) solution for 10 minutes and then left to dry before planting to increase germination success. In addition, the seed coats of all *Datura* sp. were mechanically nicked with a scalpel after drying to increase their germination rates. Plants were sowed in April of each year under greenhouse conditions supplemented with artificial light on a 16:8 LD cycle. For the first week seeds were collectively planted by species in 10 cm pots (International Greenhouse Company, Danville, IL) in metro germination mix (Sun Gro Horticulture, Agawam, MA) and placed under a humidity dome (International Greenhouse Company, Danville, IL) for one week with an initial dose of Osmocote 14-14-14 slow release fertilizer (The Scotts Miracle-Gro Company, Marysville, Ohio) at a ratio of 30 mL:1 Pot. In 2014, seeds that successfully germinated were thinned and individually separated into cell packs (5.97 x 3.94 x 5.92 cm traditional inserts for standard 1020 flats; International Greenhouse Company, Danville, IL) and allowed to grow

under greenhouse conditions supplemented with artificial light on a 16:8 LD cycle until they were transplanted into the field six weeks later. Plants for 2015 were grown similarly except thinned and transplanted into 10 cm pots (International Greenhouse Company, Danville, IL) before being transplanted into the field after four weeks. Transplanted plants were supplemented with the same dose of fertilizer as when they were sowed.

For both years, plants were transplanted into the field the first week of June to acclimate, establish, and grow. Locally, tobacco hornworms typically appear in July with two broods per year (Ingwell et al. 2017). I began scouting plants once a week beginning the first week of July and continued for 13 subsequent weeks each year. All plants were visually examined for a maximum of 2 minutes every scouting session over the entirety of their foliar growth. The number of hornworm eggs and larvae by instar was recorded at every scouting event. It is extremely likely that the lepidopteran eggs and larvae were *M. sexta* and not the closely related species *M. quinquemaculata* (the tomato hornworm), which has rarely been observed in our area (Ingwell et al. 2017). In addition to tracking the abundance of hornworms, *J. wickhami* and parasitized hornworm larvae with *C. congregata*, as denoted by egressed parasitoid larvae that had spun cocoons on their cuticle, were counted and recorded. Both of these insects are known natural enemies of hornworms.

2.2.2.2 Herbivore Performance

Larval growth rate was taken as a proxy to measure hornworm performance across the 18 plant species by taking *M. sexta* larvae from the laboratory colony that were less than 24 hours old and bagging them on a single fully expanded leaf of plants in the field using mesh netting material. This was done August 1-3 in 2015, with three replicates for each plant species (one per block). Larvae were allowed to consume plant tissue for 7 days before being recaptured to measure their mass (Mettler Toledo, Greifensee, Switzerland). Variation in starting weights was assumed to be negligible.

2.2.3 Laboratory Experiments

2.2.3.1 Moth Oviposition Trials

To validate field preferences and control for inherent variation in plant size and flowering (i.e., adult moths may be more likely to place eggs on larger plants and/or ones on which they nectar), adult *M. sexta* oviposition preference was examined in the laboratory using two plant species (*N. tabacum* and *D. wrightii*) that were frequently colonized by hornworms in the field and two other plant species (*S. dulcamara* and *C. annuum*) that rarely contained hornworms. Plants were grown as previously described except under artificial light until they were 3 months old at which point they were used in experiments. Moths used in laboratory oviposition trials were taken from the colony and isolated 24 hours post-eclosion in male/female pairs of two or more for 72 hours to facilitate mating and to control for age. Females were then exposed to the four different plant species the following evening in a 1 m^3 mesh cage and allowed to oviposit (BioQuip Products, Rancho Dominguez, CA). To standardize plant biomass, 2.5 – 3.0 grams of tissue from full leaf cuttings at the stem were taken from plants and placed into water picks the same day moth choice was assayed. Moths were provided a 20%:10% sucrose:honey solution (w/v) to nectar at in all cages. The following morning, the number of eggs placed on each plant species was recorded. Only trials containing females that laid >20 total eggs across all plant treatments were used for statistical analysis (n = 16).

2.2.3.2 Wasp Olfactory Trials

Wasp host-plant preference was also examined in the laboratory to control for hornworm abundance/presence across the different plant species used in the field experiment. In other words, wasps may just forage more intensely on plants containing more hosts, which would not be a ‘true’ plant preference. This was conducted using a y-tube olfactometer bioassay to isolate the role of plant volatiles. Wasps were presented with either plants that had received hornworm feeding damage for 24 hours or undamaged plants within a species. The same four plant species were examined for wasp preference as in the previous moth oviposition experiment. The first fully expanded leaf of each plant species (cultivated as described above) was infested with *M. sexta* larvae for 24 hours and controlled for feeding damage across plant species by manipulating the number of caterpillar individuals (1 hornworm on *C. annuum* and *S. dulcamara*, 2 to 3

hornworms on *D. wrightii* and *N. tabacum*) on a plant leaf to produce 50% leaf area removal. Plant pots and soil were then wrapped in aluminum foil before being placed into glass bell jars to prevent the release of soil and plastic volatiles from the pot. Using a positive pressure system, clean air at a rate of 1 m per minute was pumped through the glass jars before entering the y-tube at a speed of ~0.5 m/s. Female wasps were isolated from the laboratory colony at least ten minutes before the bioassay in 30 mL plastic cups. The glass y-tube was elevated at an incline of 25˚ above vertical with an energy efficient full spectrum daylight bulb placed above to encourage movement of the parasitoids. Wasps were gently placed inside the y-tube with a paint brush and their treatment choice was recorded after the wasps passed the y juncture and remained for a minimum of 10 seconds in one of the glass arms. Wasps were given a total of five minutes to choose a treatment before being removed and recorded as not responding. The glass y-tube and bell jars were washed with Alconox detergent (Alconox Inc., White Plains, NY) and dried between new plant species assays, and the tygon tubing (Saint-Gobain, Courbevoie, France) leading from the bell jars to the y-tube was replaced every 30 replications. All of these precautions were taken to prevent plant volatiles that may be adhering to the plastic and glass from influencing wasp choice. Six sets of each plant species were used during these y-tube olfactory choice tests, with a minimum of 70 wasps assayed per plant treatment (i.e., at least 12 wasps per each plant set).

2.2.3.3 Wasp Performance Across Plants

To determine potential plant-mediated caterpillar toxicity, wasp performance was investigated on hosts reared from three of the plants from the common garden experiment: *N. tabacum* cv. Keller (smoking tobacco), *N. alata* cv. Lime Green (winged tobacco), *N. sylvestris* (flowering tobacco), and *S. lycopersicum* cv. Better Boy (tomato). These species were chosen either because of high wasp abundance in the field (tomato and smoking tobacco) or lack of realized parasitism on hosts, but low/high documented nicotine levels (for winged tobacco and flowering tobacco, respectively) (Saitoh et al. 1985). Plants were germinated as previously described in the common garden experiment and then grown under greenhouse conditions (~27 ˚C ± 5; 16:8 LD) for 12 weeks before use in experiments. Ten *M. sexta* neonates (< 24 hours old) were placed on the first fully expanded leaf of each plant individual (4 plant individuals were used per plant species, resulting in 40 caterpillars per treatment), and allowed to roam freely on

their host plant. Each plant individual was placed in a 34.3 x 33.3 x 61 cm rearing cage (Bioquip, Rancho Dominguez, CA) to contain caterpillars and then randomly distributed in the greenhouse (16 cages total, 4 per plant treatment). Caterpillars were weighed (Mettler Toledo, Greifensee, Switzerland) after eight days to measure their performance on plants and then returned to their respective plants to continue feeding and growing. After the caterpillars reached their 4th instar they were parasitized by *C. congregata* using individuals from the laboratory colony. Parasitization was achieved by presenting caterpillars to wasps in a 197 cm^3 rearing cage (Bioquip, Rancho Dominguez, CA) and allowing two unique females to sting caterpillars for no more than 5 seconds sequentially to ensure parasitism. Caterpillars were then returned to their assigned treatment plant until death or wasp larvae egressed out of their bodies upon which wasp cocoons were collected. Wasps were held in 96 mL cups until emergence from cocoons after which they were provided a 20%:10% sucrose:honey solution (w/v) until they died. Wasps were frozen to preserve them until they were sexed and weighed in subsets of five by plant species (Mettler Toledo, Greifensee, Switzerland). The length of hind tibias were also measured using a Lecia M125 dissection microscope with Leica Photogrammetry Suite 8.7 imaging software in subsets of five calibrated to 0.1mm.

2.2.4 Statistical Analyses

All statistical analyses were conducted using the open source R statistical software 3.3.3. Generalized linear mixed effects models (GLMM) using the glmmADMB package were conducted on *M. sexta* and *J. wickhami* cumulative population densities across plants using a zero-inflated poisson distribution. For *C. congregata* a binomial logistic regression was applied to the data to determine presence/absence of parasitoids across plant genera. Pairwise multiple comparisons between genera were then conducted using least square means in the lsmeans package with the Tukey Method *p* value adjustment. For herbivore performance, an analysis of variance was performed across plants, with species nested in genus using the anova function in the car package specifying the calculation with type two sum of squares. Mass was log transformed to meet assumptions of normality. *A priori* contrasts using least square means in the lsmeans package with the Tukey Method *p* value adjustment were then run to compare differences in herbivore mass across genera. Two linear regressions using the lm function were also conducted; the first on average hornworm physiological performance by average total

hornworm abundance across plant species in 2014 and 2015, and the second on the average incidence of parasitized hornworms in 2015 with hornworm abundance in 2015.

For laboratory preference bioassays of *M. sexta* adults, two analyses were conducted on the proportion of eggs laid on plants per female moth. First, the nonparametric Kruskal–Wallis H-test followed by a multiple comparison test between plant species using the kruskalmc function in the pgirmess R package to establish hierarchical rankings among tested plants. Then, a Bayesian preference model was built to corroborate these findings (Fordyce et al. 2011). Plant preference in this model was unconstrained (e.g., there was no underlining assumption that herbivores would prefer all plant species equally). This Bayesian approach is preferred since it is robust for small replication sizes and returns plant preference percentages in pairwise comparisons among food plants assayed (e.g., plant 1 is preferred 94% of the time over plant 2). Wasp preference in olfactory trials between herbivore-induced and control plants within a species was analyzed with a one-sided binomial test to determine preference. For the wasp performance assays, an initial survival analysis was conducted across plants using a binomial logistic regression with pairwise contrasts using least square means with the Tukey Method p value adjustment. Wasp clutch size by plant species was analyzed with a one-way ANOVA using the aov function. Wasp mass was analyzed with a two-way ANOVA to account for sex and plant-mediated effects using the aov function. Likewise, wasp tibia length was analyzed with a two-way ANOVA to account for sex and plant-mediated effects with *a priori* contrasts using least square means in the lsmeans package with the Tukey Method *p* value adjustment; then, conducted to compare differences in wasp tibia length by plant species within a wasp sex.

2.3 Results

2.3.1 Common Garden Field Experiment

The combined natural enemy data for stilt bugs and parasitoids of *M. sexta* for the scouting years of 2014 and 2015 are contained in Table 2.2. Plant genus strongly influenced hornworm abundance, with *Nicotiana*, *Solanum*, and *Datura*, being highly preferred while *Capsicum* and *Physalis* were not (Table 2.3). *Datura* and *Nicotiana* grouped together and had the highest abundance of hornworms. Both of these genera were statistically different from plants in the *Solanum*, which had the second highest abundance of hornworms, while plants in *Capsicum* and *Physalis* had the fewest hornworms and statistically grouped together. *Capsicum* and *Physalis* also were statistically different from the other three genera (Table 2.4 & Figure 2.1).

Stilt bug abundance was also strongly influenced by plant genus (Table 2.5 & Figure 2.2), with the highest numbers being overwhelmingly found on *Nicotiana*, followed by *Datura*, and fewer on *Solanum* with all three genera being statistically distinct from one another (Tables 2.2, 2.5, & 2.6). *Capsicum* and *Physalis* did not differ in stilt bug numbers (Table 2.6).

Incidence of parasitized caterpillars varied across genera with *Datura* being statistically different from zero and *Nicotiana* trending (Table 2.7 & Figure 2.4). *Datura* was not statistically different from *Nicotiana* or *Solanum*, but was different from *Capsicum* and *Physalis* (Table 2.8). The incidence of parasitized hornworms tracked hornworm abundance ($F_{1,16} = 7.42$, $p = 0.015$, $R^2 = 0.317$; Figure 2.4) with two notable exceptions that parasitized caterpillars were never recorded from *N. alata* or *N. sylvestris*, which were commonly colonized by hornworms (Table 2.2).

2.3.2 Hornworm Performance

Neonates had an initial mass of 2.28 mg (S.E.: ± 0.06) at <24 hours before being placed on plants in the field. Seven day mass was significantly affected by genus and plant (Table 2.9 & Figure 2.5). Caterpillars performed best on *Solanum*, followed by *Capsicum*, *Physalis*, *Datura* and finally *Nicotiana* (Table 2.10 & Figure 2.5). Of most interest is that there is a trend for these

measures of hornworm physiological performance to be inversely related to hornworm abundance ($F_{1,16} = 3.33$, $p = 0.087$, $R^2 = 0.172$; Figure 2.6).

2.3.3 Laboratory Oviposition Preference

Plant species significantly affected moth oviposition (χ^2 (*d.f.* = 3, $n = 16$) = 19.94, $p < 0.001$), with *N. tabacum* and *D. wright* receiving the highest proportion of eggs (0.353 ± 0.029 and 0.277 ± 0.033, respectively) and *S. dulcamara* and *C. annum* (0.204 ± 0.021 and 0.144 ± 0.036, respectively) the fewest (Table 2.11 & Figure 2.7). The Bayesian model returned similar estimates of hawkmoth oviposition preference with the proportion of eggs placed on each plant being: *N. tabacum*, 0.365; *D. wrightii*, 0.288; *S. dulcamara*, 0.231; *C. annuum*, 0.121 (Figure 2.8). In pairwise comparisons of plants based on this model *N. tabacum* was highly preferred over all other plants (> 0.94), followed by *D. wrightii* and then *S. dulcamara* and *C. annuum* (Table 2.12).

2.3.4 Wasp Preference

Female wasps preferentially chose *N. tabacum* plants damaged with insect herbivory over undamaged plants in y-tube olfactometery choice tests (*d.f.* = 1, $x = 44$, $n = 71$, $p = 0.028$; Figure 2.9). Herbivory did not affect wasp preference in any of the other tested plants (Figure 2.9). This approximately mirrors the adult hawkmoth oviposition preference rankings among these assayed food plants (Table 2.11 & Figure 2.7).

2.3.5 Wasp Performance

Parasitoid success (percent of hosts with wasp emergence) greatly varied by plant species: *S. lycopersicum*, 88.24% (15/17); *N. alata*, 19.23% (5/26); *N. tabacum*, 34.62% (9/26); *N. sylvestris*, 0% (0/20) (Table 2.13). Hornworms reared on *S. lycopersicum* produced the most wasp clutches and were statistically different from the *Nicotiana* species (Table 2.13 & 2.14, Figure 2.10). Although *N. tabacum* was also statistically different from zero it was not different from the other *Nicotiana* species, which produced low to no clutches (Table 2.13). Of the wasps that did egress from caterpillars, plant species did not significantly impact wasp clutch size ($F_{2,26} = 1.87$, $p = 0.175$) or tibia length ($F_{2,205} = 1.15$, $p = 0.320$) but did affect dry mass ($F_{2,205} = 4.86$, p

= 0.009; Figures 2.11, 2.12, & 2.13). Male wasps reared from hornworms on *S. lycopersicum* had longer tibias than conspecifics from *N. alata* (Figure 2.13). The female to male wasp sex ratio by plant species (± SE) from the wasp performance assay was: *N. alata*, 0.618 ± 0.177; *N. tabacum*, 1.612 ± 0.950; *N. sylvestris*, N/A; *S. lycopersicum*, 0.758 ± 0.208.

2.4 Discussion

Hawkmoths overwhelmingly preferred plants in *Nicotiana* and *Datura* compared to *Capsicum*, *Physalis*, and *Solanum*. Individual plant species largely mirrored these genus trends, except for the tomatoes, *S. lycopersicum* and *S. pimpinellifolium*, which were as preferred as many *Nicotiana* and *Datura* species. Further, the laboratory preference rankings among plants paralleled field observations, illustrating that oviposition preference is not affected by plant size. Overall, the most preferred plants for adult female oviposition were inversely correlated with plant species that provided the best growth for natal offspring (Figure 2.6). My results imply that this negative preference-performance relationship is maintained in part because by utilizing these noxious food plants *M. sexta* garners protection against natural enemies in the environment, specifically parasitoids. Even though the proportion of parasitism between tobacco and tomatoes in the field was not significantly different, and hornworm preference correlated with parasitized hornworm abundance, laboratory rearing of hornworms parasitized with *C. congregata* showed wasp survival to be significantly higher on *S. lycopersicum* than *Nicotiana* sp. Field observations of parasitism may not truly represent the whole interaction between enemy and prey because the parasitism failure rate on hornworm caterpillars in the field was not capable of being observed. More hornworms on tobacco might be parasitized than appear but wasps fail to establish in these individuals due to the toxic effects of nicotine (Barbosa et al. 1991). Molecular analysis measuring polydnavirus gene expression has been used to track parasitoid outbreaks in lepidopteran pests and could provide a more accurate measure of the true parasitoid burden in field patches (Traugott et al. 2006; Gariepy et al. 2007). Further, in laboratory olfactometer assays with *C. congregata*, naïve wasps showed only a preference for *N. tabacum* plants damaged with *M. sexta*, although my field data imply a preference for *D. wrightii* as well. Preference for *N. tabacum* might be innate while other plant preferences could be learned which might be why the laboratory and field results conflict since *C. congregata* exhibits pre/post learning to orient to herbivore hosts in the field (Kester and Barbosa 1991). Our wasp colony was founded from a population collected from *N. tabacum*, where these wasps are believed to have undergone cryptic speciation, with a change in behavioral preference in addition to physiological resistance to nicotine toxicity (Kester et al 2015).

The toxic effects of nicotine on parasitoid success in *M. sexta* are well documented (Gilmore, 1938b; Thurston & Fox, 1972; Thorpe & Barbosa, 1986; Barbosa et al. 1991). Interestingly though, *N. alata*, the tobacco species with the lowest nicotine levels of any member in the genus (Saitoh et al., 1985), had no observed parasitized hornworms. Whether this plant is unattractive to wasps or if defensive secondary metabolites like nicotine are highly inducible upon herbivore damage will need subsequent investigation, but given that in laboratory parasitism trials survivorship of *C. congregata* on *M. sexta* reared from *N. alata* was similar to that of hornworms reared from *N. tabacum* I believe the latter prediction to be more likely. I also could not replicate other studies documenting an effect of nicotine on parasitoid fitness per say – of the parasitized hornworms where wasps successfully eclosed to adults, total clutch size and mass among wasps reared on *S. lycopersicum* and *Nicotiana* sp. were not different, but there was a sex-dependent effect of nicotine on wasp tibia length for male wasps but not females.

It should be noted that the most abundant natural enemy observed in the common garden against *M. sexta*, *J. wickhami*, which was historically raised as a biological control agent for this insect pest (K. Kester, *personal communication*), seems poorly suited to suppressing *M. sexta* populations. The highest stilt bug densities were found co-occurring with some of the highest *M. sexta* densities on *Nicotiana* sp., but *M. sexta* densities were the same on other Solanaceous plants with little to no observed stilt bugs (i.e., *N. tabacum* vs *S. lycopersicum*). *Jalysus wickhami* seem to be opportunistically carnivorous, preferring to feed on plant tissues and pollen and slightly supplement their diet with animal protein (Jackson & Kester 1996).

Other work documenting negative preference-performance relationships in the Lepidoptera (reviewed in Berenbaum & Feeny, 2008) have predominantly been able to explain this result as a means for defense and protection against enemies in the environment. However, another reason for why this might occur is because of a plant-pollinator mutualism whereby females prefer ovipositing on plants that they nectar (Smith et al 2018); namely, tobacco and *Datura* may be preferred because these plants have flowers with long tubular corollas that attract hawkmoths. Although *M. sexta* does prefer to oviposit on plants from which they nectar and are only capable of nectaring at plants in *Nicotiana* and *Datura* in my common garden (Reisenman et al 2010), this cannot explain their preference for tomatoes since these flowers produce no

available nectar. Also, in the southwestern United States, hawkmoths also nectar at *Agave palmeri*, which provides a superb energy resource for flight, until flowers of *D. wrightii* bloom even though the nectar quality from *D. wrightii* per flower is far inferior to *A. palmeri* (Riffell et al. 2008; Alarcón et al. 2010).

To conclude, my work coincides with others findings documenting a negative relationship between oviposition preference and physiological performance in the Lepidoptera as a means for defense and protection against natural enemies in the environment, but there are still unresolved questions, such as how this association began. Laboratory reared hawkmoths displayed the same preference hierarchy as their wild counterparts even though they have been removed from natural selection forces in the environment, implying this behavior is genetically fixed in the species. This behavior might have arisen initially due to a sensory bias in *M. sexta* chemically mediated by plant secondary metabolites. Thus, future research should focus on examining the foliar and volatile chemical similarities between the tomatoes, tobaccos, and the *Datura*.

2.5 Figures and Tables

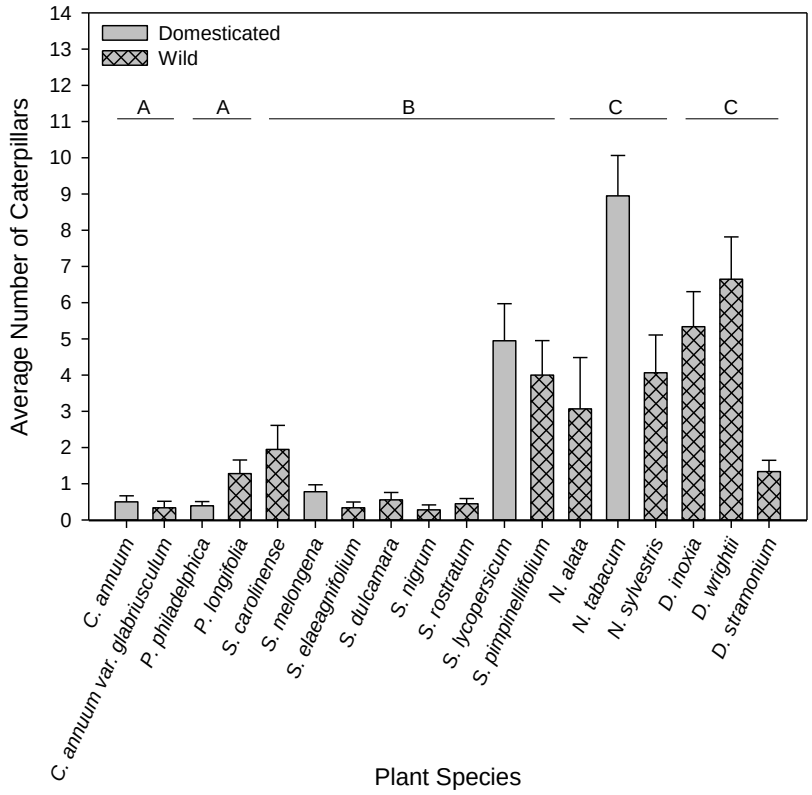

Figure 2.1 Cumulative Hornworm Density by Plant Species (+ S.E.)

Letters denote significant differences between genera.

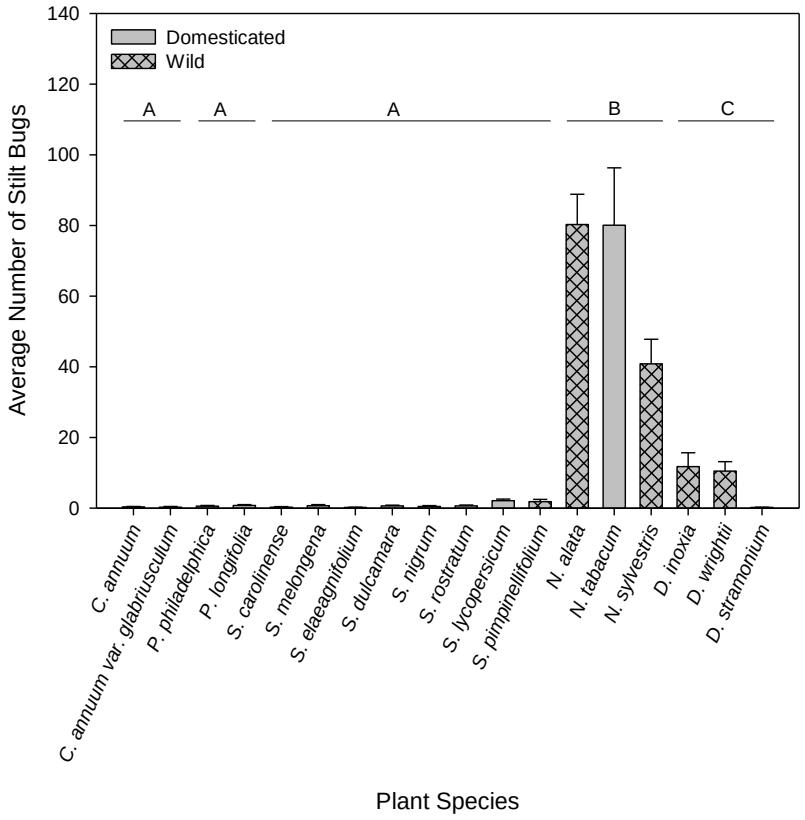

Figure 2.2 Cumulative Stilt Bug Density by Plant Species (+ S.E.)

Letters denote significant differences between genera.

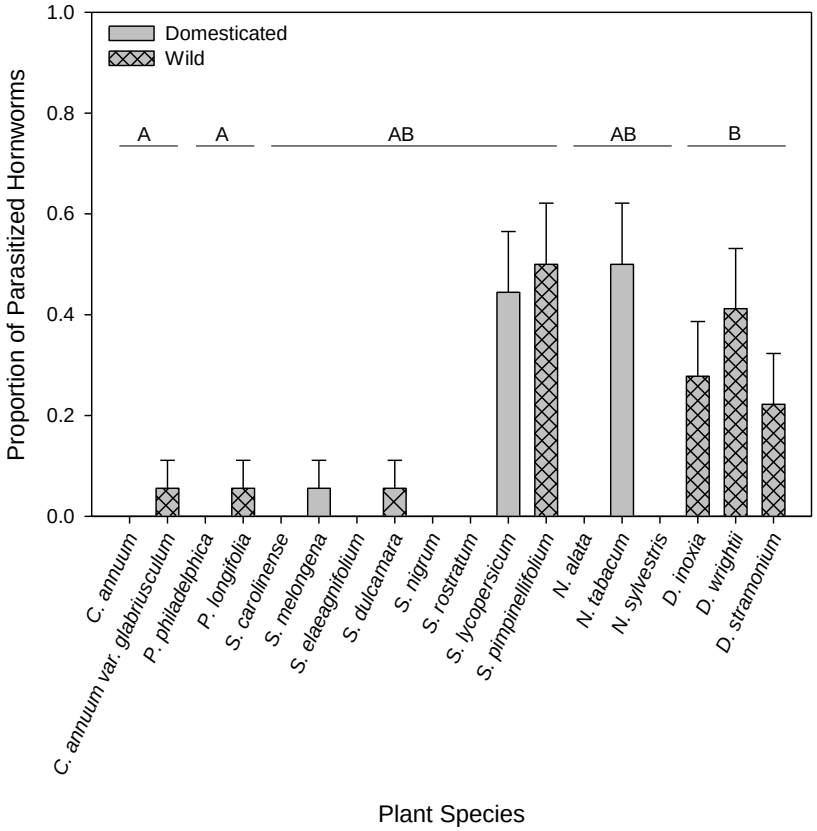

Figure 2.3 Presence/Absence of Parasitized Hornworms with *C. congregata* across Plants (+ S.E.)

Letters denote significant differences between genera.

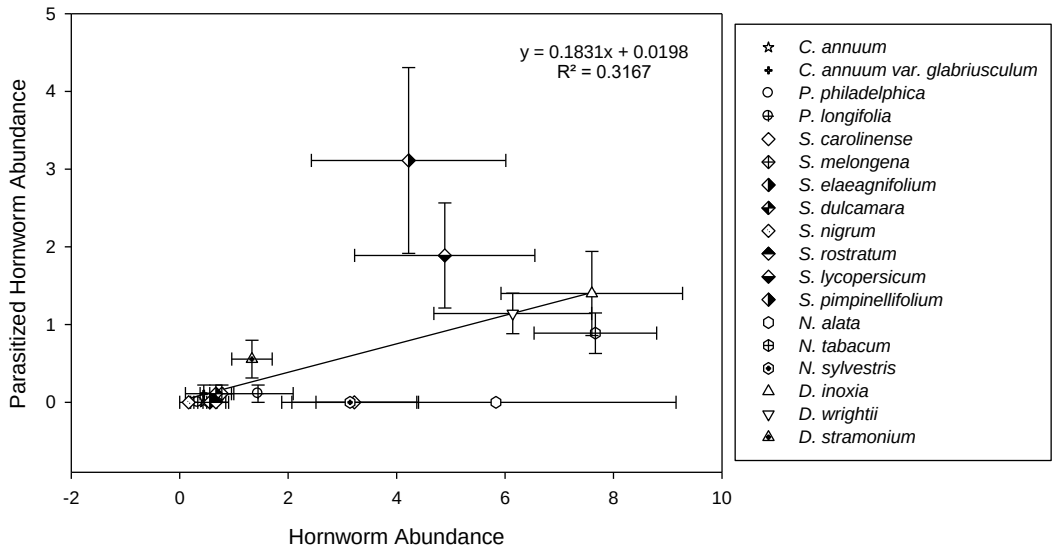

Figure 2.4 Regression of Hornworm Preference & Parasitoid Preference 2015 (± S.E.)

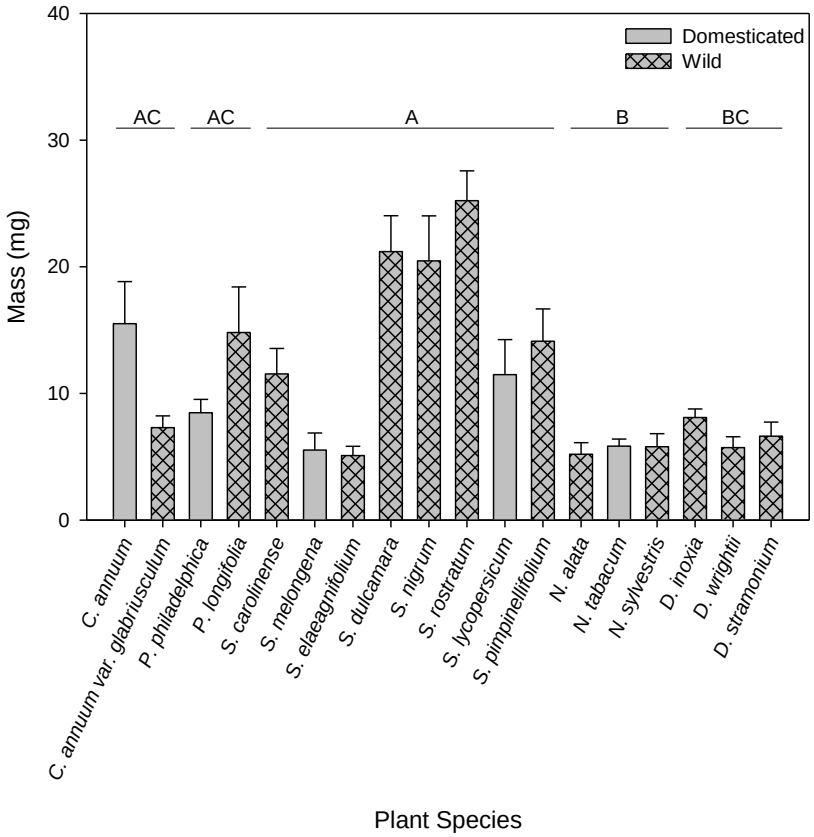

Figure 2.5 Caterpillar Wet Mass by Plant Species (+ S.E.)

Letters denote significant differences between genera.

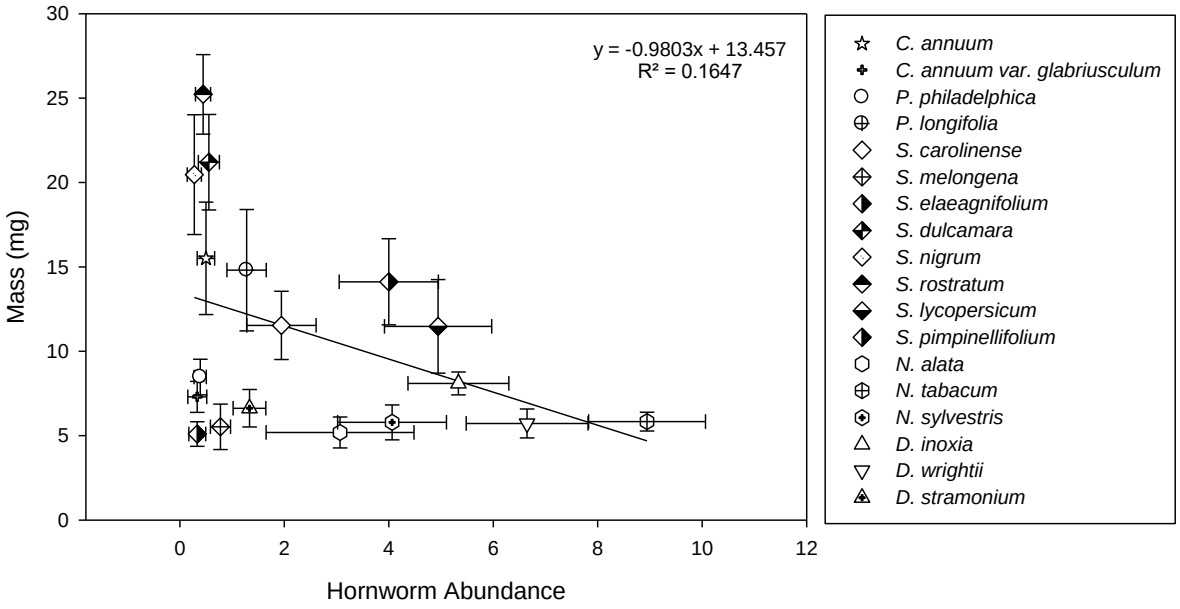

Figure 2.6 Regression of Hornworm Performance & Preference (± S.E.)

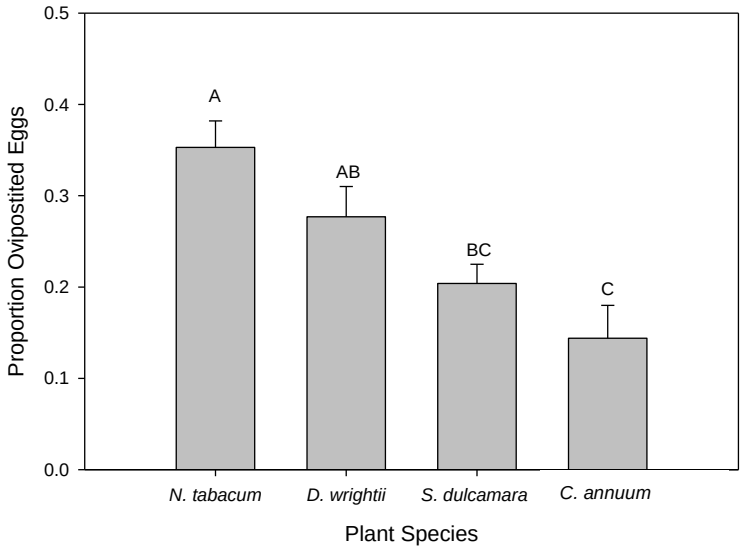

Figure 2.7 *M. sexta* Oviposition Preference by Plant Species in Laboratory Trials (+ S.E.)

Letters denote significant differences between plant species.

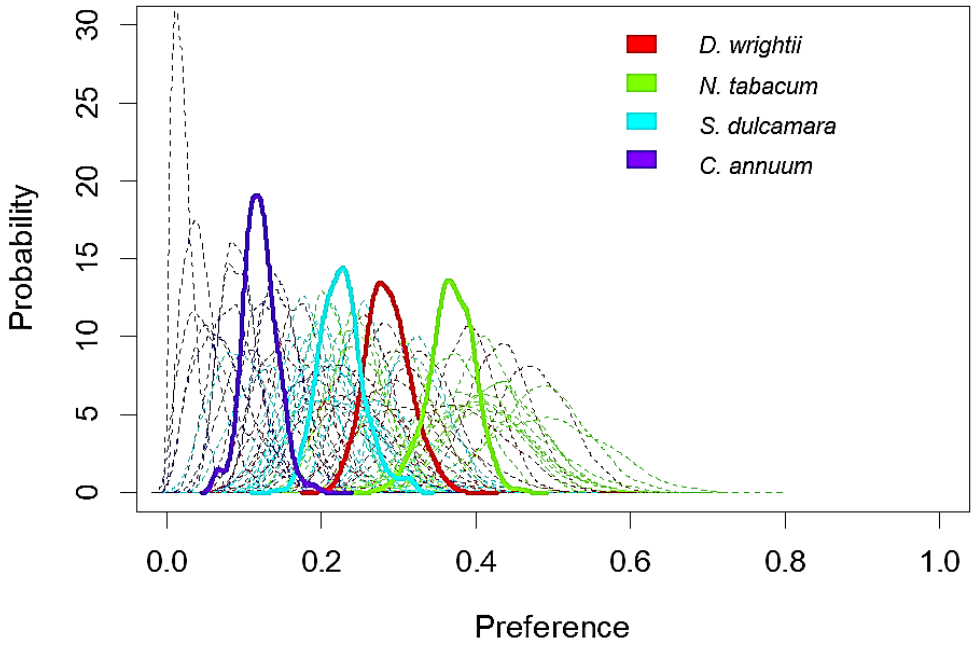

Figure 2.8 Bayesian Model of Hawkmoth Preference across Food Plants

Solid lines indicate the global average hawkmoth predicted preference across plant species. Dashed lines denote predictions for individual hawkmoths across plant species.

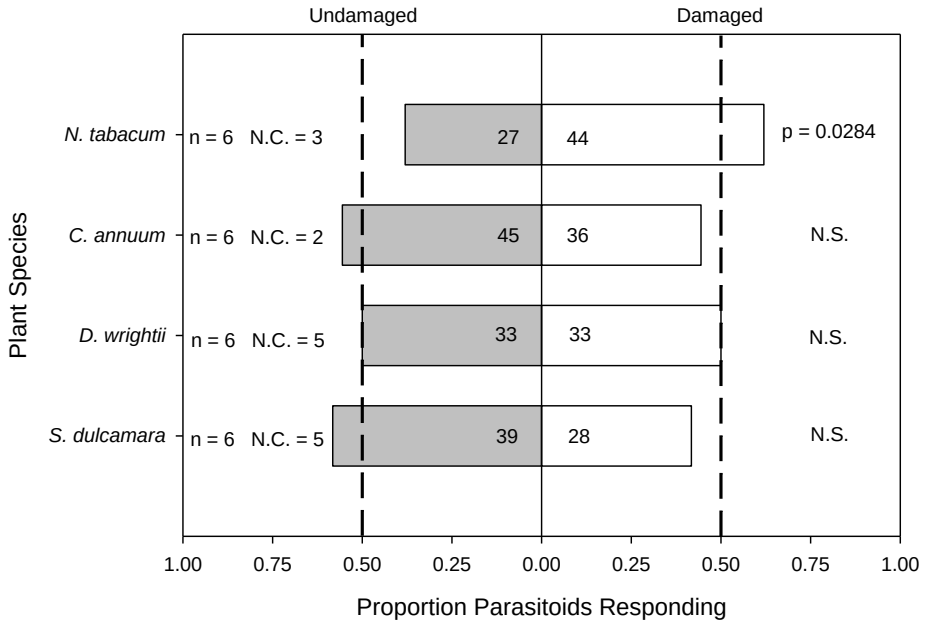

Figure 2.9 Parasitoid Preference in a Y-Tube Olfactometer.

Numbers in bars represent the total number of wasps responding to the given treatment. The number of plant replicate pairs within a species are denoted by n. Abbreviations: No choice (N.C.), Not significant (N.S.).

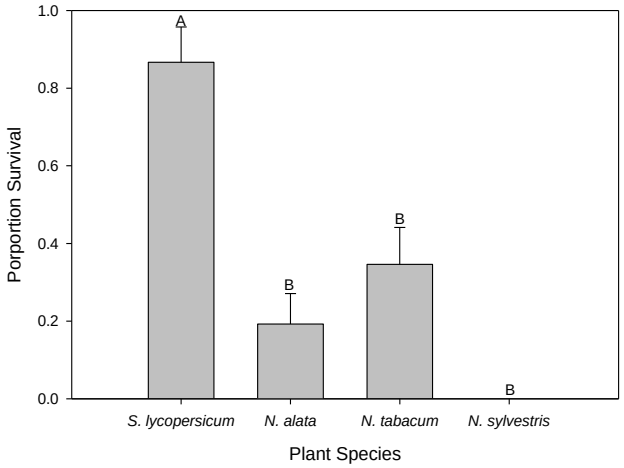

Figure 2.10 *Cotesia congregata* Survival by Plant Species (+ S.E.)

Letters denote significant differences between plant species.

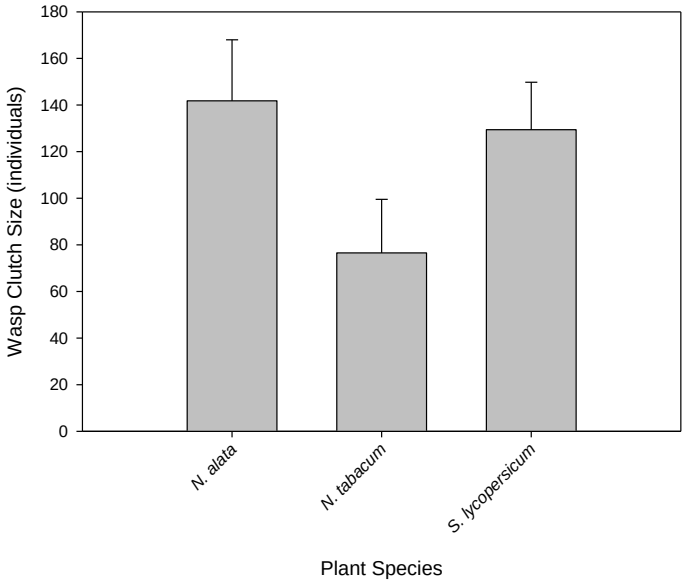

Figure 2.11 *Cotesia congregata* Clutch Size by Plant Species (+ S.E.)

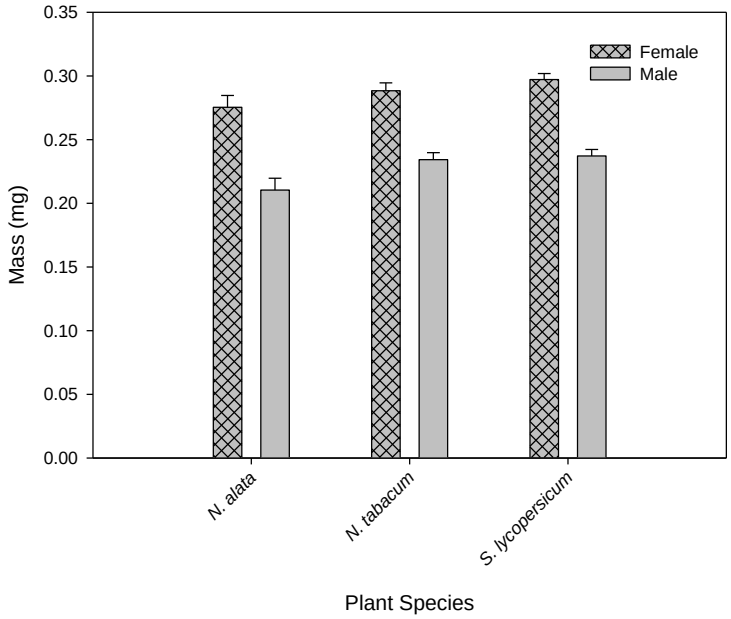

Figure 2.12 Wasp Mass by Plant Species (+ S.E.)

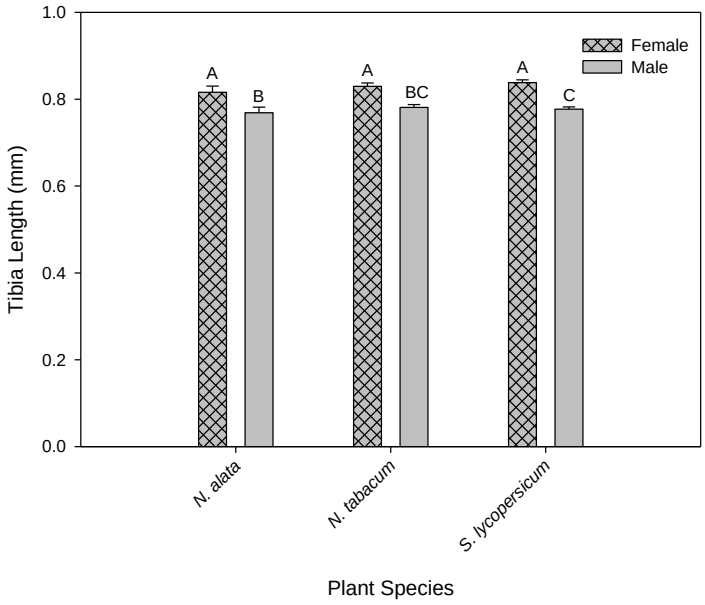

Figure 2.13 Wasp Tibia Length by Plant Species (+ S.E.)

Letters denote significant differences between plant species.

Table 2.1 Plant Species List Including Common Name, Crop vs. Wild, & Origin

Species Name	Common Name	Cultivation	USDA Accession Number/Seed Provider/Collection Origin
Datura stramonium	Jimsonweed	Wild	40.425130, -86.918136
Datura inoxia	Moonflower	Wild	Thompson & Morgan Seed Company, Poplar Lane Ipswich, Suffolk, IP8 3BU United Kingdom
Datura wrightii	Sacred thorn-apple	Wild	J. L. Hudson Seedsman, P.O. Box 337 La Honda, CA 94020
Physalis longifolia	Ground cherry	Wild	40.289219, -86.880595
Physalis philadelphica	Tomatillo	Domesticated	40.422253, -86.935336
Capsicum annuum cv. Golden California Wonder	Sweet pepper	Domesticated	NE Seed 122, Park Ave, Building H, East Hartford, CT 06108
Capsicum annuum var. glabriusculum	Chiltepin pepper	Wild	PI 224405 01 SD/631139 02 SD
Solanum carolinense	Horsenettle	Wild	40.437343, -86.934940
Solanum elaeagnifolium	Silverleaf nightshade	Wild	30.264239, -97.737444
Solanum rostratum	Buffaloburr	Wild	PI 420997 01 SD
Solanum dulcamara	Bittersweet nightshade	Wild	40.431354, -86.929015
Solanum ptycanthum	Black nightshade	Wild	France
Solanum lycopersicum cv. Better Boy	Tomato	Domesticated	NE Seed, 122 Park Ave, Building H, East Hartford, CT 06108
Solanum pimpinellifolium	Currant tomato	Wild	PI 634844 03GI
Solanum melongena cv. Black Beauty	Eggplant	Domesticated	NE Seed, 122 Park Ave, Building H, East Hartford, CT 06108
Nicotiana tabacum cv. Keller	Smoking tobacco	Domesticated	New Hope Seed Company, P.O. Box 443, Bon Aqua, TN 37025
Nicotiana alata cv. Lime Green	Winged tobacco	Wild	Select Seeds, 180 Stickney Hill Road, Union, CT 06076
Nicotiana sylvestris cv. Woodland tobacco	Flowering tobacco	Wild	Select Seeds, 180 Stickney Hill Road, Union, CT 06076

Table 2.2 Combined Enemy Data for 2014 & 2015

Plant Species	Parasitism: $\frac{\text{Parasitized Individuals}}{\text{Nonparasitized Individuals}}$	Mean Stilt bug Abundance
C. annuum	0/9	0.333 ± 0.140
C. annuum var. glabriusculum	1/6	0.278 ± 0.177
P. philadelphica	0/7	0.556 ± 0.202
P. longifolia	1/23	0.722 ± 0.240
S. carolinense	0/35	0.278 ± 0.135
S. melongena	1/14	0.667 ± 0.313
S. elaeagnifolium	0/6	0.167 ± 0.090
S. dulcamara	1/10	0.611 ± 0.216
S. nigrum	0/5	0.467 ± 0.236
S. rostratum	0/8	0.611 ± 0.231
S. lycopersicum	19/89	2.111 ± 0.435
S. pimpinellifolium	34/72	1.833 ± 0.633
N. alata	0/46	80.267 ± 8.572
N. tabacum	12/161	80.056 ± 16.253
N. sylvestris	0/65	40.813 ± 6.986
D. inoxia	9/96	11.722 ± 3.929
D. wrightii	13/113	10.471 ± 2.674
D. stramonium	5/24	0.167 ± 0.090

Table 2.3 GLMM Output for Hornworm Abundance by Plant Genus

Plant Genus	Estimate	S.E.	Z value	Pr(>|z|)
Capsicum	-0.527	0.294	-1.79	0.074
Physalis	0.122	0.220	0.56	0.578
Solanum	0.997	0.113	8.84	< 0.001
Nicotiana	1.787	0.099	17.98	< 0.001
Datura	1.643	0.102	16.10	< 0.001

Table 2.4 Pairwise Comparison for Hornworm Abundance by Plant Genus

Contrast	Estimate	S.E.	Z value	Pr(>|z|)
Capsicum – Physalis	-0.650	0.365	-1.79	0.387
Capsicum – Solanum	-1.524	0.299	-5.10	< 0.001
Capsicum – Datura	-2.171	0.297	-7.32	< 0.001
Capsicum – Nicotiana	-2.314	0.310	-7.47	< 0.001
Datura – Nicotiana	-0.144	0.127	-1.13	0.790
Datura – Physalis	1.521	0.221	6.85	< 0.001
Datura – Solanum	0.646	0.142	4.52	0.001
Nicotiana – Physalis	1.665	0.179	9.32	< 0.001
Nicotiana – Solanum	0.790	0.128	6.16	< 0.001
Physalis – Solanum	-0.875	0.216	-4.05	< 0.001

Table 2.5 GLMM Output for Stilt Bug Abundance by Plant Genus

Plant Genus	Estimate	S.E.	z value	Pr(>|z|)
Physalis	0.292	0.33	0.88	0.376
Solanum	0.552	0.22	2.50	0.012
Nicotiana	3.822	0.19	20.43	< 0.001
Datura	2.156	0.20	10.79	< 0.001
Capsicum	-0.554	0.42	-1.32	0.187

Table 2.6 Pairwise Comparison for Stilt Bug Abundance by Plant Genus

Contrast	Estimate	S.E	Z value	Pr(>|z|)
Capsicum – Datura	-2.710	0.425	-6.38	< 0.001
Capsicum – Nicotiana	-4.376	0.455	-9.62	< 0.001
Capsicum – Physalis	-0.846	0.542	-1.56	0.521
Capsicum – Solanum	-1.106	0.440	-2.51	0.088
Datura – Nicotiana	-1.666	0.207	-8.05	< 0.001
Datura – Physalis	1.864	0.302	6.17	< 0.001
Datura – Solanum	1.604	0.251	6.40	< 0.001
Nicotiana – Physalis	3.530	0.149	23.76	< 0.001
Nicotiana – Solanum	3.270	0.199	16.43	< 0.001
Physalis – Solanum	-0.260	0.274	-0.95	0.878

Table 2.7 GLMM Output for Parasitized Hornworm Presence/Absence by Plant Genus

Plant Genus	Estimate	S.E.	z value	Pr(>|z|)
Capsicum	0.010	0.046	0.21	0.837
Physalis	0.010	0.046	0.21	0.837
Solanum	0.048	0.036	1.33	0.183
Nicotiana	0.075	0.046	1.65	0.099
Datura	0.153	0.049	3.09	0.002

Table 2.8 Pairwise Comparison for Parasitized Hornworm Presence/Absence by Plant Genus

Contrast	Estimate	S.E.	Z value	Pr(>|z|)
Capsicum – Datura	-0.143	0.052	-2.76	0.046
Capsicum – Nicotiana	-0.066	0.048	-1.35	0.657
Capsicum – Physalis	< 0.001	0.049	0.00	1.000
Capsicum – Solanum	-0.038	0.040	-0.97	0.869
Datura – Nicotiana	0.078	0.050	1.55	0.532
Datura – Physalis	0.143	0.052	2.76	0.046
Datura – Solanum	0.105	0.042	2.48	0.096
Nicotiana – Physalis	0.066	0.048	1.35	0.657
Nicotiana – Solanum	0.027	0.038	0.71	0.954
Physalis – Solanum	-0.038	0.040	-0.97	0.869

Table 2.9 ANOVA Table (Type II Tests) for Hornworm Mass by Food Plant

Factor	*d.f.*	Sum Sq.	Mean Sq.	*F* value	Pr(>F)
Genus	4	3.270	0.818	13.34	< 0.001
Genus/Plant	13	4.167	0.320	5.23	< 0.001
Residuals	151	9.252	0.061		

Table 2.10 Pairwise Comparison for Hornworm Mass by Food Plant

Contrast	Estimate	S.E.	T value	Pr(>|z|)
Capsicum – Datura	0.156	0.071	2.19	0.188
Capsicum – Nicotiana	0.239	0.072	3.31	0.010
Capsicum – Physalis	-0.041	0.079	-0.52	0.984
Capsicum – Solanum	-0.079	0.058	-1.37	0.651
Datura – Nicotiana	0.083	0.073	1.13	0.788
Datura – Physalis	-0.197	0.079	-2.48	0.100
Datura – Solanum	-0.235	0.059	-3.97	0.001
Nicotiana – Physalis	-0.281	0.080	-3.48	0.006
Nicotiana – Solanum	-0.318	0.061	-5.24	< 0.001
Physalis – Solanum	-0.038	0.068	-0.57	0.981

Table 2.11 Multiple Pairwise Comparison for Hawkmoth Oviposition Preference by Plant

Contrast	Observed Difference	Critical Difference	Difference
C. annuum – D. wrightii	17.781	17.367	TRUE
C. annuum – N. tabacum	27.625	17.367	TRUE
C. annuum – S. dulcamara	7.844	17.367	FALSE
D. wrightii – N. tabacum	9.844	17.367	FALSE
D. wrightii – S. dulcamara	9.938	17.367	FALSE
N. tabacum – S. dulcamara	19.781	17.367	TRUE

Table 2.12 Bayesian Plant Pairwise Comparison for Hawkmoth Oviposition Preference

	D. wrightii	*N. tabacum*	*S. dulcamara*	*C. annuum*
D. wrightii		0.05875	0.91225	1.000
N. tabacum	0.94125		0.99850	1.000
S. dulcamara	0.08775	0.00150		0.994
C. annuum	0.00000	0.00000	0.00600	

Table 2.13 GLMM Output for Wasp Survival by Plant Species

Plant Species	Estimate	S.E.	Z value	Pr(>|z|)
N. alata	0.087	0.058	1.50	0.134
N. tabacum	0.206	0.073	2.82	0.005
N. sylvestris	0.000	0.057	0.00	1.000
S. lycopersicum	0.945	0.072	13.18	< 0.001

Table 2.14 Pairwise Contrasts for Wasp Survival by Plant Species

Contrast	Estimate	S.E.	Z value	Pr(>|z|)
N. alata – N. sylvestris	0.086	0.081	1.07	0.711
N. alata – N. tabacum	-0.120	0.092	-1.31	0.558
N. alata – S. lycopersicum	-0.858	0.092	-9.28	< 0.001
N. sylvestris – N. tabacum	-0.206	0.093	-2.22	0.117
N. sylvestris – S. lycopersicum	-0.945	0.092	-10.31	< 0.001
N. tabacum – S. lycopersicum	-0.738	0.103	-7.14	< 0.001

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CHAPTER 3. PLANT AND ENEMY MEDIATED EFFECTS ON THE PERFORMANCE AND IMMUNE RESPONSE OF *MANDUCA SEXTA*

3.1 Introduction

Why are some individuals in a population more susceptible to infections than others? It is widely accepted that genetic variation for immune capacity affects an organism's vulnerability to parasites, but individuals that mount strong immune responses are not necessarily the fittest (Viney et al. 2005; Lazzaro & Little 2009; Graham et al. 2011). This is because resistance to parasites is costly, energetically by shunting resources from other physiological processes into immune activation (Freitak et al. 2003; Ardia et al. 2012; Catalan et al. 2012), and ecologically by compromising life history traits (Schulenburg et al. 2009; van der Most et al. 2011). However, uncovering the tradeoffs associated with increased parasite resistance has been challenging (Lochmiller & Deerenberg 2000; Boughton et al. 2011).

Some of the strongest evidence for tradeoffs and plasticity in immune responses comes from insects. For example, *Drosophila melanogaster* can be selected for resistance to a larval parasitoid within five generations, but resistance costs are only manifested when food resources are limited (Kraaijeveld & Godfray 1997). Suceptible phenotypes outcompete resistant individuals when food is scarce, suggesting an evolutionary cost to maintaining this response. Activation of the immune system can similarly impair locomotor activity, attractiveness to mates, and associative learning (Moret & Schmid-Hempel 2000; Siva-Jothy 2000; Mallon et al. 2003; Adamo et al. 2008). Plasticity in the insect immune response is also known to occur when individuals are crowded. Insects reared in crowded environments exhibit density-dependent prophylaxis, having an upregulated immune response compared to conspecifics reared in solitary conditions (reviewed in Wilson & Cotter 2009). History of parasite exposure affects immunity as well, where on secondary challenge to the same parasite insects display a primed immune response to the pathogen or transgenerational immune priming of their offspring (Sadd et al. 2005; Sadd & Schmid-Hempel 2006). Although insects only have what has been termed an "innate" immune system and lack the adaptive features found in vertebrates (e.g., T-cells), the

immune systems of insects are by no means “simple” (Chambers & Schneider 2012). Recent work corroborates that insects exhibit highly specific responses to pathogens and alter their immune investment based on infection risk (Sadd et al. 2005; Sadd & Schmid-Hempel 2006; Wilson & Cotter 2009). In addition, insects can alternatively splice antimicrobial peptides, thereby making them more effective depending on the type of infectious bacterial species or strain present (Watson et al. 2005).

Given the costs of employing the immune system, it is not surprising that insects have also evolved unique parasite avoidance behaviors and other non-classical forms of defense (reviewed in Parker et al. 2011), which perhaps mitigate the costs imposed by immune activity and maintenance. One such example of nontraditional defense commonly cited is sequestration of secondary metabolites from the diet for protection against macro (e.g., parasitic wasps and nematodes) and micro (e.g., pathogens) parasites (Price et al. 1980; Demain & Fang 2000; Nishida 2002). Caterpillars that feed on more toxic host plants are better defended against parasites, but, counterintuitively, exploiting more toxic plants compromises their immune system (Haviola et al. 2007; Klemola et al. 2007; Karimzadeh & Wright 2008; Yang et al. 2008; Bukovinszky et al. 2009; Smilanich et al. 2009; Shikano et al. 2010; Diamond & Kingsolver 2011; Vogelweith et al. 2011; Martemyanov et al. 2012). In *Junonia coenia*, consuming plants high in iridoid glycosides lowers encapsulation (Smilanich et al. 2009). Likewise, *Manduca sexta* that use food high in secondary metabolites also have reduced encapsulation (Diamond & Kingsolver 2011). This impaired immune response might be due to a tradeoff with detoxification. But ultimately, it may be to the insect’s advantage to feed on more noxious food plants because they confer resistance to parasites (Barbosa et al. 1991; Nishida 2002).

In addition to toxic secondary metabolites, variation in macronutrients—proteins and carbohydrates—among food plants may be important for immunocompetence to parasitoids. Different protein-to-carbohydrate ratios (P:C) in an organism’s diet are known to affect immunity; consuming more protein increases phenoloxidase (PO) activity and thus melanization rates, perhaps increasing resistance to parasitoids, while ingesting more carbohydrates increases lysozyme activity, one of the main enzymes for defense against gram-positive bacteria (Cotter et al. 2011). Ingesting different macronutrients has also been shown to affect immune capacity. For

example, *D. melanogaster* fed diet supplemented with L-Arginine displayed increased resistance to parasitoids (Kraaijeveld et al. 2011). Although there is no global analysis for determining nutritional quality, it has been suggested that plant nutritional content can be gauged broadly by quantifying percent nitrogen and secondary metabolite profile (Shlichta & Smilanich 2012).

Given the strong fitness consequences associated with utilizing an inferior food plant in the absence of parasites, organisms should only consume noxious plants when infected (e.g., therapeutic medication) or the perceived threat of infection is high (e.g., prophylaxis). Self-medication in insects to pathogens has been documented in monarchs, woolly bear caterpillars, and fruit flies (Singer et al. 2009; Lefèvre et al. 2010; Milan et al. 2012), and prophylaxis to pathogens has been observed in wood ants (Castella et al. 2008). Prophylaxis to macroparasites is also reported in *D. melanogaster*, with females ovipositing in food with higher levels of alcohol when parasitic wasps are present (Kacsoh et al. 2013). Interestingly, no such example for prophylaxis to macroparasites in the Lepidoptera by ingestion of more toxic food plants is known (e.g., moths/butterflies ovipositing eggs on more toxic food plants in parasite burdened areas). This is surprising given the amount of research conducted in this group describing how food plant toxicity alters parasite susceptibility and host fitness (Ode 2006 and references therein). Top-down pressure from parasites is a key driver in herbivore diet breath, but studies investigating the evolution of food plant shifts to gain enemy free space are still lacking in the literature (but see Murphy 2004). Further, given the great level of phenotypic plasticity in their immune response, caterpillars might be able to detect certain parasites in the environment and alter their defensive physiology to increase immunocompetence.

Natural enemies often cause prey to develop faster, and express defensive behavioral and morphological traits (reviewed in Agrawal 2001, Benard 2004, Whitman et al. 2009). *Daphnia spp.* grow bigger and develop robust spines under high predation risk (Tollrian 1995; Laforsch et al. 2006), while damselflies/dragonflies grow longer caudal filaments which aid them in escaping predation by fish (Johansson & Samuelsson 1994; Dahl & Peckarsky 2002; Johansson 2002). The basis of these plastic responses is unquestionably physiological, but few studies have shown adaptive changes in physiological traits due to predation risk (e.g., how metabolism is altered to facilitate faster development; but see Thaler et al. 2012). Research examining phenotypic

plasticity in the immune response under predation stress seems to indicate that predators antagonize the immune response and increase susceptibility to parasites (Joop & Rolff 2004; Stoks et al. 2006; Ramirez & Snyder 2009; Yin et al. 2011; Adamo 2012). However, these studies mostly examine how carnivores alter immunity; cues from different types of natural enemies, such as parasitoids and pathogens, could induce different shifts in the immune response. For instance, the presence of parasitoids in the environment may upregulate the immune response, similar to how predation risk reduces development time, but they may also increase the allostatic load on the host antagonizing immune activity similar to carnivores.

I will address these knowledge gaps using the tobacco hornworm, *Manduca sexta* (Lepidoptera: Sphingidae), its main parasitoid, *Cotesia congregata* (Hymenoptera: Braconidae), and plants in the family, Solanaceae, to determine how diet quality and the threat of parasitism and predation affect host plant preference and parasite susceptibility. Most studies in this area have only investigated how a few secondary metabolites tradeoff with immune function, by augmenting artificial diet with the compounds of interests, and even fewer have related those findings directly to parasitoid performance (discussed in Ode 2013). I predict that caterpillar immune resistance to parasitism will decrease when reared on more toxic host plants and individuals will have reduced fitness compared to caterpillars reared on more nutritious plants. Second, I also predict that caterpillars in the presence of parasitoids and predators will have impaired immune activity but develop faster to escapes the threat from these natural enemies.

3.2 Methods

3.2.1 Insects and Plants

3.2.1.1 Hornworm and Wasp Colonies

A more thorough explanation of insect colony husbandry is provided in chapter two of this dissertation, so a brief review is only covered here. To avoid issues associated with inbred laboratory strains, an outbred colony of *M. sexta* was established in 2013 from local collections of larvae in Lafayette, IN. The laboratory rearing regime is similar to other *M. sexta* colonies. Eggs were collected from a moth mating cage and allowed to hatch communally onto a commercially available wheat germ based diet (Southland Products Inc., Lake Village, AR). Once neonates were 1-3 days old they were isolated in individual 45 mL cups containing diet cubes. Caterpillars were provided artificial diet *ad libitum* throughout their larval stage. Upon molting to the fourth instar caterpillars were then transferred to larger 96 mL cups. Larvae were reared in a scientific incubator (Percival Scientific, Perry, IA) on a 16:8 LD cycle at 25˚C and 25% RH. Once caterpillars become pharate and began their wandering stage, they were transferred first to a pupation block and then to a climate and light controlled incubator (VWR, Radnor, PA) on a 0:24 LD cycle at 25˚C and 25% RH. Newly eclosed adults were transferred to a 61 cm^3 collapsible field cage (BioQuip Products, Rancho Dominguez, CA) kept in an insect rearing room on a 16:8 LD cycle at 25˚C. These conditions facilitated mating and egg production. Moths were provided a 20%:10% sucrose:honey solution (w/v) and a solanaceous plant for oviposition.

The laboratory *C. congregata* colony was initially derived from individuals provided by Dr. Karen Kester in 2012 (origin information contained in Bredlau et al. 2013) and was supplemented with wasps every year from a local population by collecting parasitized *M. sexta* larvae with egressed wasp cocoons on their cuticle. To maintain the parasitoid stock, wasps were presented with third to fourth instar caterpillars and watched until they were stung. Parasitized *M. sexta* were then reared as described above until wasp larvae egressed from the caterpillar's body (ca. two to three weeks). Following cocoon spinning by the wasps, pupae were carefully harvested from the hornworm's body and placed in a 30.5 cm^3 mesh rearing cage (BioQuip

Products, Rancho Dominguez, CA). Adult wasps were provided with cotton macerated in DI water and a 20%:10% sucrose:honey solution (w/v), and maintained in a scientific growth chamber (Percival Scientific, Perry, IA) on a 16:8 L:D cycle at 25˚C and 65% RH.

3.2.1.2 Plant Species and Accession Lines

Plants included a subset of species from the common garden experiment (Table 2.1) and encompassed: *Datura stramonium* (jimsonweed), *Capsicum annuum* (sweet pepper), *Physalis pruinosa* (ground cherry), *Solanum carolinense* (horsenettle), *Solanum dulcamara* (bittersweet nightshade), *Solanum ptycanthum* (black nightshade), *Nicotiana tabacum* (tobacco), *Solanum lycopersicum* (tomato), *Solanum melongena* (eggplant). Potato, *Solanum tuberosum* cv. Yukon Gold (Burpee Seed Company, Warminster, PA) was also added in this experiment to include another agricultural crop. To increase germination, all seeds except for *N. tabacum* and *S. tuberosum*, were treated with a 10% bleach (NaOCl) solution for 20 minutes and then dried before sowing. As in the common garden experiment (chapter 2 of this dissertation), the seed coat of *D. stramonium* was mechanically nicked with a scalpel after drying to increase germination rates.

Plant mutants varying in anti-herbivore defense were obtained to examine how plant defenses affect immunity. Tomato lines varying in jasmonic acid signaling were provided by Dr. Gregg Howe (Michigan State University): *Solanum lycopersicum* cv. Castlemart (wt), *jasmonic acid–insensitive1* (*jai1*), and *35S:Prosystemin* (*35S:PS*) (Howe et al. 1996; Li et al. 2004). *jai1* is insensitive to prosystemin, the signaling phytohormone that initiates the jasmonic acid pathway coordinating plant defense responses to herbivore damage, and thus does not mount a defensive response upon herbivore feeding. In contrast, *35S:PS* constitutively produces prosystemin overexpressing defensive traits signaled by the jasmonic acid pathway making it systemically resistant to herbivores. Tobacco germplasm varying in nicotine synthesis (low alkaloid: LAB21, intermediate alkaloid: LIB21, and high alkaloid: HAB21) were provided by the Kentucky Tobacco Research and Development Center. These Burley 21 tobacco lines are isogenic and display varying levels of nicotine from 0.20/0.50% for LAB21 to 3.5/4.0% for HAB21 (total alkaloid content per dry weight) with LIB21 being intermediate due to mutations in one or both genes, *Nic1* and *Nic2*, for nicotine synthesis (Legg & Collins 1971; Hibi et al. 1994). Nicotine is

one of the most acutely toxic compounds known to man with one of its main functions in nature being a feeding deterrent/toxicant that is upregulated upon attack from insect herbivores (Steppuhn et al. 2004).

3.2.2 Insect Immune Responses

3.2.2.1 Encapsulation Response

The encapsulation response is one of the main immune responses insects deploy to combat parasites (Lavine & Strand 2002; Strand 2008). Objects incapable of phagocytosis via circulating hemocytes are encapsulated by insects, which entails the deposition of melanin and hemocytes on the surface of a foreign object suffocating the invader and isolating it from the rest of the body cavity (Lavine & Strand, 2002; Strand 2008). Implanted beads were used to assess encapsulation responses. The procedure for production of these beads and methodology for implanting them is described in Laughton et al. (2011), Smilanich et al. (2009), and Lavine & Beckage (1996), but briefly, beads were placed in a 0.1% (w:v) Congo Red Dye (Sigma-Aldrich, St. Louis, MO) solution at a ratio of 1:10 for 24 hours to absorb the dye before being air dried. Following this, beads were washed with 70% EtOH and then submerged in sterile Insect Ringer's Solution (7.48 g NaCl, 2.00 g $CaCl_2$, 0.10 g KCl, 0.19 g $NaHCO_3$ in 1000 mL DI H_2O, pH: 6.7; Sigma-Aldrich, St. Louis, MO) before being injected into the hemocoel of larvae using a zero-point syringe (Hamilton Company, Reno, NV, USA). At 24 hours post injection, caterpillars were freeze killed, and beads were removed. The encapsulation immune response to Congo Red dyed Sephadex beads was then scored on two qualitative scales (Figure 3.1): melanin deposited on the bead surface (Low, Medium, & High) and encapsulation of beads by hemocytes (Low, Medium, & High) also established in Lavine & Beckage (1996).

The encapsulation protocol was then modified from Laughton et al. (2011) based off of Smilanich et al. (2009) and Lavine & Beckage (1996) for Sephadex beads to quantify encapsulation of red nylon filament implants in a subsequent experiment to examine the effect of nicotine on the encapsulation response. Caterpillars that survived and molted into the 4^{th} stadium had pieces of red nylon filament measuring 0.356 mm in diameter by 0.3 mm in length (Cajun Line Red Lightnin' Monofilament) inserted into their fourth proleg and sealed with New Skin

liquid bandage (Moberg Pharma, Cedar Knolls, NJ). Insects were freeze-killed 24 hours later. The filaments were then assessed for encapsulation efficiency by dissecting them from specimens under PBS and photographing images of implants on an Olympus SZ Microscope attached with an Infinity-1 Analyze Digital Camera at 20x magnification and maximum luminosity. Control filaments that were not implanted were photographed simultaneously with treatment filaments. These images were then processed in ImageJ 1.x (Schneider et al. 2012) by converting them into digital 8-bit channel .jpeg file format pictures to determine the mean change in red values (based on the RGB color model) due to the deposition of melanin and hemocytes on the filament's surface vs. controls (Smilanich et al. 2009; Laughton et al. 2011). For digital 8-bit channel images, the red/*r* value of an image varies on a scale from zero to 255, where 0 represents an image that is completely black while 255 comprises a pure red image. Thus, since melanin is generally black to brown in color, a lower *r* value represents higher melanization of filaments. *r* values were converted to a percentage of melanization using the following formula: 1 − (treatment filament *r* value/control filament *r* value), as described in Smilanich et al. (2009) for statistical analysis.

3.2.2.2 Hemocyte counts

Hemocytes are circulating insect cells in the hemocel that contribute to immunity by encapsulating pathogens and parasites, synthesizing immune related defense enzymes such as proPO, and clotting wounds (Lavine & Strand, 2002; Strand, 2008). *M. sexta* has four distinct types of hemocytes: plasmatocytes, granulocytes, oenocytoids, and spherule cells (Willott et al., 1994; Strand, 2008 and references therein). To determine the total number of hemocytes, 10 µl of hemolymph was diluted at a ratio of 1:2 (v/v) in chilled Anti-Coagulant Saline Solution (0.23 g NaCl, 2.98 g KCl, 2.98 g EDTA, 1.99 g Citric Acid-Monohydrate, 7.98 g Sodium-Citrate, 50.00 g Sucrose, 1.00 g PVP, 0.58 g PIPES in 1000 mL DI H_2O, pH: 6.5; Sigma-Aldrich, St. Louis, MO) and aliquots of 10 µl were transferred to an improved Neubauer Phase Hemacytometer (Hausser Scientific, Horsham, PA) (Willott et al. 1994; Beetz et al. 2008). The cells were then counted using a phase contrast light microscope (Olympus XI 51) at 40x magnification. The number of cells per cubic millimeter equaled the number of cells counted per square millimeter, times the dilution, times 10.

3.2.2.3 Total Phenoloxidase

Phenoloxidase is a ubiquitous enzyme found in all insects that contributes to pigment formation in the insect cuticle and melanization in addition to immune defense (Kanost et al. 2008; González-Santoyo & Córdoba-Aguilar, 2012). Upon immune challenge, hemocytes release PO to form melanin and reactive oxygen species to kill parasites and pathogens (Kanost et al. 2008; González-Santoyo & Córdoba-Aguilar 2012). PO is also involved in the encapsulation response, melanizing invaders after hemocytes have adhered and suffocated them to further defend the insect (Lavine & Strand, 2002; Strand, 2008). Total PO activity of hemolymph samples was measured using a spectrometric PO assay modified from Söderhäll & Unestam (1979), Brookman et al. (1989), and Hall et al. (1995). Dopamine (3 mg/mL; Sigma-Aldrich, St. Louis, MO) dissolved in a 1.00 M phosphate buffer (1.141 g K2HPO4 in 500 mL DI H2O, pH 5.9; Sigma-Aldrich, St. Louis, MO) was used as the substrate for enzymatic activity since it is believed to be the natural substrate for the enzyme (Sugumaran, 2002; Kanost et al., 2008; Gonzalez-Santoyo & Cordoba-Aguilar 2012). PO is synthesized in insects as a proenzyme and then activated upon immune challenge from a pathogen/parasite (Kanost et al. 2008; González-Santoyo & Córdoba-Aguilar 2012). Here, a 10% cetylpyridinium chloride (CPC:1 mg/10 mL; Sigma-Aldrich, St. Louis, MO) solution, a powerful detergent that induces a confirmation change in proPO allowing its activation site to become exposed and display PO activity (Hall et al. 1995), also dissolved in 1.00 M phosphate buffer was used to activate the pro-PO enzyme by incubating samples with CPC at room temperature for 30 minutes. This assay indirectly measures PO activity by quantifying the change in sample absorbance due to the formation of dopaminochrome (Gonzalez-Santoyo & Cordoba-Aguilar 2012). Total PO for each sample, along with positive/negative controls containing Tyrosinase from mushroom (Sigma-Aldrich, St. Louis, MO) with/out dopamine, and PBS as a blank, were measured on a 96 well plate by running in triplicate 20 μL of activated sample with 130 μL of dopamine added phosphate buffer (described above). Tyrosinase from mushroom (Sigma-Aldrich, St. Louis, MO) is generally accepted as a control because of similar enzymatic activity (S. Adamo, *personal communication*). The kinetic absorbance for formation of dopaminochrome in each sample was then quantified at 492 nm every minute for one hour using an Eon Biotek microplate spectrophotometer (BioTek Instruments Inc., Winooski, VT). The maximum change in kinematic

absorbance for each sample was calculated using Biotek Gen5 All-In-One Microplate Reader Software (BioTek Instruments Inc., Winooski, VT), where a Δ 0.001 Optical Density/minute is defined as one unit (U) of enzymatic activity.

3.2.2.4 Lysozyme-like activity

Lysozyme-like activity was estimated similarly using the same microplate spectrophotometer with a turbidity assay modified from Dunn & Drake (1983), de Azambuja et al. (1991), and Adamo et al. (2016). Lysozymes are essential enzymes used by animals for defense against bacteria because of this enzyme's ability to cleave the β-(1,4)-glycosidic bond between N-acetylmuramic acid and N-acetylglucosamine in bacteria peptidoglycan cell walls (Callewaert & Michiels 2010). To quantify Lysozyme-like activity of each sample, 20 μL of diluted hemolymph (1:5) in 6.24 pH PBS solution was added to a 180 μL suspension of lysed *Micrococcus lysodeikticus* (9 mg/15 mL; Sigma-Aldrich, St. Louis, MO) in phosphate buffer (6.24 pH: as described above) and measured on a 96 well plate with the kinetic decrease in absorbance of each sample and controls being recorded at 450 nm every minute for 10 minutes using an Eon Biotek microplate spectrophotometer (BioTek Instruments Inc., Winooski, VT). The maximum change in kinematic absorbance for each sample was then calculated using Biotek Gen5 All-In-One Microplate Reader Software (BioTek Instruments Inc., Winooski, VT) where a Δ 0.001 Optical Density/minute is defined as one unit (U) of enzymatic activity. All samples were run in triplicate along with positive/negative controls containing Lysozyme from chicken egg white (Sigma-Aldrich, St. Louis, MO) with/out a suspension of *M. lysodeikticus* as substrate and PBS as a blank.

3.2.3 Experiments

3.2.3.1 Immune Defense across Differentially Resistant Host-Plants

Encapsulation capacity of caterpillars was assayed by rearing them on different plant food sources, which included: *Datura stramonium* (jimsonweed), *Capsicum annuum* (sweet pepper), *Physalis pruinosa* (ground cherry), *Solanum carolinense* (horsenettle), *Solanum dulcamara* (bittersweet nightshade), *Solanum ptycanthum* (black nightshade), *Nicotiana tabacum* (tobacco), *Solanum lycopersicum* (tomato), *Solanum melongena* (eggplant) and *Solanum*

tuberosum cv. Yukon Gold. Plants were sowed under greenhouse conditions and for the first week seeds were collectively planted by species in 10 cm pots in metro germination mix (Sun Gro Horticulture, Agawam, MA) and placed under 5 cm clear humidity domes (International Greenhouse Company, Danville, IL) for one week with an initial dose of Osmocote 14-14-14 slow release fertilizer (The Scotts Miracle-Gro Company, Marysville, Ohio) at a ratio of 30 mL: 1.84 L. Seeds that successfully germinated were then thinned and individually separated into 15 cm pots (Kord Traditional Std., 15.24 cm x 14.605 cm; International Greenhouse Company, Danville, IL) and allowed to grow under greenhouse conditions until they were used at two months of age. One day old neonates were then placed on plants in 1 m^3 mesh cages under greenhouse conditions at an initial density of 10 caterpillars per cage with 10 plants in a cage. Caterpillars were allowed to freely forage for 7 days on plants until they were recaptured to be weighed (Mettler Toledo, Greifensee, Switzerland). Larvae were then returned to their respective plant treatments and reared on plants until the mid-third instar, after which they were injected with Sephadex beads (Sigma-Aldrich, St. Louis, MO), which approximate parasitoid wasp eggs (Lavine & Beckage 1996). Each treatment of this experiment was replicated twice.

I performed a second experiment with a smaller subset of plant species to examine differences in *M. sexta* immunity across host-plants varying in specific plant defenses: *S. lycopersicum jai1*, *S. lycopersicum* wt, *S. lycopersicum 35S:PS*, *N. tabacum* HPB21, *N. tabacum* LIB2, and *N. tabacum* LAB21. These tomato and tobacco mutant plants were collectively planted by line in 10 cm pots (International Greenhouse Company, Danville, IL) in metro germination mix (Sun Gro Horticulture, Agawam, MA) and placed under a humidity dome (International Greenhouse Company, Danville, IL) for the first week after sowing with an initial dose of Osmocote 14-14-14 slow release fertilizer (The Scotts Miracle-Gro Company, Marysville, Ohio) at a ratio of 30 mL: 1 Pot in a plant growth chamber (Percival Scientific, IA) with a 16:8 LD cycle, 50% humidity, and ambient temperature of 25˚C. Seeds that successfully germinated were then thinned and individually separated into 10 cm pots (International Greenhouse Company, Danville, IL) 4 weeks later and moved to a semi-controlled bioclimatic room (~ 25˚C) and allowed to grow under artificial light at a 16:8 LD cycle until they were 3 months old after which they were used. One day old neonates were reared on these plants in the same bioclimatic room at a density of 5 caterpillars per plant. Caterpillars were bagged on the

first fully expanded leaf of every plant replicate, using 10 replicates per treatment, and allowed to forage for 7 days until they were weighed (Mettler Toledo, Greifensee, Switzerland). Larvae were then returned to their respective plant treatments and reared until the early fourth instar after which hemolymph was collected to quantify total PO and lysozyme-like activity (described above).

A third experiment was conducted to investigate differences in hemocyte density of *M. sexta* across host-plants varying in defense using the tomato and tobacco lines: *S. lycopersicum* cv. Better Boy, *S. lycopersicum jai1*, *N. tabacum* cv. Keller, and *N. tabacum* LAB21. Plants for this experiment were also sowed under greenhouse conditions and for the first week seeds were collectively planted by species in 10 cm pots (International Greenhouse Company, Danville, IL) in metro germination mix (Sun Gro Horticulture, Agawam, MA) and also then placed under a 5 cm clear humidity dome (International Greenhouse Company, Danville, IL) for one week with an initial dose of Osmocote 14-14-14 slow release fertilizer (The Scotts Miracle-Gro Company, Marysville, Ohio) at a ratio of 2 TBS: 1.84 L. Seeds that successfully germinated were then thinned and individually separated into 15 cm pots (Kord Traditional Std., 15.24 cm x 14.605 cm; International Greenhouse Company, Danville, IL) and allowed to continue and grow under greenhouse conditions until they were used at three months of age after which they were moved to a climatic controlled rearing room on a 16:8 LD cycle set to ~ 27˚C. Caterpillars were reared on these different plants in the same climatic controlled rearing room by placing one day old neonates at an initial density of 10 per plant, with two plant individuals per treatment, and allowed to freely forage for 7 days until they were weighed (Mettler Toledo, Greifensee, Switzerland). Larvae were then returned to their respective plant treatments and reared on plants until the mid-fourth instar after which caterpillar hemolymph was collected to quantify total circulating hemocytes (described above).

3.2.3.2 Immune Defense & Performance of Hornworms across Defined Nutritional Diet Mixes

A simplified artificial diet based on Ahmad et al. (1989) was used as the foundation for constructing nine artificial diet treatments varying in macronutrient concentration and toxicity (Table 3.1). This diet stoichiometry approach is used to study the nutritional contribution to physiological performance and immunity by construction of nutritional rails (Behmer 2009;

Cotter et al. 2011). All diets contained 900 mL of DI water, 18.00 g of agar (Bio-Serv, Flemington, NJ), 13.50 g Wesson's Salt (Bio-Serv, Flemington, NJ), 3.60 g of cholesterol (Sigma-Aldrich, St. Louis, MO), 4.50 g of ascorbic acid (Sigma-Aldrich, St. Louis, MO), and 9.00 grams of a commercially available vitamin mix (Southland Products Inc., Lake Village, AR). Different protein and carbohydrate amounts were added to each mix to vary the total amount of digestible macronutrients and ratio of protein to carb. The sole source of protein in each diet was derived from casein while the only source of carbohydrates was provided by sucrose. Cellulose (Bio-Serv, Flemington, NJ) was used as an additive to bulk diet mixes to maintain a constant volume. Diets also had pure nicotine (Sigma-Aldrich, St. Louis, MO) added to them to create diet treatments at either 0% or 0.5% nicotine of the total diet volume. Nicotine was added once they cooled to 60˚C but before they solidified. Ten one-day old neonates per treatment were placed on diets individually in 30 mL mini soufflé cups and reared in a climate-controlled incubator (Percival Scientific, Perry, IA) at 25˚C, 25% relative humidity, and a 16:8 LD cycle. Caterpillars were provided fresh diet every other day. On the 7th day caterpillars were weighed (Mettler Toledo, Greifensee, Switzerland) and then reared on diet treatments until the early fourth instar after which caterpillar hemolymph was collect to quantify total PO activity (described above). This experiment was replicated twice. Lysozyme-activity was omitted from analysis in this set of experiments due to the nonsignificant results displayed in the other plant-mediated immunity experiments.

3.2.3.3 Encapsulation of Nylon Filaments across Diets Varying in Nicotine

To isolate the effect of a specific resistance chemical, nicotine, on the immune response I tested the impact of dietary nicotine variation on encapsulation. Neonates, 48 hours post hatching, were assigned different nicotine (Sigma-Aldrich, St. Louis, MO) diets of 0%, 0.5%, and 1.0% (v:w), using 20 individuals per diet treatment, using a proprietary available commercial diet mix for hornworms as the base (Great Lakes Hornworm, Romeo, MI). Individuals were then reared under the methodology used to maintain the hornworm colony (described above). Caterpillars that survived and molted into the 4th stadium had pieces of red nylon filament (Cajun Line Red Lightnin' Monofilament) inserted into their fourth proleg and sealed with New Skin liquid bandage (Moberg Pharma, Cedar Knolls, NJ) before being freeze killed 24 hours later and

their encapsulation response to the filament measured (described above). This experiment was replicated twice.

3.2.3.4 Non-Lethal Risk Assays

To investigate the impact of natural enemies on *M. sexta* performance and immunity I exposed caterpillars to two of their main enemies: the spined soldier bug, *Podisus maculiventris*, and the parasitoid wasp *C. congregata* under laboratory conditions. Newly hatched hornworms were placed individually on an artificial diet in 30 mL soufflé cups with mesh secured over the cup opening. The mesh allowed volatile compounds from the enemies to enter the rearing cups but did not permit the enemies to interact with the caterpillars directly. These caged hornworms were then placed in clear plastic containers (30 x 25 x 10 cm LWH) at a density of 10 caterpillars per container and five enemies per container, separated by enemy gender or a control container containing no enemies, resulting in a total of five containers per replicate at any given time housed in the rearing room. This experiment was replicated twice. Eight week old *Nicotiana alata* cv. Lime Green plants along with cotton soaked in water or a 20% sucrose: 10% honey solution was also provided to enemies as a food source in each container. Containers were placed in a controlled rearing room on a 16:8 LD cycle at ~25˚C and checked daily with enemies being replaced as they died to maintain a constant level of risk. Hornworms were weighed one week later and development time to the 4^{th} instar was recorded. Upon transitioning to the 4^{th} instar hemolymph samples were collected and assayed for PO and lysozyme-like activity (described above).

3.2.4 Statistical Analyses

All statistical analyses were run using the open source R statistical software 3.3.3 (R Core Team, 2017). Mass for caterpillars in the bead encapsulation experiments by plant was log transformed to meet assumptions of normality and then analyzed with a one-way ANOVA. Multiple comparisons between plants were subsequently analyzed using the Tukey HSD test. Encapsulation and melanization by plant species were analyzed separately by constructing 2-way contingency tables using the MASS package and then analyzed with χ^2 tests. Mass for caterpillars in the different *S. lycopersicum* and *N. tabacum* defensive line experiments was also log transformed to meet assumptions of normality and then analyzed with a one-way ANOVA by

plant species. Multiple comparisons between plant defensive lines were subsequently analyzed using the Tukey HSD test. Caterpillar development on these different species defensive lines was investigated using a survival analysis with the survminer package followed by pairwise comparisons between plant defensive lines with a log-rank test. PO activity, lysozyme activity, and total hemocyte density on *N. tabacum* and *S. lycopersicum* plant defensive lines were analyzed with a one-way ANOVA by species initially, and then immune parameters were examined with a two-way ANOVA to compare differences across species with an interaction with plant defense.

Mass for caterpillars in the nonconsumptive effects experiment was analyzed with a one-way ANOVA by enemy treatment with multiple comparisons between treatments subsequently analyzed using the Tukey HSD test. Caterpillar development in the presence of different natural enemies was investigated using the same survival analysis methodology with the survminer package followed by pairwise comparisons between enemy treatments with a log-rank test. PO activity and lysozyme activity in the nonconsumptive effect experiment was also analyzed with a one-way ANOVA by natural enemy treatment and then subsequently analyzed using the Tukey HSD test.

A three-way ANOVA with interactions was also run on caterpillar mass and PO activity for the nutritional diet stochiomentery test, but the three way interaction was not significant so the condensed model is presented below. A three-way ANOVA with interactions was also originally run on hornworm PO activity for the nutritional diet stochiomentery test, but all interactions were not significant so the condensed model with only main effects is presented below. Melanization and encapsulation of nylon filaments across diets containing different amounts of nicotine was analyzed with a one-way ANOVA by treatment with multiple comparisons between diets subsequently analyzed using the Tukey HSD test.

3.3 Results

3.3.1 Plant Mediated Effects on Hornworm Performance and Immunity

3.3.1.1 Across Solanaceous Host-Plants

Plant species significantly affected hornworm mass at day 7 ($F_{9,442} = 21.90$, $p < 0.0001$; Figure 3.2). Plant species, however, did not predict encapsulation (χ^2 (*d.f.* = 9, *n* = 58) = 11.637, $p = 0.235$; Table 3.2) or melanization (χ^2 (*d.f.* = 9, *n* = 58) = 7.30, $p = 0.606$; Table 3.3).

3.3.1.2 Across *N. tabacum* and *S. lycopersicum* Defensive Lines

Tobacco defensive lines affected caterpillar mass at day 7 ($F_{2,91} = 13.87$, $p < 0.001$; Figure 3.3) and development time to the 4th instar stage (χ^2 (*d.f.* = 2, *n* = 76) = 39.80, $p < 0.001$; Figure 3.4 A), with both being reduced with increasing nicotine content, particularly from low to intermediate levels. However, nicotine did not affect PO activity ($F_{2,34} = 1.96$, $p = 0.156$; Figure 3.5), lysozyme activity ($F_{2,34} = 0.71$, $p = 0.500$; Figure 3.6), or total hemocyte density ($F_{1,15} = 0.28$, $p = 0.602$; Figure 3.7).

Tomato defensive lines also affected hornworm larval mass at day 7 ($F_{2,61} = 4.70$, $p = 0.013$; Figure 3.3) and development time to the 4th instar stage (χ^2 (*d.f.* = 2, *n* = 63) = 31.60, $p < 0.001$; Figure 3.4 B). However, plant defense line did not affect PO activity ($F_{2,28} = 0.14$, $p = 0.868$; Figure 3.5), lysozyme activity ($F_{2,30} = 0.58$, $p = 0.564$; Figure 3.6), or total hemocyte density ($F_{1,10} = 0.002$, $p = 0.966$; Figure 3.7).

When analyzed together, PO activity was higher on tobacco compared to tomato ($F_{1,62} = 9.32$, $p = 0.003$), while lysozyme activity and total hemocyte density were not different ($F_{1,64} = 1.15$, $p = 0.287$ and $F_{1,25} = 0.46$, $p = 0.506$, respectively).

3.3.2 Nutritional Diet Stochiomentery Effects on Hornworm Performance & Immunity

Only PO activity and hornworm mass were investigated in this experiment given the nonsignificant results plant defense had on lysozyme-like activity and total hemocyte density in the previous experiments. Protein and the addition of nicotine to the diet significantly affected

hornworm mass ($F_{1,137}$ = 104.22, p < 0.001; $F_{1,137}$ = 17.67, p < 0.001; Table 3.4 & Figure 3.8). Carbohydrates did not affect hornworm mass ($F_{1,137}$ = 0.05, p = 0.826), but there was a significant interaction effect between carbohydrates and nicotine ($F_{1,137}$ = 11.55, p < 0.001; Table 3.4 & Figure 3.8). Protein and carbohydrate did not affect PO activity ($F_{1,85}$ = 0.07, p = 0.787; $F_{1,85}$ = 2.44, p = 0.122; Table 3.5); however, nicotine did affect PO activity ($F_{1,85}$ = 8.79, p = 0.004; Table 3.5 & Figure 3.9). No hornworms survived to the fourth instar stage on the 4:1 carbohydrate skewed diets so no samples were collected.

3.3.3 Effects of Nicotine on Encapsulation of Nylon Filaments

Diet significantly affected encapsulation and melanization of nylon filaments ($F_{2,60}$ = 3.92, p = 0.025; Figure 3.10). Caterpillars reared on diet supplemented with 1.0% and 0.5% nicotine were not different from individuals reared on control diet containing no nicotine, but hornworms reared on 1.0% and 0.5% nicotine diets were different from each other.

3.3.4 Nonconsumptive Effects of Natural Enemies on *M. sexta* Performance & Immunity

Enemy treatment significantly affected hornworm mass at day 7 ($F_{4,81}$ = 6.16, p < 0.001). Notably, in pairwise comparisons hornworms in the presence of female soldier bugs and female wasps were larger compared to individuals without natural enemies (p < 0.001 and p < 0.004, respectively; Figure 3.11), while there was no difference for male natural enemies compared to controls (Control vs *C. congregata* (Male), p = 0.378; Control vs *P. maculiventris* (Male), p = 0.962). Enemy treatment also affected hornworm development to the fourth instar stage (χ^2 (*d.f.* = 4, n = 42) = 10.40, p = 0.035). Wasps separated by sex were not significantly different from each other so they were grouped together, and the data reanalyzed (Figure 3.12). No enemy treatments were significantly different from controls (Control vs *P. maculiventris* (Male), p = 0.170; Control vs. *P. maculiventris* (Female), p = 0.170; Control vs *C. congregata*, p = 0.470). However, hornworm development was significantly slower in the presence of male soldier bugs compared to female soldier bugs and wasps (*P. maculiventris* (Male) vs. *P. maculiventris* (Female), p = 0.030; *P. maculiventris* (Male) vs. *C. congregata*, p = 0.030; Figure 3.12).

Similar results on performance also manifested in differences in prey immune activity under the threat of natural enemies. Predator treatment significantly affected PO activity ($F_{4,39}$ =

5.15, $p = 0.002$). Male and female wasps were not significantly different from each other in pairwise comparisons so wasps of both sexes were grouped together, and the data reanalyzed ($p = 0.994$; Figure 3.13). The control group was not significantly different from male or female soldier bugs ($p = 0.508$; $p = 0.577$, respectively) but was significantly different from wasps which had lower PO activity ($p = 0.031$; Figure 3.13). Wasps were not different from female soldier bugs ($p = 0.520$), but were different from male soldier bugs ($p < 0.001$; Figure 3.13). There was also a strong trend between male and female soldier bugs implying that sex for this natural enemy might induce differences in PO activity ($p = 0.060$). However, enemy presence did not affect lysozyme-like activity, ($F_{4,39} = 0.53$, $p = 0.716$; Figure 3.14).

3.4 Discussion

Plant species assayed caused drastic differences in growth of *M. sexta*, but there was no evidence that plant species affected the encapsulation response of the immune system conflicting with my original prediction. However, with a more targeted examination of immune activity varying by plant defensive qualities in the plant species *N. tabacum* and *S. lycopersicum*, differences in insect immune function caused by plant nutritional quality became evident. This result suggests that it is not overall plant toxicity, or lack thereof, that moderates changes in resource allocation among physiological systems, from growth and development to immune defense, but specific secondary metabolites that initiate these shifts (i.e., nicotine). Although caterpillars reared on the different *S. lycopersicum* defensive lines performed equivalently to individuals reared on the comparable *N. tabacum* defensive line, caterpillars did not exhibit the same PO activity.

Contrary to my original hypothesis, these results together suggest that nicotine is immunotherapeutic, enhancing immune defense against parasites by increasing PO activity while not affecting total hemocyte concentrations, lysozyme-like activity, or encapsulation. However, since all immune defense parameters measured are inducible responses upon pathogen/parasite challenge, immune activity was not expected to increase but to be lower on more chemically defended food plants given an underlying assumption of a resource allocation cost from increased investment in the detoxification system over the immune system. This finding parallels work from Del Campo et al. (2013) where *M. sexta* fed diet supplemented with the plant phenolic chlorogenic acid had a higher number of circulating hemocytes, which increased their resistance to bacterial infections. Other research in *Junonia coenia* showed that caterpillars had higher survival and more positive PO activity when reared on *Plantago lanceolata*, a high iridoid glycoside containing plant, compared to *Plantago major*, a low iridoid glycoside containing species (Smilanich et al. 2017). My work adds to this growing body of literature illustrating that plant secondary metabolites can be immunotherapeutic to herbivores.

In addition, even though nicotine also had negative effects on larval development, these effects were mitigated by increased dietary protein concentration. Protein and carbohydrate

levels did not affect PO activity per say, a result that differs from Cotter et al. (2011) reporting the importance of protein for PO activity. Further, *M. sexta* does not sequester any plant secondary metabolites, which is why my results might differ from the majority of work demonstrating a tradeoff between immune activity and food plant toxicity (Haviola et al. 2007; Klemola et al. 2007; Karimzadeh & Wright 2008; Yang et al. 2008; Bukovinszky et al. 2009; Smilanich et al. 2009; Shikano et al. 2010;Vogelweith et al. 2011; Martemyanov et al. 2012). Many of these cases examined insects that sequester plant chemical compounds in their body and sequestration of plant secondary metabolites might compromise immune activation, while tolerance of plant chemicals, metabolism, and excretion may not be as energetically costly and burdensome on an insect, such as in the case for *M. sexta* (A. Smilanich, *personal communication*).

The mechanism by which nicotine might cause altered PO activity is unknown, but could work directly on the PO enzyme, changing the binding dynamic with its substrate or cause gene upregulation of proPO. Since nicotine is a neurological toxin and stressor that acts on the nicotinic acetylcholine receptor, the effect of increased PO might be modulated through the neuroendocrine system. McMillan et al. (2017) found that detoxification of a diet supplemented with the synthetic insecticide permethrin induced not only upregulation of detoxification genes but also upregulation of immune related genes in *M. sexta*. McMillan et al. (2017) hypothesized that this effect is mediated through the neuroendocrine system since chemical toxins and pathogens both induce specific and general oxidative stresses and there is overlap for the molecular pathways in the immune and detoxification system to respond through anti-oxidative mechanisms (Kodrík et al. 2015). This could tentatively explain the immunotherapeutic effects found in this study and by Del Campo et al. (2013) and Smilanich et al. (2017).

The risk of parasitism and predation also had alternative effects on immune activity for *M. sexta* demonstrating that the identity of the natural enemy inducing stress is important to *M. sexta* when altering investment across its physiological systems. First, the presence of either sex of *C. congregata* lowered PO activity while *P. maculiventris* did not affect PO activity. This would imply that having parasitoids in the environment make their hosts more permissive to parasitism, and thus would be beneficial to the parasitoid, but also shows that *M. sexta* does not

preemptively invest in its immune response to thwart off a possible future parasitoid attack. Further, chronic long term stress and acute short term stress might manifest different results on individuals. Adamo et al. (2017) demonstrated that in *M. sexta* acute short term stress from a single mock predation event had neutral effects on immune activity, while I present evidence of the role of chronic long term stress (i.e., continuously over the whole lifespan of the prey) from multiple natural enemies of *M. sexta*, which might explain the differences in our results. How *M sexta* is detecting these signals from parasitoids, whether through volatile chemical cues or detection of wing beat vibrations from wasps, is unclear and would require further study. Second, the presence of female predators and parasitoids increased caterpillar mass but did not alter development time, while male wasps did not alter mass, but notably male *P. maculiventris* slowed *M. sexta* development compared to wasps and female soldier bugs. I could not find evidence of predation from *P. maculiventris* accelerating the development of *M. sexta* (Thaler et al. 2012), but my experimental design preventing predators from interacting with prey and rearing *M. sexta* on an artificial diet as opposed to *S. lycopersicum* to exclude plant effects might account for these differences. Interestingly, this does corroborate a sex effect of *P. maculiventris* on *M. sexta* development such as described by Hermann & Thaler (2014) for *Leptinotarsa decemlineata*, the Colorado potato beetle, implying that the male produced pheromone of this predator is likely detectable in the environment to numerous prey species. Overall, *M. sexta* did not necessarily develop faster in the presence of natural enemies and had neutral to depressed immune activity, which is consistent with other research reporting that prey in the presence of predators are more susceptible to pathogen infection (Joop & Rolff 2004; Stoks et al. 2006; Ramirez & Snyder 2009; Yin et al. 2011). Increasing the allostatic load chronically on an individual seems to push an organism beyond what it can compensate for physiologically (Adamo 2012).

This work globally highlights how life history strategies are impacted by the environment one inhabits and the necessity to adequately respond to different stressors to balance the competing physiological tasks of development and immune defense. Utilizing a food resource in parasite burdened habitats that increases immune activity to mitigate the nonconsumptive effect the presence of parasitoids have on an insect's physiology (e.g., impairing immune function) can be vital for survival. *M. sexta* can accomplish this by feeding on *N. tabacum*, an

immunotherapeutic plant due to its nicotine content, for defense against the parasitoid *C. congregata*, the presence of which lowers immune activity. While my data illustrates the importance that food nutritional quality and stress have on physiological performance, further examination of these interactions in a whole community context is needed to untangle how plant chemistry shifts prey physiology balanced against numerous other commensal and antagonistic interactions. Indeed, the benefits conferred by plant secondary metabolites, which make these compounds effective against ones' parasites and predators while still allowing the prey to develop to adulthood is a grand challenge that all organisms face.

3.5 Figures and Tables

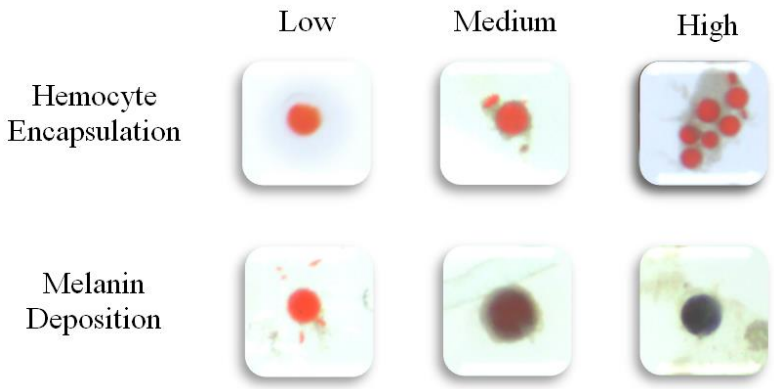


Figure 3.1 Semi-Quantitative Hornworm Immune Response Scale to Implanted Beads

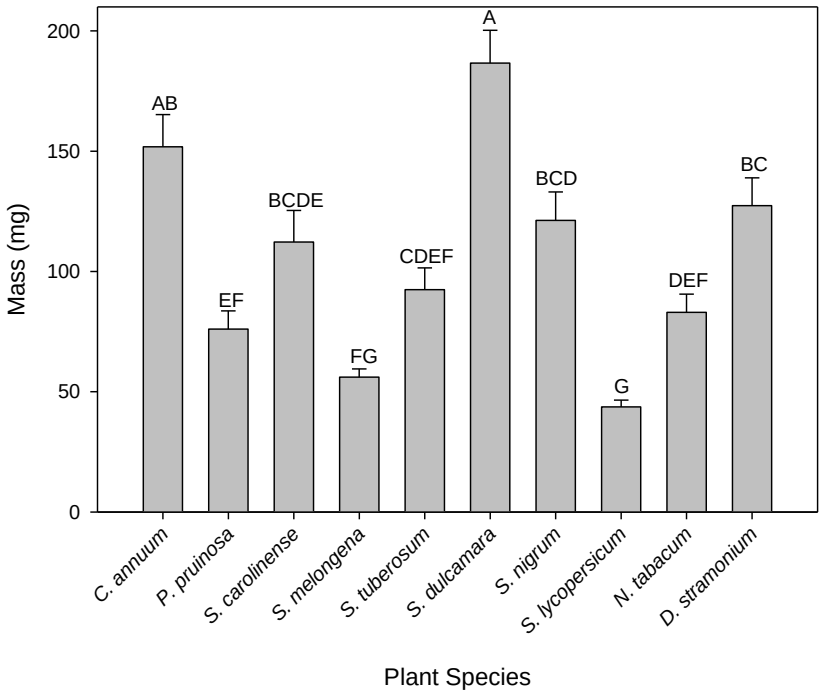


Figure 3.2 Mean 7 Day Caterpillar Mass by Plant Species (+ S.E.)

Letters indicate significantly different treatments.

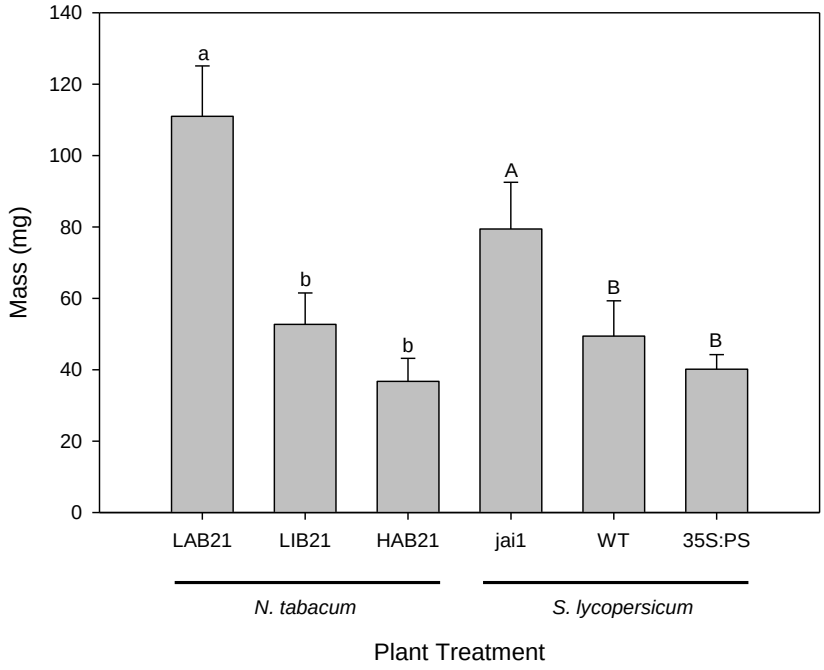
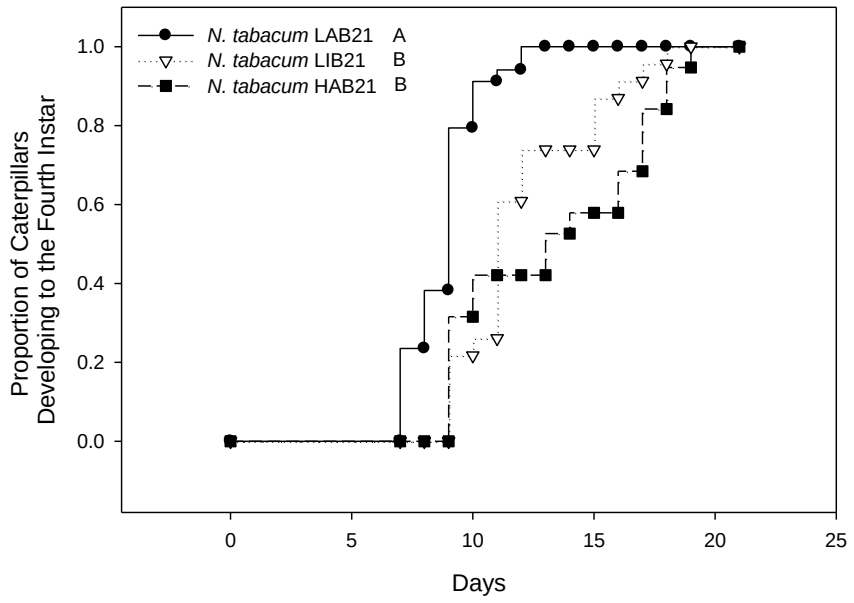


Figure 3.3 Mean 7 Day Caterpillar Mass by Plant Defense Treatment (+ S.E.)

Lowercase letters indicate significantly different groups within N. tabacum. Uppercase letters indicate significantly different groups in S. lycopersicum.

A)



B)

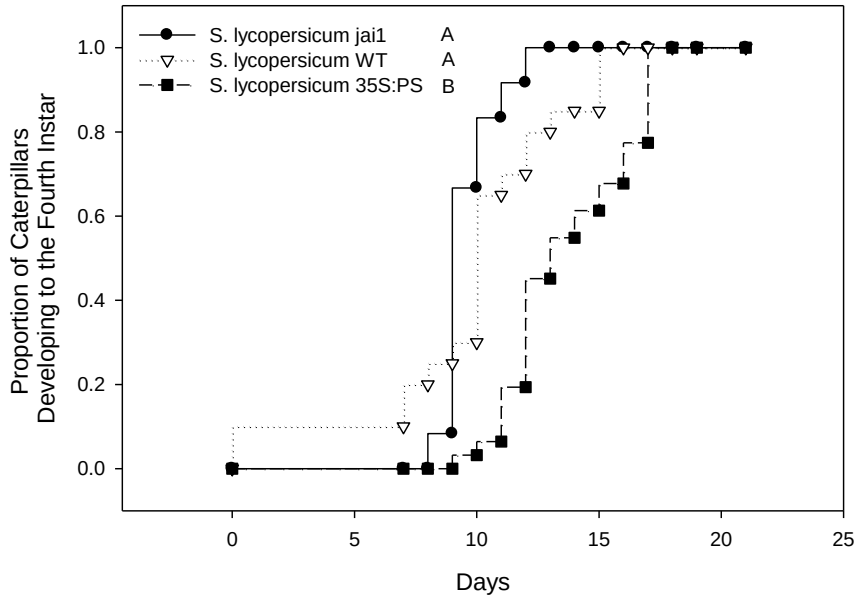


Figure 3.4 Caterpillar Development on Different Plant Defensive Lines.

A) N. tabacum. B) S. lycopersicum. Letters indicate significantly different groups.

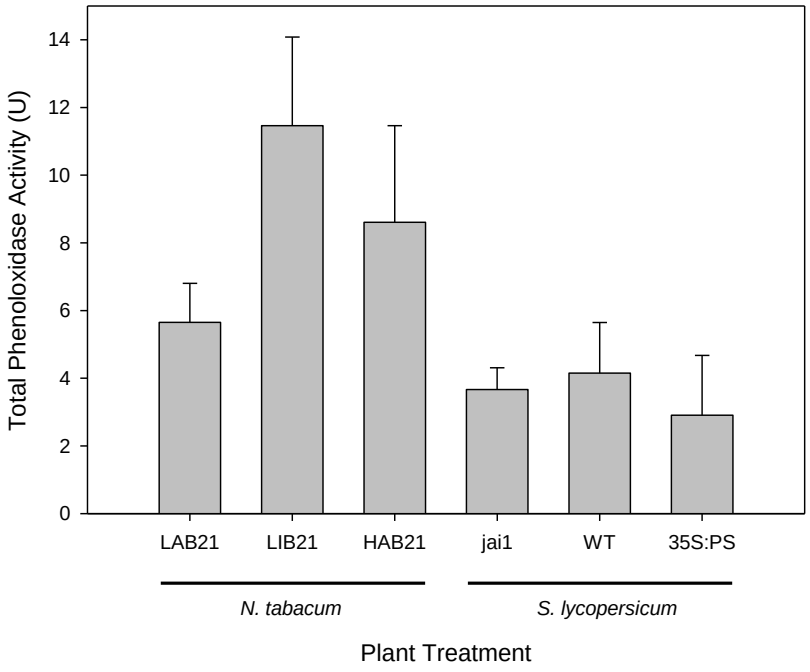


Figure 3.5 Mean Total Phenoloxidase Activity by Plant Defensive Treatment (+ S.E.)

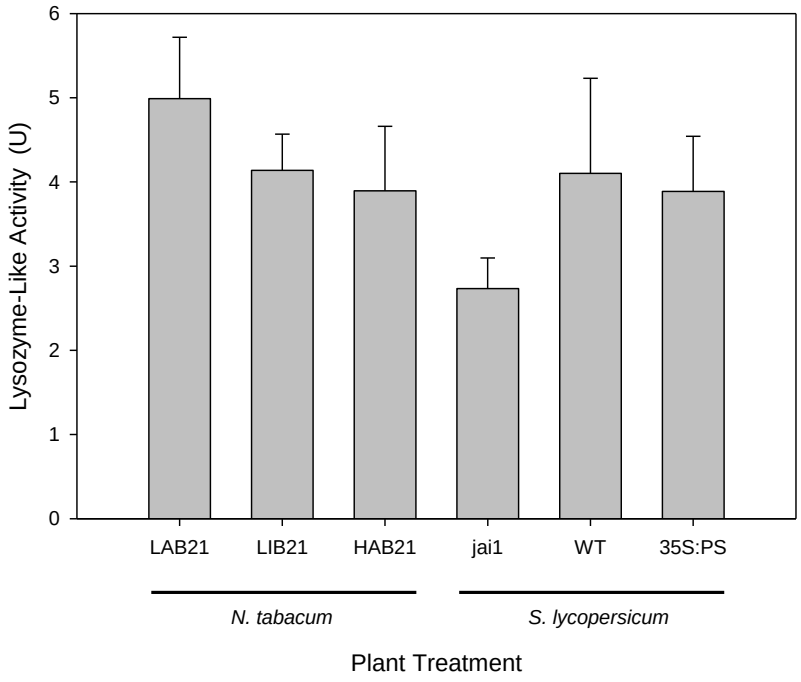


Figure 3.6 Mean Lysozyme-Like Activity by Plant Defensive Treatment (+ S.E.)

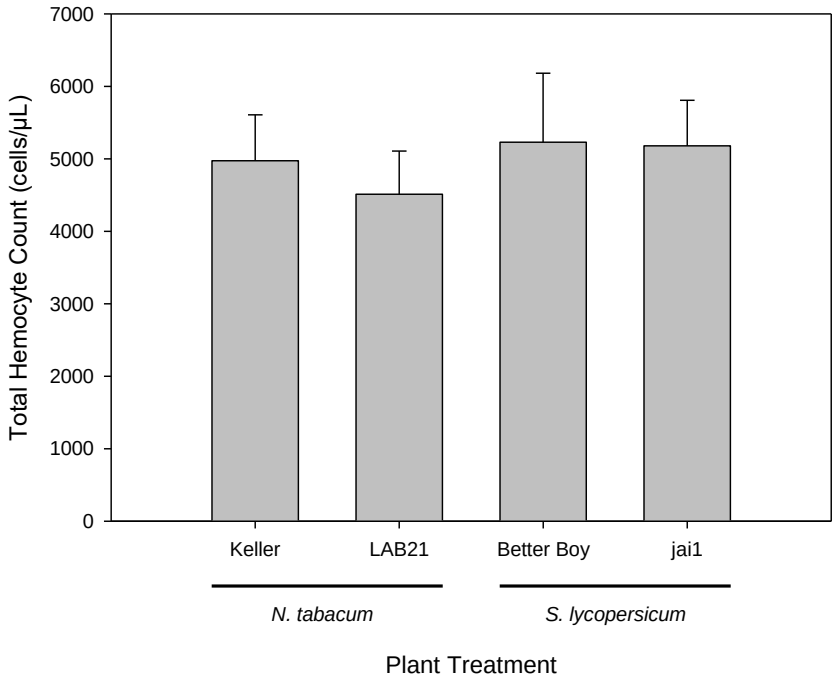


Figure 3.7 Mean Total Hemocyte Count by Defensive Plant Treatment (+ S.E.)

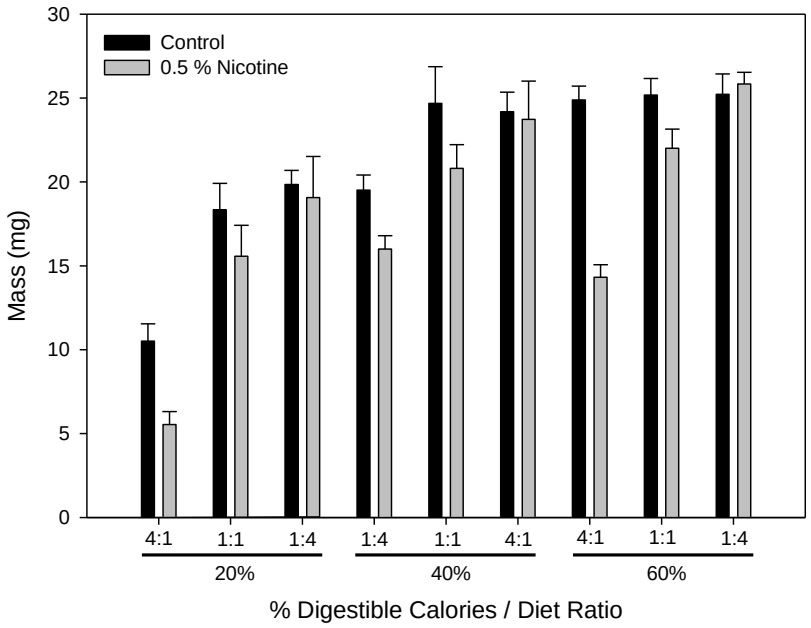


Figure 3.8 Mean Hornworm Mass across Nutritional Space (+ S.E.)

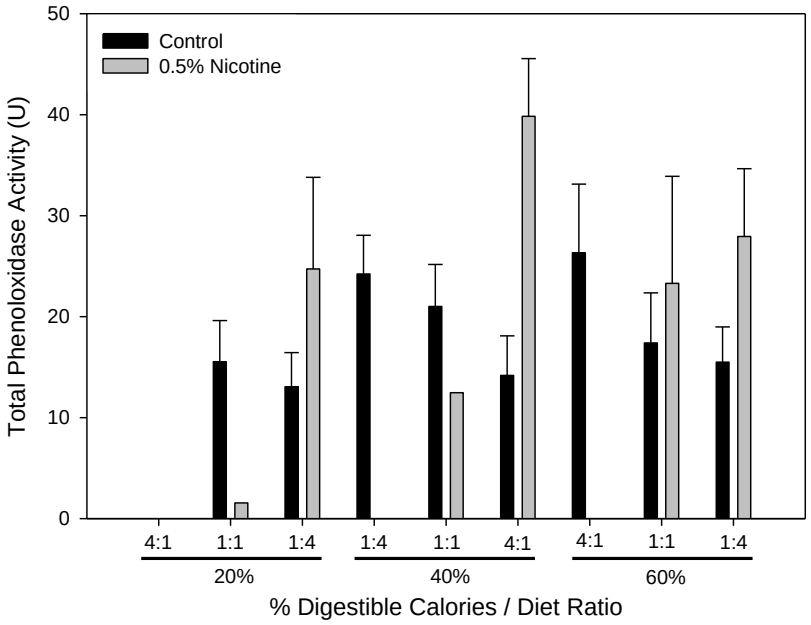


Figure 3.9 Mean Total Phenoloxidase Activity across Nutritional Space (+ S.E.)

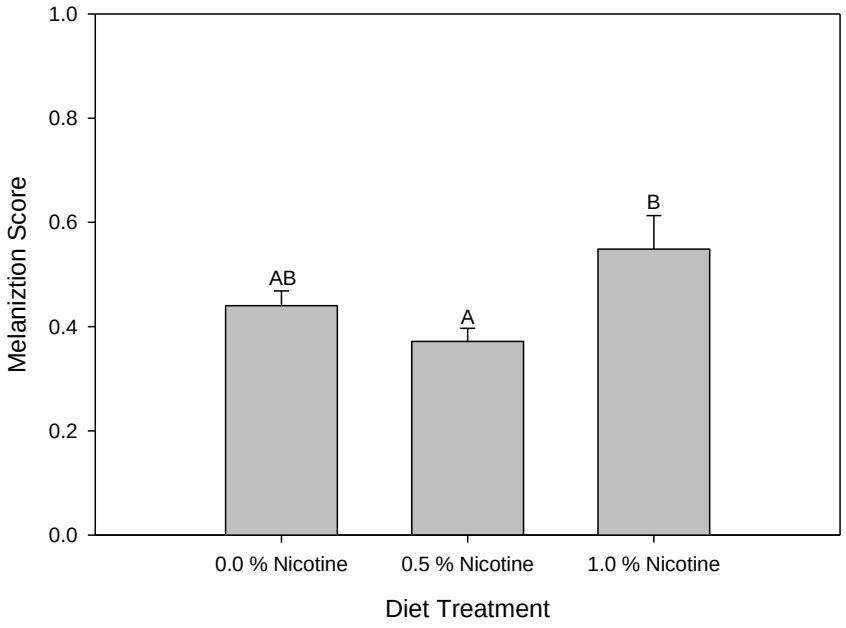


Figure 3.10 Mean Melanization Score of Nylon Filaments across Diet Treatments (+ S.E.)

Letters denote significant differences between treatments.

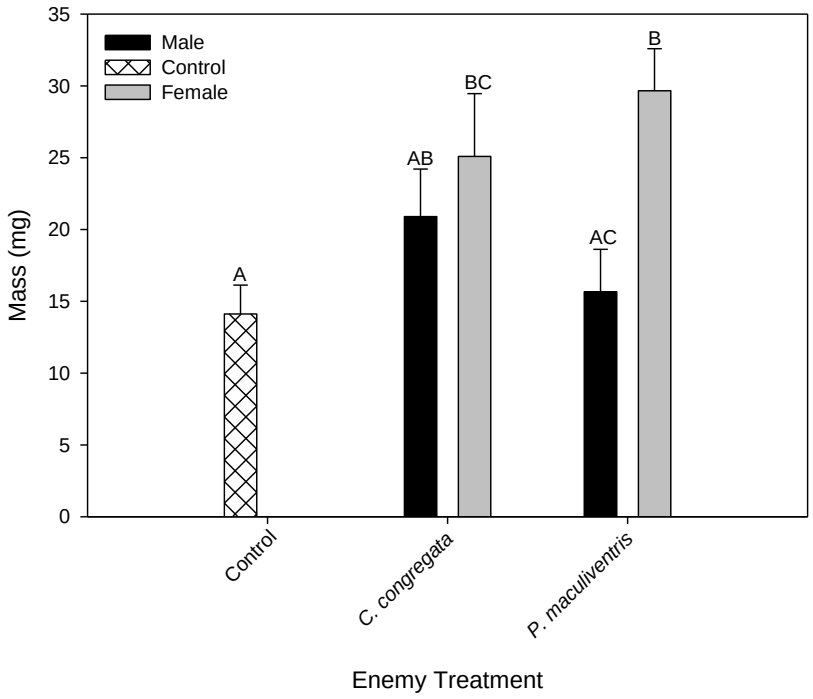


Figure 3.11 Mean 7 Day Caterpillar Mass by Predator Treatment (+ S.E.)

Letters denote significant differences between treatment.

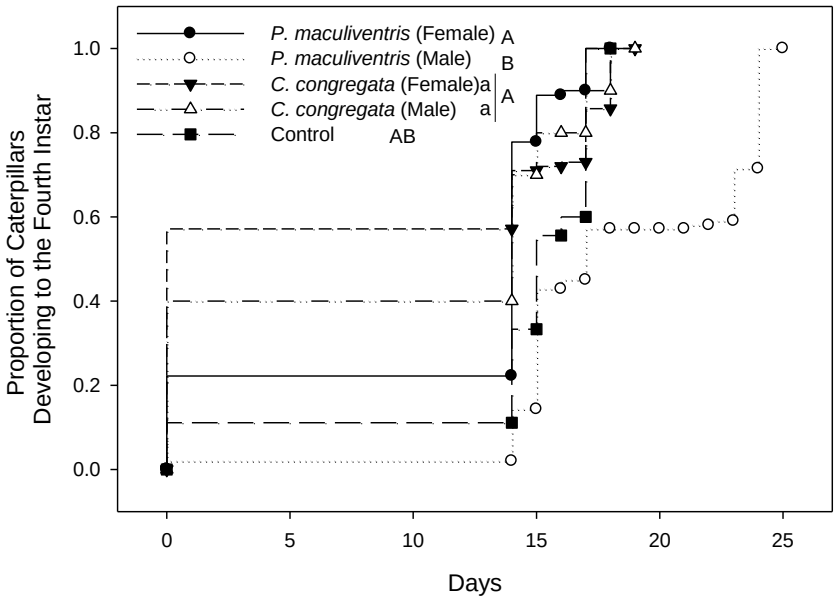


Figure 3.12 Caterpillar Development in the Presence of Natural Enemies

Letters denote significant differences between enemy treatment.

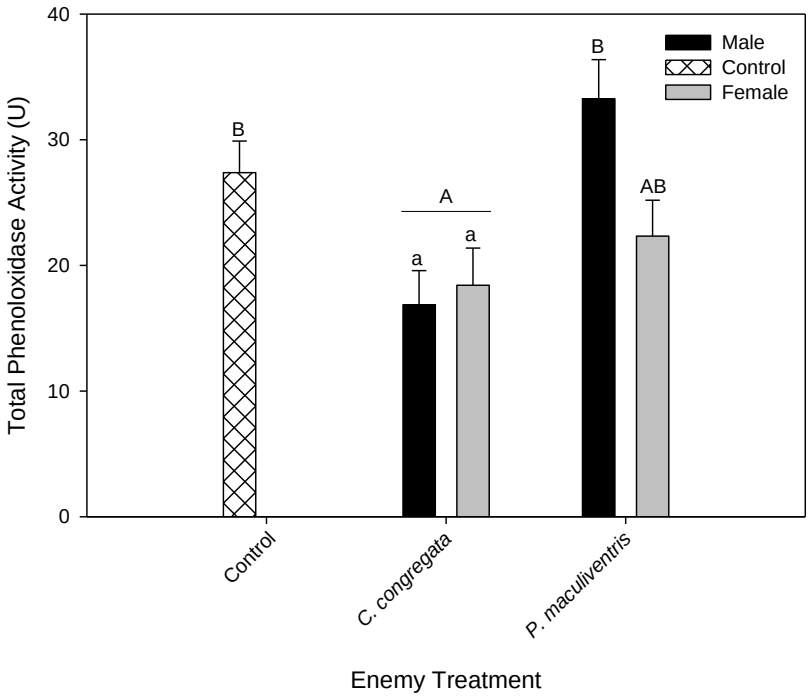


Figure 3.13 Mean Total Phenoloxidase Activity by Enemy Treatment (+ S.E.)

Letters denote significant differences between enemy treatments.

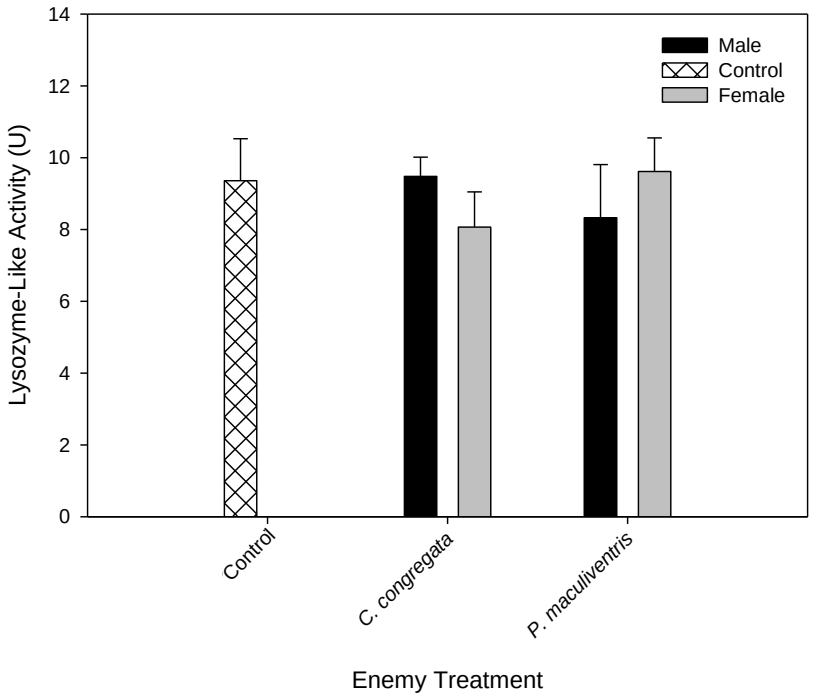


Figure 3.14 Mean Lysozyme-Like Activity by Enemy Treatment (+ S.E.)

Table 3.1 Protein (P), Carbohydrate (C), and Cellulose (Cell)
Composition of Nutritional Stoichiometry Diets

	Percent Total Digestible Nutrients in Diet (g)					
Protein:Carbohydrate	20%		40%		60%	
1:4	P:	9.12	P:	18.24	P:	27.36
	C:	36.48	C:	72.96	C:	109.40
	Cell:	133.40	Cell:	87.80	Cell:	42.20
1:1	P:	22.80	P:	45.60	P:	68.4
	C:	22.80	C:	45.60	C:	68.40
	Cell:	133.40	Cell:	87.80	Cell:	42.20
4:1	P:	36.48	P:	72.96	P:	109.40
	C:	9.12	C:	18.24	C:	27.36
	Cell:	133.40	Cell:	87.80	Cell:	42.20

Table 3.2 2 Contingency Table of Sephadex Bead Encapsulation Across Solanaceous Host-Plants

Plant Species	Low	Medium	High	**Total**
C. annuum	0	0	6	6
P. pruinosa	0	1	4	5
S. carolinense	0	0	5	5
S. melongena	0	0	4	4
S. dulcamara	0	0	6	6
S. nigrum	0	1	5	6
S. tuberosum	0	0	8	8
S. lycopersicum	0	2	4	6
N. tabacum	0	0	6	6
D. stramonium	0	2	4	6
Total	0	6	52	58

Table 3.3 Contingency Table of Sephadex Bead Melanization Across Solanaceous Host-Plants

Plant Species	Low	Medium	High	**Total**
C. annuum	4	2	0	6
P. pruinosa	5	0	0	5
S. carolinense	5	0	0	5
S. melongena	3	1	0	4
S. dulcamara	5	1	0	6
S. nigrum	5	1	0	6
S. tuberosum	5	3	0	8
S. lycopersicum	4	2	0	6
N. tabacum	5	1	0	6
D. stramonium	6	0	0	6
Total	47	11	0	58

Table 3.4 ANOVA Table for Nutritional Diet Stochiomentery on Hornworm Mass

	DF	Sum Sq	Mean Sq	*F* value	P (>F)
Carbs	1	1.0	1.0	0.05	0.826
Protein	1	2187.1	2187.1	104.24	< 0.001
Nicotine	1	370.7	370.7	17.67	< 0.001
Carbs:Protein	1	0.7	0.7	0.04	0.852
Carbs:Nicotine	1	242.4	242.4	11.56	< 0.001
Protein:Nicotine	1	31.0	31.0	1.48	0.226
Residuals	137	2874.5	21.0		

Table 3.5 ANOVA Table for Nutritional Diet Stochiomentery on Hornworm PO Activity

	DF	Sum Sq	Mean Sq	*F* value	P (>F)
Carb	1	466	465.9	2.44	0.122
Protein	1	14.0	14.0	0.07	0.787
Nicotine	1	1667	1676.6	8.78	0.004
Residuals	85	16224	190.9		

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CHAPTER 4. DOMESTICATION OF PEPPER, *CAPSICUM ANNUUM*, ENHANCED NATURAL ENEMY RECRUITMENT TO HERBIVORE-DAMAGED PLANTS

4.1 Introduction

The plant domestication-reduced defense hypothesis proposes that domesticated crops are more susceptible to insect herbivores than their wild relatives (Gaillard et al. 2017). This change in susceptibly is thought to have occurred in two ways: first, through an inadvertent loss of direct plant defenses, likely due to selection by crop breeders for yield or palatability for human consumption; and second, due to inadvertent changes in plant volatile organic compounds (VOCs hereafter), impairing plant signaling of natural enemies. These unintended consequences due to anthropomorphic selection that lowers plant resistance to herbivores are now well documented in the literature (reviewed in Chen et al. 2015a; Whitehead et al. 2017); however, despite the presumed generality of this hypothesis, results are highly crop dependent. In one of the most thorough examinations of this topic to date, 29 wild-domesticated crop pairings were experimentally challenged by generalist herbivores from different feeding guilds (Turcotte et al. 2014). This study found that crops, overall, were more vulnerable to insects compared to their wild relatives, but certain wild-crop pairings showed the opposite pattern. For example, aphid reproduction was significantly higher on wild than domesticated lettuce. A limitation of this study, however, is that it uses generalist herbivores, which cause less damage and are typically less ecologically relevant than coevolved specialists (Harvey et al. 2015). Importantly, a recent series of experiments found that in maize generalist insects are far more vulnerable to reductions in performance on wild progenitors (teosinte) than specialists. These data collectively indicate that wild-crop comparisons should ideally be performed using specialized herbivores and consider the possibility that insects sometimes perform *worse* on the crop.

The results of the few studies that have examined the effect of crop domestication on tritrophic interactions are also mixed and highly plant-herbivore-enemy specific. In sunflower, for instance, domestication increased the abundance of the seed-feeding moth, *Homoeosoma*

electellum, compared to wild populations due to differences in attack rates by its main parasitoid, *Dolichogenidea homoeosomae* (Chen et al. 2007). Agriculturally produced sunflowers have tougher seed coats than their wild counterparts, preventing the wasp ovipositor from penetrating and providing a structural refuge for the pest. The evolutionary lag in parasitoid traits due to rapid selection on plants resulted in an ecological mismatch, destabilizing this plant-herbivore-parasitoid interaction. However, other studies have reported the opposite pattern (i.e., higher natural enemy attack on domesticated relatives) in comparable systems. In wild/cultivated bean seeds (*Phaseolus vulgaris*), the wasp *Stenocorse bruchivora* performed better and was more likely to parasitize hosts (the beetle *Zabrotes subfasciatus*) on cultivated than wild seeds (Campan & Benrey 2004). This was attributed to the fact that hosts from cultivated beans were larger and developed faster. These outcomes illustrate that the specific plant traits responsible for differences in enemy attack are variable across crops and depend in large part on the life history of the insects involved.

Although the plant traits (e.g., toxins, VOCs, trichomes) responsible for domestication-mediated differences in enemy attack rates are unknown in most instances (Chen et al. 2015b; Whitehead et al. 2017), functional differences in enemy orientation to prey due to changes in plant volatile signaling is considered an overwhelmingly crucial aspect of tri-trophic relationships. The plant domestication-reduced defense hypothesis stipulates changes in plant VOCs impairing recruitment of natural enemies, but *only a few systems (e.g., mustards, maize) have been evaluated. In Brassica sp., the parasitoid Cotesia glomerata* alighted more frequently on domesticated mustard, *Brassica nigra*, over wild accessions of this species and the co-occurring wasp *Diadegma semiclausum* also preferred the domesticated *Brassica juncea* (Gols et al. 2009). Both parasitoids seemed to prefer plants on which their hosts performed the best, independent of domestication history. Work in a companion system using the parasitoid *Cotesia rubecula* on *Brassica oleracea* showed the opposite result: *C. rubecula* was more attracted to wild over cultivated cabbage, with headspace analysis implicating differences in isothiocyanates as the chemical mechanism underlying changes in wasp recruitment (Gols et al. 2011).

Maize domestication is the most well-studied model system investigating the impact of domestication on crop VOCs. This work has shown remarkably compromised volatile signaling

due to crop breeding. European varieties of maize produce the sesquiterpene (E)-β-caryophyllene while their North American counterparts lost the ability to do so. As a result, maize varieties unable to synthesize (E)-β-caryophyllene received higher rates of rootworm, *Diabrotica virgifera virgifera*, damage compared to crop lines able to produce this chemical (Rasmann et al. 2005; Köllner et al. 2008; Degenhardt et al. 2009). This was due to the inability of the VOC-deficient lines to recruit entomopathogenic nematodes to infect beetle larvae. Experimentally augmenting the soil near these impaired varieties with (E)-β-caryophyllene rescued this ecological interaction, decreasing beetle emergence. Corn volatile signaling is also impaired aboveground. Oviposition by the stemborer moth, *Chilo partellus*, induces VOC responses that attract the egg and larval parasitoid, *Trichogramma bournieri* and *Cotesia sesamiae*, respectively, but this tri-trophic interaction occurs mainly on landraces rather than commercial hybrid varieties (Tamiru et al. 2011; Tamiru et al. 2015; Tamiru et al. 2017).

Last, recent work in tomato provides evidence that diverse natural enemies, including the parasitoid *Cotesia congregata* and egg predator *Jalysus wickhami*, are more attracted to damage-induced VOCs from wild *Solanum* sp. than landrace or domesticated tomatoes (Li et al. 2017). However, a shortcoming of this study is the lack of data on functional differences in VOC blends from different plant-types, which prevents a mechanistic interpretation of the causative factors mediating this interaction. Here, I expand on this work to include other species of nightshades in the Solanaceae—one of the most agriculturally important plant families worldwide—focusing on a poorly studied group, the peppers (*Capsicum* sp.). *C. annum* has a relatively clear domestication history with numerous wild accession lines available and cultivars that have specifically been selected for their pungency because of varying capsinoid content. I tested attraction of the parasitoid, *Cotesia congregata*, to plants damaged by its host, *Manduca sexta*, in small-scale lab behavioral assays, and large cage foraging enclosures to measure parasitoid efficiency. Per the plant domestication-reduced defense hypothesis, I predicted that hornworms would perform better on domesticated compared to wild plants and wasps would have reduced attraction and parasitism efficiency on domesticated compared to wild peppers.

4.2 Materials and Methods

4.2.1 Study System

Wild *C. annuum* is a perennial shrub that prefers shaded highland areas and ranges from the southern United States of America to Columbia (Bosland & Iglesias 1992; Hayano-Kanashiro et al. 2016 and references therein). This plant is notable for its pungent fruits containing capsinoids, mainly capsaicin and dihydrocapsaicin, which are used as a culinary flavoring globally (Hayano-Kanashiro et al. 2016; Pickersgill 2016). These compounds also have antibacterial and antioxidative properties (Luo et al. 2011; Zimmer et al. 2012), functioning in nature as a defense against fungal pathogens and a directed defense and selective seed filter for preferential dispersal by birds over mammals (Tewksbury & Nabhan 2001; Tewksbury et al. 2008). Birds lack the chemoreceptors for capsinoids and passage through their intestinal track greatly increases the rate of seed germination compared to mammals (Tewksbury & Nabhan 2001; Tewksbury et al. 2008). Interestingly, natural polymorphisms exist for fruit capsinoid content in wild peppers due to a resource allocation cost for maintaining this chemical plant defense against fungal pathogens and mammalian herbivores when water is scarce (Haak et al. 2012). All cultivated peppers comprise the species *C. annuum* var. annuum while the accepted wild progenitor given current ecological, genomic, and anthropological evidence of this crop is *Capsicum annuum* var. glabriusculum (Kraft et al. 2014; Qin et al. 2014). *C. annuum* was domesticated multiregionally in central-east Mexico during the mid-Holocene with most varieties arising by the time of the conquest of Latin America by the Conquistadors (Clement et al. 2010; Qin et al. 2014; Pickersgill 2016).

Manduca sexta (Lepidoptera: Sphingidae), the tobacco hornworm, ranges from Canada to South America and overlaps with all of the native range of wild *C. annuum*. Adults in natural populations have a high affinity for tomatoes and tobacco, making *M. sexta* a common pest in home gardens and agricultural fields (Ashmead 1887; Madden & Chamberlin 1945). This lepidopteran however is a broad specialist on plants in the Solanaceae and capable of feeding on over 30 plant species (Madden & Chamberlin 1945; Yamamoto & Fraenkel 1960; chapter 2 of this dissertation) including *Capsicum* sp. The native host plant of *M. sexta* is believed to be *Nicotiana attenuata* (Kessler & Baldwin 2001) but in the southwestern United States and

Mexico, where wild tobacco is scarce or absent, this insect is abundant on *Datura wrightii* (Riffell et al. 2008; Reisenman et al. 2010).

Upon herbivore egg oviposition and subsequent feeding by insect larvae, plants induce chemical defenses and emit specific plant VOCs that attract natural enemies, like predators and parasitoid wasps (Paré & Tumlinson 1999; Thaler 1999; Van Poecke et al. 2001; Kessler & Baldwin 2001; Turlings & Wäckers 2004; Heil 2008). One of the main natural enemies of *M. sexta* is the parasitoid wasp *Cotesia congregata*. *C. congregata* is a gregarious parasitoid wasp that attacks mid-late larval instars of *M. sexta*, and has been reported to parasitize 12 sphingid species (Gilmore 1938a, b; Fulton 1940; Lawson 1959). This parasitoid has been recorded over most of the Midwest, Eastern, Southern, and Gulf Coast of the United States in addition to Canada, Brazil, and Jamaica. From personal observations, *C. congregata* does not survive long in arid climates, which might explain its absence in some areas of Mexico and the Southwestern United States. A common garden experiment (chapter 2 of this dissertation) using wild field population of *M. sexta*, wild and domesticated peppers, and a natural *C. congregata* population did observe caterpillar parasitism on peppers, although rates were low.

4.2.2 Plants and Growing Conditions

Plant accession lines and cultivars used during experiments are listed in Table 4.1 with accompanying demographic information and providers. Wild plant accessions over a latitudinal gradient were taken to represent different plant populations. Domesticated pepper cultivars were chosen because of their agricultural importance and variation in fruit capsinoid content representing an anthropomorphic selection gradient. To increase germination, all seeds were treated with a 20% bleach (NaOCl) solution for 10 minutes and then left to dry before being sowed. Plants for herbivore performance and initial parasitoid preference assays were grown under greenhouse conditions (~27 ˚C ± 5; supplemented with a 16:8 LD cycle). Seeds were collectively planted by cultivar/accession in 10 cm pots (International Greenhouse Company, Danville, IL) in metro germination mix (Sun Gro Horticulture, Agawam, MA) and placed under a humidity dome (International Greenhouse Company, Danville, IL) for one week with an initial dose of Osmocote 14-14-14 slow release fertilizer (The Scotts Miracle-Gro Company, Marysville, Ohio) at a ratio of 15 mL:1 pot in a scientific incubator (Percival Scientific, Perry,

IA) on a 16:8 LD cycle at 25˚C and 50% RH. Seeds that successfully germinated were then thinned and individually separated into 15 cm pots (International Greenhouse Company, Danville, IL) four weeks later and allowed to grow under greenhouse conditions (~27 ˚C ± 5; supplemented with a 16:8 LD cycle) until they were used in experiments at three months of age. Plants for parasitoid efficiency experiments were grow as previously described above except under artificial light with a 16:8 LD light cycle in a semi-controlled bioclimatic room (~25 ˚C) and transplanted in 10 cm pots (International Greenhouse Company, Danville, IL) until they were three months old at which point they were also used in experiments.

4.2.3 Laboratory Insect Colonies

A more thorough explanation of insect colony husbandry is documented in chapter two of this dissertation, so a brief review is only covered here. For *Manduca sexta*: An outbred colony of *M. sexta* was established in 2013 to avoid issues associated with inbred laboratory colonies. The laboratory rearing regime is similar to other lab colonies. Eggs were collected from a moth mating cage and allowed to hatch communally onto a commercially available wheat germ based diet (Southland Products Inc., Lake Village, AR). Once neonates were three days old they were isolated in individual 44 mL cups. Upon molting to the fourth instar caterpillars were transferred to 96 mL cups. Caterpillars were provided artificial diet *ad libitum* throughout their larval stage. Larvae were reared in a scientific incubator (Percival Scientific, Perry, IA) on a 16:8 LD cycle at 25˚C and 25% RH. Once caterpillars become pharate and began their wandering stage, they were transferred to a pupation block and then to a climate and light controlled incubator (VWR, Radnor, PA) on a 0:24 LD cycle at 25˚C and 25% RH. Once adults eclosed, the moths were transferred to a 61 cm cube collapsible field cage (BioQuip Products, Rancho Dominguez, CA) kept in an insect rearing room on a 16:8 LD cycle at 25˚C. These conditions facilitate mating. Moths were provided a 20%:10% sucrose:honey solution (w/v) and a solanaceous plant for oviposition.

For *Cotesia congregata*: the laboratory wasp colony was initially derived from individuals provided by Dr. Karen Kester in 2012 (origin information contained in Bredlau et al. 2013) and was supplemented with wasps every field season from the local population by collecting parasitized *M. sexta* larvae with egressed wasp cocoons on their cuticle. To maintain

the parasitoid stock, wasps were presented with third to fourth instar caterpillars and watched until they were stung. Parasitized *M. sexta* were reared as described above until wasp larvae egressed from the caterpillar's body (~two to three weeks). Following cocoon spinning by the wasps, cocoons were gently harvested from the hornworm body and placed in a 30.5 cm cube mesh rearing cage (BioQuip Products, Rancho Dominguez, CA). Adults were provided with cotton macerated in DI water and a 20%:10% sucrose:honey solution (w/v), and maintained in a scientific growth chamber (Percival Scientific, Perry, IA) on a 16:8 LD cycle at 25˚C and 65% RH.

4.2.4 Hornworm Development and Performance on Domesticated and Wild Peppers

Hornworm growth rate was taken as a proxy for performance, while development time was measured as the number of days until caterpillars molted into the 4th instar. The 4th instar is ecologically relevant because after the 4th instar *M. sexta* becomes much less vulnerable to parasitism by *C. congregata*. To accomplish this, 12 week old crop (Sweet pepper, Golden California Wonder) and wild (Chiltepin pepper, Mexico PI 281372) plants were infested at an initial density of 8 newly hatched (< 24 hours old) neonates/plant, with nine plant individuals each for the crop and wild pepper. Due to resource constraints, only the two aforementioned pepper varieties were studied in this experiment and the subsequent parasitoid preference assay. These two plants were chosen because they represent the most derived selected pepper (containing almost no capsinoids in its fruit) and the accession line closest to the area of origin of domestication for this crop, thereby encompassing the broadest amount of variation in the wild-domesticated plant pair. Caterpillars were able to feed *ad libitum* and the development time to the 4th instar and weight at 8 days post-hatching were recorded (Mettler Toledo, Greifensee, Switzerland).

4.2.5 Parasitoid Preference on Domesticated and Wild Peppers

Parasitoid preference was assessed using a modified methodology from Fatouros et al. (2008) measuring the cumulative retention time of parasitoids in a 4-way choice test on excised leaf samples from crop and wild peppers that had been undamaged or damaged by feeding from *M. sexta*. To do so, 10 mm leaf discs were collected immediately before use from locally damaged leaves of plants used in the herbivore performance experiment and control plants. Leaf

discs were then arranged equidistant from one another on lightly moistened filter paper in an open air area consisting of a 100 x 15 mm petri dish, and placed in a shallow Styrofoam container to provide a uniform background from all sides except above. Female wasps from the laboratory colony that were ca. 1-2 weeks old were presented leaf samples in this open air arena (Figure 4.1) and allowed 5 minutes to forage on leaf samples. The cumulative time spent on each plant by damage treatment was recorded for each wasp replicate. These behavioral choice assays were performed in the laboratory under artificial florescent lighting, one meter in front of a chemical fume hood to provide directional airflow and between the hours of 10 am and 6 pm. Plant discs were rotated randomly between each wasp replicate and new plant tissue from different plant individuals was collected after every 10 wasp trials (n = 9 plant/damage pairings and n = 96 wasps assayed total). Wasps that did not choose any plant discs in the assay were censured and removed from the statistical analysis (n = 8). This data was collected over the course of four consecutive days with 24 wasps measured each day.

4.2.6 Parasitoid Efficiency on Domesticated and Wild Peppers

The above short-term behavioral foraging experiment was complimented with a more realistic large scale trial where wasps could fly between intact plants (vs. walking between neighboring leaf discs). Plants used in these tests include all 14 peppers listed in Table 4.1. For each replicate, one of each of the 14 pepper cultivars/accessions lines were moved into a 2 x 3 x 2 m chiffon mesh cage and placed in shallow 2 L plastic containers (Rubbermaid, Atlanta, GA) filled with water housed within the Entomology Environmental Laboratory Greenhouse complex. Plants were spaced ~60 cm equidistantly apart so individuals were not touching and infested at a density of three newly molted 3rd instar caterpillars per plant. Caterpillars were able to feed *ad libitum* for 24 hours before the assay to induce plant volatiles. Female wasps that were 1-2 weeks old were released during the evening (8 to 10 pm) while temperatures were cooler at a density of 1 wasp: 7 caterpillars (6 wasps total) in the cage and allowed to forage for the subsequent 60 hours. The plastic containers with water prevented caterpillars that fell off of plants from migrating between pepper individuals; those that did fall were removed from the statistical analysis. Caterpillars were collected at the end of the trials and reared on artificial diet per the methodology outlined above until parasitoids egressed, became pharate, or died. All

caterpillars where wasps did not egress were dissected to check for parasitism. This experiment was replicated twice.

4.2.7 Statistical Analyses

All statistical analyses were run using the open source R statistical software 3.3.3 3 (R Core Team, 2017). For herbivore performance, the nonparametric Kruskal–Wallis H-test was administered to determine differences between treatments. Differences between hornworm development was examined by conducting a survival analysis with the survminer package followed by pairwise comparisons between cultivation history with a log-rank test. For wasp preference, the nonparametric Kruskal–Wallis H-test followed by a multiple comparison test between plant cultivation history and herbivore induction using the kruskalmc function in the pgirmess R package to establish hieratical rankings among choices was conducted to assess significance. For wasp efficiency, an ANOVA was conducted on the proportion of parasitized caterpillars by domestication history crossed with cultivar/accession identity; however, cultivar/accession returned non-significant results so all cultivars and accession were pooled by domestication history and a nonparametric Kruskal–Wallis H-test was again administered to test for differences between treatments.

4.3 Results

4.3.1 Herbivore Performance on Wild and Domesticated Peppers

Herbivore mass did not vary by pepper cultivation history (χ^2(*d.f.* = 1, n = 18) = 0.09, p = 0.757; Figure 4.2). Likewise, herbivores did not vary in their development time on wild vs domesticated peppers (χ^2 (*d.f.* = 1, n = 56) = 0.12, p = 0.731; Figure 4.3).

4.3.2 Wasp Preference and Efficiency on Wild and Domesticated Peppers

Wasp preference in 4-way choice tests revealed significant differences among treatments (Figure 4.4). Notably, the cumulative proportion of time spent on domesticated herbivore damaged leaf discs was different from the other three treatments (χ^2(*d.f.* = 3, n = 88) = 11.06, p = 0.011). This outcome mirrored the result from the parasitism efficiency assay where hornworms reared on domesticated pepper plants had a higher proportion of individuals parasitized as opposed to individuals which fed on wild pepper accession lines (χ^2(*d.f.* = 1, n = 18) = 3.92, p = 0.048). Hornworms on domesticated pepper had more than twice the proportion of individuals parasitized compared to caterpillars on wild plants (Table 4.2 & Figure 4.5).

4.4 Discussion

Hornworm larval performance was not different on wild compared to crop peppers suggesting that domestication in peppers did not result in a loss of direct resistance to this herbivorous insect. This implies that wild and domesticated peppers are phytochemically similar in their foliar tissues, and plant signaling pathways of domesticated peppers are still capable of responding to herbivore damage. However, plant nutritional quality and defensive pathways, such as protease inhibitors and polyphenol oxidases, were not measured directly in this study. These findings might be due to selection on fruit chemical properties, such as capsinoid content. As a result, foliar tissue quality might not be directly impacted, although there is some evidence suggesting the chemical profile of these tissues are connected in other plant species.

Whitehead & Bowers (2013) tested the competing evolutionary hypotheses that secondary metabolites in fruit are adaptive for selective seed dispersal (such as in *C. annuum* as described in Tewksbury & Nabhan 2001 and Tewksbury et al. 2008) vs. a pleiotropic effect due to selection on leaves from herbivores for plant defense in *Lonicera* × *bella* (Belle's bush honeysuckle), a plant that also has vertebrate dispersed fruit. The authors concluded that secondary metabolite concentrations in the fruit of *Lonicera* × *bella* could not be explained only by foliar defense but that these compounds were also not entirely independent between the reproductive and foliar tissues. Borrowing from the literature detailing changes in secondary metabolite allocation between foliar and fruit tissue upon herbivore attack also illustrates the interconnectedness of these plant tissues. In *Hamelia patens* (Rubiaceae), plants that received herbivore damage or a methyl jasmonate treatment had decreased fruit removal over controls due to higher secondary metabolite concentrations, which decreased fruit palatability to frugivores (Whitehead & Poveda 2011). Experiments using *Piper reticulatum* (Piperaceae), a wild species related to the economically important black table pepper (*P. nigrum*), however, showed no correlation between foliar and reproductive plant defenses (Whitehead et al. 2013). Activation of foliar plant defenses against herbivores did not change secondary metabolite allocation in fruits, nor did activation of plant defenses against granivores impact the chemical compounds in leaves, which would imply independent selection between reproductive and foliar tissues in *P. reticulatum* (Whitehead et al. 2013). In other related solanaceous species foliar herbivory causes

increased secondary metabolite concentrations in reproductive tissues. Both *Nicotiana tabacum* and *Nicotiana attenuata* increase nicotine concentration in reproductive tissues (flower nectaries and capsules) upon leaf damage from *M. sexta* (Baldwin & Karb 1995; Adler et al. 2006). This indicates that secondary metabolite concentrations between plant tissues are linked in *Nicotiana* sp. but this phenomenon likely operates as a plant defense against granivores (Stanton et al. 2016) since their seeds are not vertebrate dispersed as in the previously described systems. *C. annuum* is unique among the mentioned plant systems in that the fruit secondary metabolites, capsaicin and dihydrocapsaicin, in peppers act as a pathogen defense and selective seed filter for vertebrate dispersal (Tewksbury & Nabhan 2001; Tewksbury et al. 2008) and the foliar tissue of wild and crop peppers contain no capsinoid content (B. Benrey, *personal communication*). This could uncouple selection on secondary metabolite concentrations in the foliar and reproductive tissues, but a dedicated study such as Whitehead & Bowers (2013) should be undertaken for confirmation.

In addition, domestication in peppers improved attraction and recruitment of natural enemies. Wasps had higher retention time on damaged domesticated peppers over undamaged plants and damaged wild peppers. Further, parasitism in laboratory efficiency assays was higher on domesticated compared to wild peppers. The mechanism facilitating improved wasp recruitment and efficiency is most likely changes in VOCs (Gols et al. 2009), so future studies should focus on determining the functional differences of the HIPV blends from wild and domesticated peppers as was conducted in the maize and *Brassica* systems (Rasmann et al. 2005; Köllner et al. 2008; Degenhardt et al. 2009; Gols et al. 2009; Gols et al. 2011; Tamiru et al. 2011; Tamiru et al. 2015; Tamiru et al. 2017). This would elucidate the causative factors altering this plant-insect-parasitoid interaction to determine if specific VOCs have been added to the HIPV profile, the ratio or abundance of compounds are different, or if both are altered causing these improved fitness benefits of indirect plant defenses on signaling and natural enemy recruitment. Evidence from a current meta-analysis by Rowen & Kaplan (2016) suggest that crops release more VOCs upon herbivore damage but have a reduced volatile profile compared to their wild counterparts. Thus, speculatively, crop peppers might produce more VOCs but have no changes in their volatile profile composition in order to increase natural enemy recruitment.

Given these findings I reject the plant domestication-reduced defense hypothesis in this system. For this plant domestication complex it seems that domestication resulted in increased parasitism of *M. sexta* by *C. congregata*. Whether this applies to other insect pests of peppers and their assemblage of natural enemies should be investigated to examine the generality of this finding. Currently the only crop to have been investigated for multiple pest-enemy pairs is maize (Rasmann et al. 2005; Köllner et al. 2008; Degenhardt et al. 2009; Tamiru et al. 2011; Tamiru et al. 2015; Tamiru et al. 2017); thus, future work in peppers and other crops should expand to focus on guilds of insects such as phloem-feeders like aphids and raspers like thrips to determine pest performance on host plants and attraction of the pests' natural enemies.

4.5 Figures and Tables

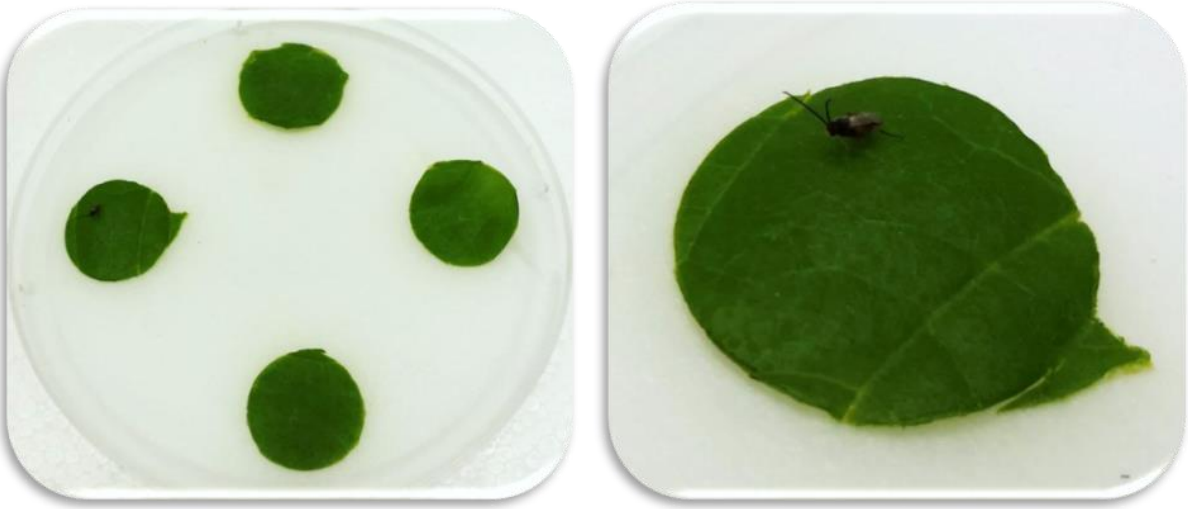

Figure 4.1 Wasp Choice Arena

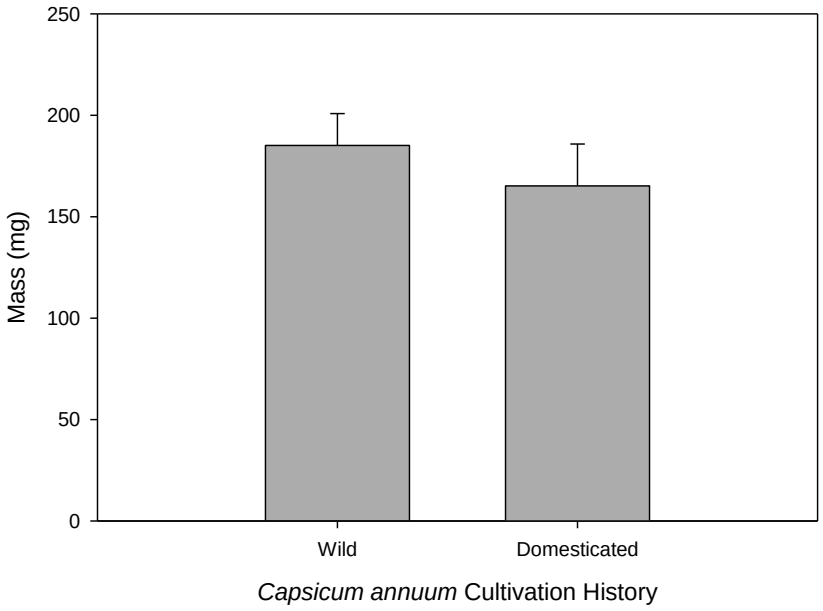

Figure 4.2 Mean 8 Day Caterpillar Mass by Pepper Treatment (+ S.E.)

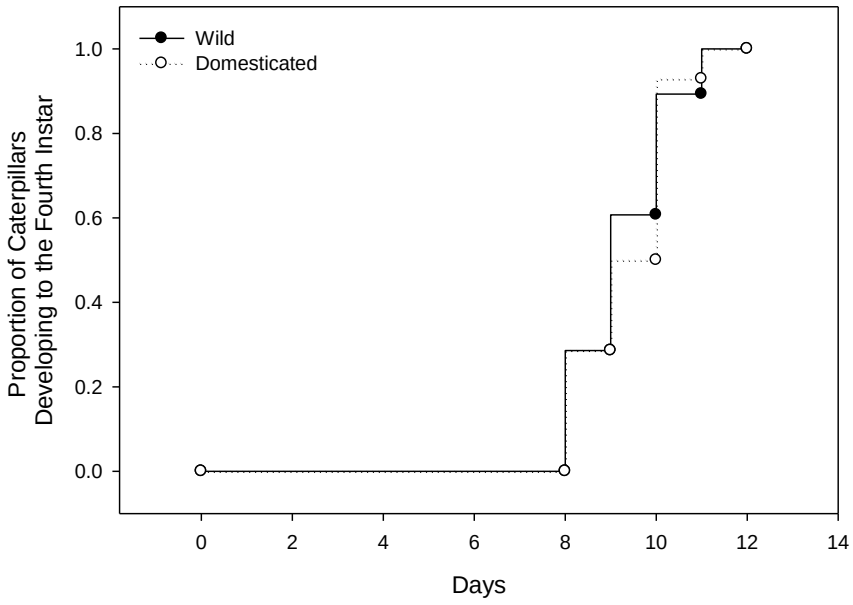

Figure 4.3 Caterpillar Development on Wild vs Domesticated *C. annuum*

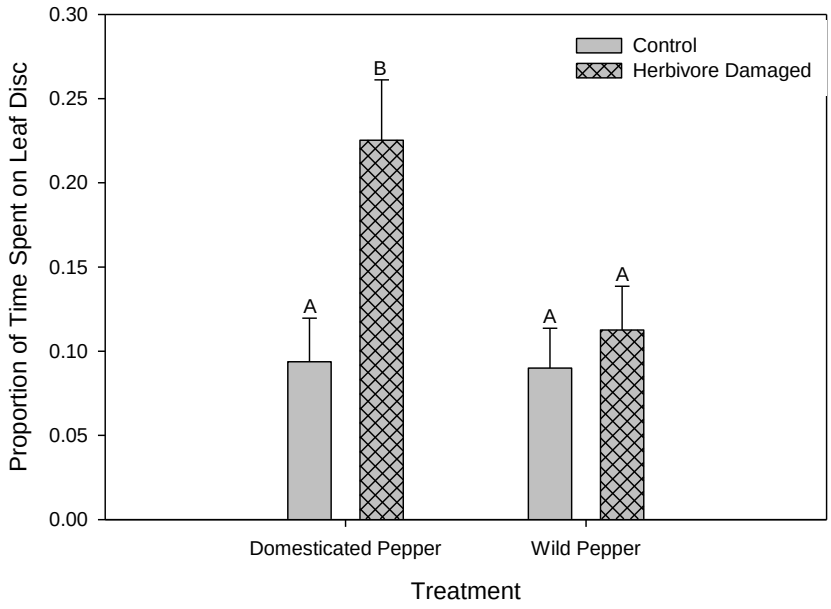

Figure 4.4 Cumulative Proportion of Time Spent on Leaf Disc Treatments by Wasps (+ S.E.)

Letters denote significant differences between treatments.

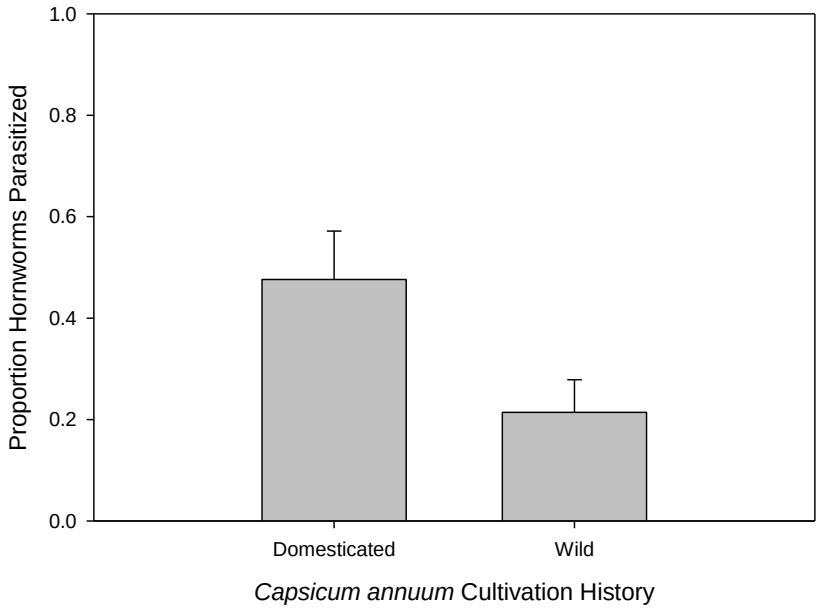

Figure 4.5 Proportion Parasitized Hornworms by Pepper Cultivation (+ S.E.)

Table 4.1 Pepper Species including Location of Origin/Common Name, Cultivation, & Provider

** denotes varieties used in commercial production*.

Species Name	Origin or Common Name (Company Catalogue #)	Cultivation	USDA Accession # or Seed Provider
Capsicum annuum var. annuum	Hot pepper, Ancho (52498A)	Domesticated*	Burpee Seed Company W. Atlee Burpee & Co., 300 Park Ave, Warminster, PA 18974
Capsicum annuum var. annuum	Hot pepper, Cayenne (62195A)	Domesticated*	Burpee Seed Company W. Atlee Burpee & Co., 300 Park Ave, Warminster, PA 18974
Capsicum annuum var. annuum	Sweet pepper, Golden California Wonder	Domesticated*	NE Seed 122, Park Ave, Building H, East Hartford, CT 06108
Capsicum annuum var. annuum	Hot pepper, Jalapeno (62687A)	Domesticated*	Burpee Seed Company W. Atlee Burpee & Co., 300 Park Ave, Warminster, PA 18974
Capsicum annuum var. annuum	Sweet pepper, Pimento (64605A)	Domesticated*	Burpee Seed Company W. Atlee Burpee & Co., 300 Park Ave, Warminster, PA 18974
Capsicum annuum var. annuum	Hot pepper, Serrano (50534A)	Domesticated*	Burpee Seed Company W. Atlee Burpee & Co., 300 Park Ave, Warminster, PA 18974
Capsicum annuum var. annuum	Hot pepper, Thai Hot (62600A)	Domesticated*	Burpee Seed Company W. Atlee Burpee & Co., 300 Park Ave, Warminster, PA 18974
Capsicum annuum var. glabriusculum	Chiltepin pepper, Guatemala, Retalhuleu Department	Wild	PI 631135
Capsicum annuum var. glabriusculum	Chiltepin pepper, Guatemala, Zacapa Department	Wild	PI 631139
Capsicum annuum var. glabriusculum	Chiltepin pepper, Numex Bailey Piquin, USA	Wild*	PI 640726
Capsicum annuum var. glabriusculum	Chiltepin pepper, Mexico	Wild	PI 281372
Capsicum annuum var. glabriusculum	Chiltepin pepper, Navassa Island, USA Territory	Wild	PI 639127
Capsicum annuum var. glabriusculum	Chiltepin pepper, Nicaragua	Wild	PI 311126
Capsicum annuum var. glabriusculum	Chiltepin pepper, Nicaragua	Wild	PI 406948

Table 4.2 Parasitoid Efficiency on Domesticated and Wild Peppers

Pepper Cultivar/ Accession Line	Parasitism: $\frac{\text{Parasitized Individuals}}{\text{Total Individuals}}$
Ancho	3/6
Cayenne	3/6
Golden California Wonder	2/5
Jalapeno	3/5
Pimento	2/6
Serrano	2/5
Thai Hot	2/5
Guatemala (PI 631135)	1/4
Guatemala (PI 631139)	3/5
Mexico (PI 281372)	1/6
Nicaragua (PI 311126)	2/6
Nicaragua (PI 406948)	1/5
USA (PI 639127)	0/6
USA (PI 640726)	0/6

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CHAPTER 5. CONCLUSION

You are what you eat. But what you choose to ingest, at what time, and the benefits these foods bestow, given your current physiological state, can be challenging to explain. Here, I presented evidence that natural enemies help maintain a negative preference-performance relationship for the specialist herbivore, *Manduca sexta* and its solanaceous host plants. This relationship is maintained through plant-mediated effects altering *M. sexta*'s physiology that increases resistance to natural enemies. I then incorporate human intervention as a fourth selective force on trophic interaction to explore how anthropomorphic selection altered plant interactions with insect herbivores and parasitoids.

Positive ovipositional preference-physiological performance relationships are inherently more obvious because they increase growth and development of offspring. Here I add to the few examples that demonstrate the contrary and reinforce other researcher's findings supporting the argument that negative preference-performance relationships can be maintained by increasing survival and protection against natural enemies in the environment. Through inter-disciplinary research connecting the fields of insect ecology and physiology I was then able to provide a subsequent explanation for the adaptive value of this interaction. While secondary metabolites from plants in herbivores can be co-opted as a defense to deter attack from natural enemies, I showed that specific secondary metabolites affect herbivore physiology differently by demonstrating nicotine to be immunotherapeutic in *M. sexta*, a finding which currently has only been recorded in one other insect, *Junonia coenia*. Further, upon studying non-consumptive effects of natural enemies on *M. sexta* in the presence of *C. congregata* and the spined soldier bug, *Podisus maculiventris*, my work showed that *M. sexta* generally accelerates their development in the presence of natural enemies at the cost of some immune defenses, mainly lowered phenoloxidase activity but not lysozyme activity, implying a resource allocation tradeoff to physiological development and immunity. Few studies have shown changes in physiological traits due to predation risk so this work greatly expands on our understanding of the burden that stress places on insect herbivores. These results taken together in a community level context transform our view of plant-insect-enemy interactions by showing how utilizing a food resource

in parasite burdened habitats increases direct resistance to parasites while also improveing one's own immune activity, even at the cost of development. As a result, herbivores can mitigate the consumptive and nonconsumptive effects natural enemies have on their physiology.

Last, I expanded on our current understanding of tritrophic interaction by incorporating an obvious fourth selective force, humanity, to explore the consequences of human intervention through crop domestication on plant-insect-enemy interactions centered on the chili pepper (*Capsicum annuum*), *M. sexta,* and the parasitoid wasp *C. congregata*. This work showed that anthropomorphic selection through plant domestication in peppers enhanced parasitoid recruitment to *M. sexta* damaged plants with no loss of plant direct resistance to this herbivorous pest, a finding contrary to the plant domestication-reduced defense hypothesis. My work in peppers thus highlights work in other crops supporting a context-dependent nature that domestication has on trophic interactions and emphasizes the need for dedicated investigation in each unique crop system.

My work globally highlights the importance of studying plant-herbivore and herbivore-enemy interactions under natural and anthropomorphic selection forces in a tritrophic framework via inter-disciplinary research, such as through utilizing physiological mechanisms to help explain the maintenance of observed ecological interactions.