

Functional response and the effects of insecticidal seed treatment on the soybean aphid
parasitoid, *Binodoxys communis*.

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Dedication

To my loving parents Phillip and Deborah Carter who always taught me to believe in myself and no matter how hard things get, I can work hard to make tomorrow better. You are my inspiration.

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Abstract

The soybean aphid, *Aphis glycines*, is native to Eastern Asia where populations are regulated, in part, by natural enemies such as the braconid parasitoid wasp *Binodoxys communis*. However, the establishment of *B. communis* in North America as a classical biological control agent where *A. glycines* populations have reached economic injury levels, has been unsuccessful to date. This study examined the impact of neonicotinoid seed treatment on *B. communis*, as well as determined the parasitoid's functional response. Seed treatments and biological control are often both used in a soybean aphid integrated pest management (IPM) regime. A field study was conducted to evaluate any impact seed treatment may have on the parasitoid. There were eight susceptible soybean plots, four of which were untreated and four were treated with a neonicotinoid seed treatment. Adult male and female wasps were released onto individual caged plants and mummies were collected 10 days later. Proportion of aphids that mummified, emergence rates, sex ratios, and hind tibia lengths of the offspring were measured as fitness parameters of the parasitoid and compared between treatments. The results suggest that even though there was an effect on *B. communis* fitness, the results may not be biologically significant due to inconsistent results across all parameters in the study.

Another potential hypothesis for the parasitoid's difficulty as a classical biological control agent, could be a type IV (or 'domed') functional response, i.e., increased parasitism per capita with increased host density to a threshold level, beyond which the parasitism per capita decreases. The functional response of *B. communis* on *A. glycines* was measured at six aphid densities, ranging from 10 to 1000 per soybean plant. Aphids

were placed on plants two days prior to exposure to a single, mated, one-day-old female *B. communis*. The parasitoid was left on the plant for 24 hours and 10 days later all mummies were collected. Emergence rates, sex ratios, and hind tibia lengths of the offspring were measured as fitness parameters of the parasitoid. The results of this experiment were consistent with a type IV functional response. Parasitism per capita by *Binodoxys communis* increased with number of aphids per plant and decreased between 500 and 1000 per plant. This may indicate a limit to this parasitoid's ability to control *A. glycines* populations during outbreaks. Furthermore, the more that is known about parasitoid ecology, the easier it will be to forecast successful establishment of future agents.

Table of Contents

Acknowledgements	i
Dedication	ii
Abstract	iii
List of Tables	vii
List of Figures	viii
Chapter I: Functional response and the effects of insecticidal seed treatment on the soybean aphid parasitoid, <i>Binodoxys communis</i> .	1
Introduction	2
Soybean Aphid in North America	3
Current Management Options for Soybean Aphid	7
Classical Biological Control of <i>Aphis glycines</i>	11
Functional Response of <i>Binodoxys communis</i>	16
Summary	18
Chapter II: Effect of neonicotinoid seed treatment on the soybean aphid parasitoid <i>Binodoxys communis</i> .	21
Introduction	22
Methods	25
Results	28
Discussion	29

Chapter III: Type IV functional response in the soybean aphid parasitoid <i>Binodoxys</i> <i>communis</i> .	33
Introduction	34
Methods	37
Results	41
Discussion	42
Comprehensive Bibliography	49

List of Tables

Chapter II

Table 2-1: Results from 2010. ^a Anova, ^b Wilcoxon rank sums test. Standard errors are left out due to sample size of one. 31

Table 2-2: Results from 2011. Number of aphids at release and proportion Of aphids mummified were left blank due to missing data. 32

Chapter III

Table 3-1: Comparison of the different models used to describe type II, III, and IV functional responses for *Binodoxys communis*. * = $p < 0.05$; ** = $p < 0.01$. 48

List of Figures

Chapter I

Figure 1-1. Type I, II, III and IV functional response. 20

Chapter III

Figure 3-1: Average number of *B. communis* mummies per one parasitoid per 24 hour period as a function of densities of *A. glycines*. Treatment means fitted to a type II functional response

$$\text{(Model 1) } y = \frac{(0.976)x}{1 + (0.976)(0.015)x}, \text{ type III (Model 2) } y = \frac{(62.109)x^2}{x^2 + 4325.09},$$

as well as the best fitting type IV functional response (Model 6)

$$y = \frac{(0.497)(x)}{1 + (0.497)(0.005)(x) + (0.497)(1.2E - 5)(x^2)}.$$

Error bars represent standard errors of the means. 46

Figure 3-2: Average *B. communis* hind tibia lengths (mm) as they relate to densities of *A. glycines* (number/plant). Error bars represent standard errors of the means. Fig 2a male hind tibia length. Fig 2b female hind tibia length. 47

Chapter I: Functional response and the effects of insecticidal seed treatment on the soybean aphid parasitoid, *Binodoxys communis*.

Introduction

The soybean aphid, *Aphis glycines* Matsumura (Hemiptera: Aphididae), is responsible for extensive economic damage in soybeans via crop yield loss (Diaz-Montano et al. 2007, Ragsdale et al. 2007), reduction of seed quality (Beckendorf et al. 2008) and increased insecticide use (Ragsdale et al. 2011). The soybean aphid is native to eastern Asia and was first detected in the United States in 2000 (Ragsdale et al. 2011). Currently, the most widely used management tools for control of the soybean aphid are pyrethroids, organophosphates, and neonicotinoid insecticides. Although insecticides have the potential to be successful at managing populations of soybean aphids if applied at the correct time, they can also have negative effects on the environment and non-target organisms (Fox et al. 2005). One potential alternative or addition to insecticides is classical biological control, in which the goal is to establish populations of natural enemies of the pest species imported from its native range (Murdoch et al. 1985). Using multiple management tactics risks the chance of one tactic exhibiting a negative influence on another, as is the case with host plant resistance negatively influencing biological control agents by decreasing population size and possibly reproductive capabilities (Chacon et al. 2012, Ghising et al 2012). The management of *A. glycines* is more effective when insecticides, host plant resistance, cultural control, and biological control are utilized in an integrated pest management regime (Johnson et al. 2008).

Pest density plays a big role in integrated pest management regimes as a result of the potential that it has to decrease the probability of establishment of classical biological control agents. The effect of pest density is represented by the functional response of an

agent. Functional response is a measure of how the agent's feeding rate varies with host density. If an agent responds with higher consumption rates at low or medium versus high host densities, it would have a lower probability of establishing during host outbreaks. Since the overall goal of *A. glycines* management strategies is to reduce the number of aphids, understanding an agent's response to varying aphid densities will help identify potential *A. glycines* classical control agents and inform how to release them.

The Soybean Aphid In North America

Life Cycle

The soybean aphid possesses a heteroecious, holocyclic life cycle, undergoing both sexual and asexual reproduction, and develops on at least two different host species (Dixon 1973). In the United States, overwintering by the soybean aphid occurs on buckthorn (Rhamnaceae) during the egg stage (Ragsdale et al. 2004). The first morph to hatch in the spring, the fundatrix, asexually produces one to three wingless, apterous generations on buckthorn. The following generation is comprised of winged (alate) aphids, which emigrate to the secondary host, soybean, *Glycine max* (L.) Merr. (Fabaceae). Once on soybean, both apterous and alate females are produced via parthenogenesis, giving viviparous birth to young throughout the summer, with alates ensuring dispersal once population densities get too high (Li-hua and Rui-lu 1993, Ragsdale et al 2004). Populations can grow exponentially and under controlled laboratory conditions, can double in 1.5 days (McCornack et al. 2004). The most favorable temperature for soybean aphid reproduction is 28° C with fecundity decreasing as

temperatures increase up to 35°C (McCornack et al. 2004; McCornack et al. 2005). In the fall, when the plants are senescing and there is a significant decrease in both photoperiod and temperature, male aphids and gynoparae (alate females) are produced (Ragsdale et al. 2004). The males and gynoparae emigrate to buckthorn where the latter produce sexual oviparae. The oviparae and males will mate on buckthorn and the oviparae will lay overwintering eggs (Ragsdale et al. 2011).

Host Plants

In the United States, the sexual stage of the life cycle occurs on species in the genus *Rhamnus* and the secondary hosts are species in the genus *Glycines*. The most common primary host species are common buckthorn, *Rhamnus cathartica* L. or alderleaf buckthorn, *Rhamnus alnifolia* L'Hér, although occasionally glossy buckthorn *Frangula alnus* P. Mill is used (Voegtlin et al. 2004). In the Midwestern United States, these species are not only exotic, but also invasive and widespread. One hypothesized soybean aphid control option, to eliminate the overwintering host, is improbable due to the widespread nature of buckthorn. In the US, the most common secondary host is *Glycines max*. It has been suggested that summer reproduction could take place on red clover, *Trifolium pratense* L., and violet prairie clover *Delea purpurea* Vent. (Ragsdale et al. 2004). Due to limited observation it is thought that red clover only represents a small portion of soybean aphid summer reproduction in the U.S. (Ragsdale et al. 2004).

Invasion History

The soybean aphid was first detected in Wisconsin in 2000 (Ragsdale et al. 2004). Since its invasion, it has spread to a majority of the North Central soybean growing region of North America (Ragsdale et al. 2011). It is possible that the soybean aphid was present in the United States prior to 2000 without official record (Ragsdale et al. 2004). Although an exact origin of this invasion has not been verified, Japan has been identified as a possible source (Venette and Ragsdale 2004). Data that denote the source of soybean aphid as not coming from South Korea also suggest that the invasion originated as small populations that rapidly grew parthenogenetically and eventually led to the current establishment (Michel et al. 2009). As a consequence of decreased genetic variability in laboratory colonies of soybean aphid compared to field colonies, studies looking at genetic background as well as resistance information are difficult to accurately assess (Michel et al. 2010).

The widespread invasion is due, in part, to the aphids' dispersal capabilities. Dispersal of the soybean aphid, over long distances, can be supported by the aphids' ability to enter low level jet streams. This allows the soybean aphids to move within fields using direct flight, and to other fields using jet streams (Ragsdale et al. 2004; Zhang et al. 2008). Initial spring colonization of the secondary host is often spatially highly irregular due in part to this dispersal behavior. It is hypothesized that the irregularity of colonization is due to winged aphids finding a specific soybean plant, depositing nymphs then continuing the search for optimal soybean plants (Ragsdale et al. 2004, Rhainds et al. 2008).

Economic Importance

The United States is one of the leading producers and exporters of soybeans in the world, and the soybean aphid represents the first major insect pest of this crop in the North Central growing region of the U.S. (Ragsdale et al. 2011). During the last 12 years, there were approximately 74 million acres of soybean planted each year, with the production value increasing from US \$12.5 billion in 2000 to almost US \$36 billion in 2011. Before the first soybean aphid detections in 2000, less than 0.1% of soybean acres in the North Central Region were treated with insecticides and, by 2006, this percentage increased to over 13% (Ragsdale et al. 2011).

The soybean aphid generates damage in the crop that can lead to a 10-20% yield loss, and in certain conditions, can result in yield losses of up to 40% (Ragsdale et al. 2007). Damage to the crop occurs by direct feeding on the plant phloem, which can decrease plant vigor and height, and reduce: pod number, seed size, quality and yield (Beckendorf et. al. 2008; Tilmon 2011). Aphids also produce honeydew, a sugar-rich byproduct of phloem feeding, which can promote the growth of sooty mold. The increased amount of sooty mold decreases the amount of sunlight penetrating the leaf surface and interferes with photosynthesis (Tilmon 2011). Even though it is possible for low to moderate aphid densities to cause a significant decrease in photosynthetic capabilities (Macedo et al. 2003), as aphid densities increase, potential for damage will increase as well.

Current Management Options for Soybean Aphid

Integrated Pest Management

Integrated pest management (IPM) is a pest management strategy that incorporates the biology and ecology of the pest as well as at least one control tactic, while being environmentally safe and economically feasible (Radcliffe et al. 2009). Developing a successful IPM program involves a broad knowledge base including: pest reproductive capabilities, population dynamics, response to chemical control, and response to natural enemies. Much of the recent effort for IPM of the soybean aphid has been on curative as opposed to preventative strategies (Ragsdale et al. 2011).

Economic Thresholds

The economic threshold (ET) is an important part of an IPM program. It is the population density of the pest at which a management option must be chosen to prevent economic loss. For soybean aphid, an economic threshold is in place at 250 aphids per plant from bloom through seed set to anticipate when to treat and ensure densities will not increase too quickly (Ragsdale et al. 2007). If there is no action taken, population densities can reach the economic injury level (EIL). The EIL of 674 soybean aphids per plant represents the level of infestation at which it is economically beneficial to use chemical treatments (Ragsdale et al. 2007).

Neonicotinoid Insecticide

Insecticides are currently the main management tactic that growers use in IPM strategies, to control *A. glycines*, with the use of neonicotinoids especially on the rise. This class of insecticides acts on the central nervous system of insects, binding to specific binding sites on the nicotinic acetylcholine receptors (Matsuda et al. 2001; Tomizawa and Casida 2005). Binding to these receptors promotes the sending of action potentials, overwhelming the central nervous system to the point that it is no longer able to respond to further stimuli. Neonicotinoids are typically applied as seed treatments, foliar sprays or soil drenches (Tomizawa and Casida 2005).

Thiamethoxam is a systemic neonicotinoid targeted at insects that feed directly on plant tissue. Because Thiamethoxam is highly water-soluble, it is taken up by the plant and transported into plant cells. Once in the plant cells, the insecticide is translocated and incorporated into plant tissue from the roots to the leaves (Inoue et al. 1998; Sur and Stork 2003; Tomizawa and Casida 2005). Thiamethoxam is available commercially as Crusier© for use in soybeans.

Neonicotinoids can have impacts on natural enemies in two main ways. The first is direct toxicity, either lethal or sub-lethal, which results in negative effects on natural enemies (Rosenheim and Hoy 1988; Croft 1990; Morgan and Hare 2003; van de Veire 2003). The second involves lowering prey/host densities and can result in either positive or negative effects on natural enemies (Cloyd and Bethke 2010). If the natural enemy expresses positive density dependent attack rates, these insects would experience increased relative parasitism as host density increases. The lowering of host densities

through the use of other IPM tactics could have an adverse effect due to the decrease in relative parasitism at low host densities. But if the natural enemy expresses inverse density dependent attack rates, the effect could be positive because these insects experience a decrease in percent parasitism as host density increases. Here, the lowering of host densities, due to the use of other IPM tactics, would be beneficial due to the increase in relative parasitism at low host densities.

Late in the season, the combination of declining soybean foliage quality and less favorable climatic factors results in lower aphid populations (Ragsdale et al. 2004, Rhainds et al. 2009). The critical period for aphid control measures occurs after the aphid population reaches the ET but before the aphid population hits the exponential growth stage early in the season (Van Den Berg et al. 1997), and before the population expands and results in extensive economic damage at the EIL (Ragsdale et al. 2004; Beckendorf et al. 2008). A critical period has also been identified for predatory insects of the soybean aphid, acting early in the season (Fox et al. 2005; Meihls et al. 2010).

The use of neonicotinoids has been linked to unintentional, adverse effects on the environment, as well as non-target organisms (Stapel et al. 2000; Desneux et al. 2007; Johnson et al. 2008). In the case of the soybean aphid, the application of neonicotinoids is often based on presence of aphids rather than the economic threshold density of aphids. This consistently leads to overuse of the insecticide, which increases exposure to the environment as well as non-target organisms (Pedigo and Rice 2008). Non-target effects are seen in beneficials such as pollinators, predators, and parasitoids (Smith and Krischik 1999; Stapel et al. 2000; Desneux et al. 2007; Krischik et al. 2007; Papachristos and

Milonas 2008; Moser and Obrycki 2009). Because neonicotinoids are water-soluble, there is also an increased risk of seepage and leeching that contributes to water pollution (Gupta et al. 2008). But neonicotinoids can also be applied as a seed treatment and the concentration of the insecticide decreases as the plant grows. This translates into a safer option for non-target species that target larger plants or flowering stages.

Biological Control

In Asia, where soybean aphid is a minor pest, populations are successfully regulated by resource availability and natural enemies (Chang et al. 1994, Li et al. 2000). This latter type of pest regulation is termed biological control. Biological control is an environmentally sound and often more cost effective way to control pest populations compared to insecticides. For example, in the United States from 1968 to 1983, the use of a biological control program for alfalfa weevil was able to reduce pesticide use against this pest by 95% and eliminate \$122 million in insecticide use and application (Radcliffe et al. 1998). There are four main biological control tactics: classical, augmentation, conservation, and natural. Classical biological control involves the use of a specialized natural enemy of a pest from its native range, with the aim of establishing a sustained population of the natural enemy (Murdoch et al. 1985). The augmentation strategy involves supplemental releases of a natural enemy with no requirement of establishment. Augmentation biological control can occur via inoculation, introduction of a small number of agents with the possibility of reproduction, or inundation, the introduction of a large number of agents. Conservation biological control is a non-release tactic used to

increase control potential of existing agents alone. Finally, natural biological control encompasses all naturally occurring biological control (Hajek 2004; Heimpel and Mills, in press).

The most common classes of arthropod biological control agents are predators, parasitoids and entomopathogens. Predators eat more than one other organism during a life cycle. Parasitoids develop in or on another organism, kill it, and emerge as free-living adults. Entomopathogens encompass a variety of organisms (e.g., bacteria, viruses, protozoa, fungi) which cause disease in insects (Hajek 2004). Parasitoids are the most promising biological control agents for classical biological control of *A. glycines* (Heimpel et al. 2004). In China, *A. glycines* seldom achieves populations high enough to attain pest status (Wu et al. 2004), and populations have been kept relatively low, in large part by both insect predators and parasitoids (van den Berg et al. 1997; Liu et al. 2004; Wu et al. 2004). The promise of parasitoids is also reinforced by the lack of successful natural enemies of soybean aphid in North America (Rutledge et al. 2004; Desneux et al. 2006; Kaiser et al. 2007; Noma and Brewer 2008; Noma et al 2010).

Classical Biological Control of *Aphis glycines*

Starting in 2001, an examination of parasitoids and predators of *A. glycines*, suitable for release in the United States, has been examined in China, South Korea and Japan (Ragsdale et al. 2011). The identified potential agents mostly include *Aphelinus spp.* and members of the aphidiine Braconidae (Desneux et al. 2009; Wyckuys et al. 2009). *Aphelinus atriplicis* was released against the Russian wheat aphid, *Diuraphis*

noxia, in the early 1990's (Elliot et al. 1995; Brewer et al. 1998). The parasitoid was released against *A. glycines* in 2002 after observations of it attacking and developing on *A. glycines* in the field, but proof of establishment has been absent to date (Heimpel et al. 2004; Wu et al. 2004). *Aphelinus glycinis* is another specialist that has the ability to oviposit in all aphid stages, including winged adults (Hopper et al. 2012). Federal approval was granted to release *A. glycinis*, a specialist on the soybean aphid, in 2012. Another promising parasitoid, that is not believed to be intentionally introduced but has been found to attack *A. glycines* in the United States, is *Aphelinus certus* (Frewin et al 2010; Heimpel et al. 2010). Because of its broad host range and high probability for non-target impacts, *A. certus* was deemed unacceptable for release against *A. glycines*. Even though it was not intentionally introduced, *Aphelinus certus* was first discovered in Ontario, spread to the much of the Eastern United States, and shown potential as a biological control agent of the soybean aphid.

Since development of parasitoids occurs within or on their host, understanding the life history of a target pest will help to identify a more efficient biological control agent. On soybean, *A. glycines* reproduces asexually via viviparity, resulting in the ability to increase their population exponentially (McCornack et al 2004). Due to the potential for such high growth rates, the successful establishment of the initial spring migrants and their offspring is critical to determining if the population will develop into an outbreak in any given year. The establishment of initial spring migrants is largely influenced by the number of aphids from the previous year that survived on the overwintering host

(Rhainds et al. 2010). One characteristic for a potential parasitoid classical biological control agent may be the ability to control aphid populations in early spring.

Binodoxys communis

Another parasitoid that has been released against *A. glycines* is *Binodoxys communis* Gahan (Wyckhuys et al. 2007a). *Binodoxys communis* (Braconidae: Aphidiinae) is a solitary endoparasitoid of aphids, meaning only one egg is laid and larva develops inside a single aphid host. *B. communis* is also a koinobiont, allowing its host to continue development after parasitization. *Binodoxys communis* larvae develop inside *A. glycines*, feeding on the aphid hemolymph. The female has a set of claspers on the tip of the abdomen that is used to grasp hosts during oviposition. When development is almost complete and before the adult wasp emerges, the aphid is killed and the resulting husk of the aphid is termed a mummy (Wyckhuys et al. 2008). Like most hymenopterans, *B. communis* exhibits haplodiploid sex determination where males are haploid and can develop from unfertilized egg and females are diploid and can only develop from a fertilized egg. The adult wasp is free living while the larva is parasitic inside soybean aphid. The wasps start reproducing almost immediately after emergence and females have the potential to lay up to 200 eggs (Dieckhoff and Heimpel 2010).).

Permission for release of *B. communis* in the United States was granted federal approval in 2007, after extensive investigation on host specificity and potential environmental impact (Wyckhuys and Heimpel 2007; Wyckhuys et al. 2007a; Wyckhuys et al. 2007b; Desneux et al. 2009; Wyckhuys et al. 2009; Heimpel et al. 2010; Desneux et

al. 2012). The establishment of *B. communis* in North America as a classical biological control agent, however, has been unsuccessful. The potential for success is tied to the parasitoid's ability to establish and spread throughout the introduced range of the soybean aphid in the United States.

Lack of Establishment of *B. communis* in North America

There are several reasons that may explain why establishment of *B. communis* has not been documented. One possible explanation could be the major difference in dispersal behavior between *B. communis* females and males with females dispersing more than males from release sites (Heimpel and Asplen 2011). This would not only complicate the parasitoid's mate-finding ability but also decrease successful matings and potential for establishment. Although there is a climate match between the native range of *B. communis* and the United States, little is known about the exact overwintering capabilities of the parasitoid, which could impact potential establishment. Even though it is possible for the wasp to parasitize aphid morphs that are specific to buckthorn, the overwintering host of the soybean aphid, the wasps preferentially attack apterous versus alate aphids and develop slower on alate aphids (Whyckhuys et al. 2008; Asplen et al. 2011). It is improbable that the only strategy that *B. communis* utilizes to locate buckthorn is dispersal of immature wasps through specific aphid morphs (Asplen et al. 2011). It is also plausible that the overwintering capability of the wasp has been lost in culture.

It is possible that other aphid control tactics may also interfere with *Binodoxys communis* as a classical biological control agent (Chacon et al. 2010 and 2012). The presence of predatory insects is an example of a naturally-occurring control method that will interfere with soybean aphid population growth and also reduce established populations (Van Den Berg et al. 1997). Although predators that act in a density-dependent manner can successfully limit aphid population growth, they can also limit the effect of parasitoids they will feed on aphids and parasitized aphids (mummies) alike (Chacon and Heimpel 2010; Heimpel et al. 2010). By feeding on aphids, the predators effectively reduce resource availability for parasitoids and considering parasitoids develops inside *A. glycines*, when the predator feeds on mummies it influences parasitoid growth rates by reducing adult emergence (Chacon and Heimpel 2010; Heimpel et al. 2010). Parasitoids are also affected by induced group defense behavior of aphids and increased amounts of sticky honeydew produced by high aphid densities (Wu et al. 2010). Because parasitoids are vulnerable to the effects of these biological interactions, like predators, it is important to first understand how a parasitoid will respond to these outside forces before considering them for mass release.

Since the establishment of *B. communis* in North America, where populations of the soybean aphid have exploded, has been unsuccessful, another factor to consider is the effect of host population size on the agent. The failure to establish, to date, coincides with results found in the lab where there was difficulty rearing *B. communis* on high densities of soybean aphid. Such a finding implies that over a certain threshold there is a negative relationship between aphid density and growth rate of the parasitoid. Growth rate is

defined as a change in number of individuals over time. This relationship would suggest that the population growth rate for *B. communis* would increase as aphid densities increase up to a certain threshold and above that threshold growth rates of *B. communis* will decrease. Similar results were found for the parasitoid *Aphidius ervi* and its host, the pea aphid *Acyrtosiphon pisum* where the parasitoid went extinct even though it was exposed to high pea aphid densities (Ives and Settle 1996). Deickhoff and Heimpel (2010) found that *B. communis* laid more eggs at aphid densities of 150 soybean aphids per plant as opposed to 30 soybean aphids per plant, but the densities did not exceed 150 aphids per plant. In a field setting 150 aphids per plant is well below what a soybean aphid outbreak would incorporate.

Functional Response of *Binodoxys communis*

The functional response of an insect is a mathematical model that describes the rate of feeding or parasitism of the insect and how it varies with prey or host density (Holling 1961, 1965, 1976; O'Neil 1990). There are four types of functional responses where the number of prey eaten/parasitized per predator/parasitoid per unit time is dependent on the density of prey (Figure 1-1) (Holling 1961, 1965, 1976). Type I is a response that increases linearly until reaching a critical point, after which it plateaus. Type II response increases at a decreasing rate until reaching a plateau, and type III is a sigmoidal response curve. For a type IV response curve the number of prey parasitized per parasitoid increases at a decreasing rate until it reaches a critical prey density and beyond that density, the number of prey parasitized per parasitoid decreases.

For each of the four response curves, the reason for the critical point and what happens after that point is reached can be explained by different factors. For type I, the plateau after the critical point is reached is due to parasitoid saturation. Type I response assumes that handling time for these parasitoid is negligible (Hassel 1978). Some predators that have a random searching pattern for prey and possess a very short handling time as well as sedentary filter feeders, typically possess a type I functional response (Holling 1959; Mills & Lacan 2004). The more prey or hosts there are, the more the predator will catch, until it is satiated or until the parasitoid's eggload is depleted. It is likely, though, that natural enemies will have a limit to how efficiently they can consume prey or parasitize hosts (Heimpel et al. 1998). For this reason, type II responses incorporate handling time, which accounts for predators/parasitoids capturing, killing, parasitizing, eating or digesting prey. For type II, at low prey densities the parasitoids spend most of their time searching and at high prey densities they spend most of their time on prey handling (Hassell et al. 1976). A majority of insects possess a type II functional response. Type III is similar to type II but exhibits more of a sigmoidal curve. At lower prey densities it increases at a slower rate which can be due to a learning curve of the parasitoid or a change in search activity (Hassel et al. 1977). It could be due to the parasitoid's need to create a search image before it can start to detect prey or the parasitoid switching from one prey item to another. The domed appearance in the type IV functional response could be due to increased effectiveness of group defense of prey as prey density increases. This was shown to be the case with a pentatomid bug *Podisus modestus* and their jack pine sawfly prey as well as ant-eating spiders, *Zodarion rubidum*,

and their ant prey *Tetramorium caespitum* (Liznarova and Pekar 2013; Tostowaryk 1972).

All four functional response types express inverse density dependence. The one big difference between types I, II, III and IV is that type IV has much stronger inverse density dependence. The number of prey eaten per predator increases with prey density until it reaches the critical prey density. Unlike the other three types, that sustain the same level of prey eaten per predator as prey number increases, type IV decrease in number of prey eaten per predator as prey number increase past that critical period (Collings 1997; Sabelis 1985; Taylor 1984).

Summary

For *B. communis* to be a successful biological control agent of *A. glycines*, it is critical to understand the response it has to differing *A. glycines* population concentrations and how that information can be integrated into a successful IPM program. Determining if there is a negative relationship between soybean aphid densities and growth rates of *B. communis* can be accomplished by exploring different parasitism rates. Because a decrease in parasitism rates results in decreased egg production as well as a decreased number of adult individuals produced, a connection can be made between parasitism rates, growth rates, and potential establishment of the parasitoid. Knowing how an organism responds to low, medium and high host densities is an important part of determining if the organism, specifically *B. communis*, will be an effective biological control agent. This information must be used when planning a IPM program since

neonicotinoid seed treatments are so common in north central soybean growing area. Seed treatments have the potential to affect establishment by lowering the population of *A. glycines* early in the year, which may affect the growth rate of the parasitoid. If *B. communis* has low growth rates while exposed to extremely high aphid densities, it suggests that there will be a limit to the parasitoid's ability to control aphid populations during an outbreak, as is common in the U.S. It is possible, however, that this negative relationship, in conjunction with the critical time period for management, could affect the way we use the parasitoid as a biological control agent. The functional response of a parasitoid agent has implications for a potential parasitoid release, both in selecting the time of the season, and a location based on when and where host outbreaks occur.

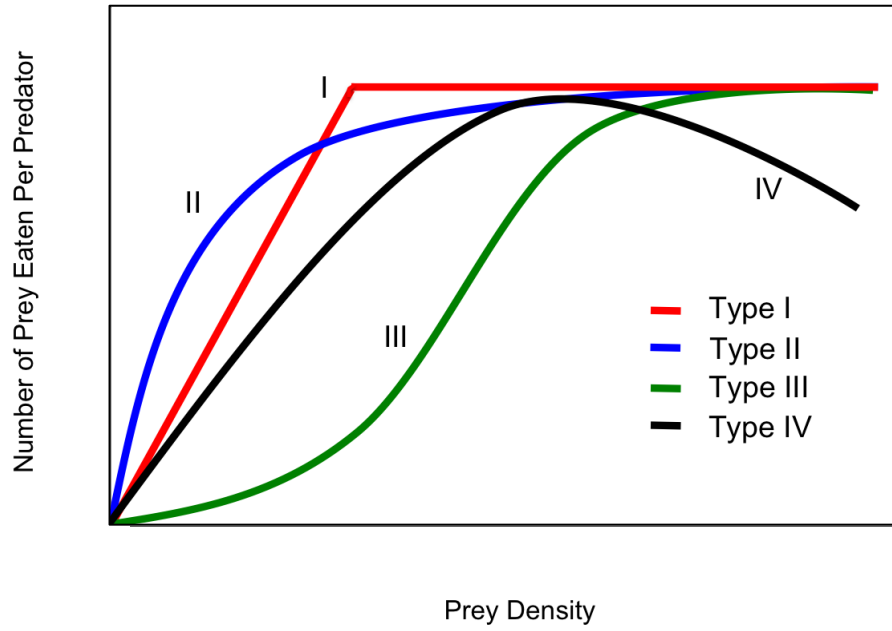


Figure 1-1. Type I, II, III and IV functional response.

**Chapter II: Effect of neonicotinoid seed treatment on the soybean aphid
parasitoid *Binodoxys communis***

Introduction

The soybean aphid, *Aphis glycines* Matsumura (Hemiptera: Aphididae), has emerged as the most important economic pest of soybean, *Glycine max* (L.) Merr. in the North Central United States (Venette and Ragsdale 2004; Ragsdale et al. 2007; Ragsdale et al. 2011; Schmidt et al. 2012). To date in the United States, classical biological control and insecticides are two of the main management options used to control *A. glycines* (Ragsdale et al. 2011). The goal of classical biological control is to establish populations of natural enemies of the pest species imported from its native range (Murdoch et al. 1985). Neonicotinoids, broad spectrum insecticides often applied as a seed treatment, are a specific class of insecticides used for control of the soybean aphid (Tomizawa and Casida 2005; Magalhaes et al. 2009; Schulz et al. 2009). Since insecticide use and biological control tactics often overlap in the field, it is important that these two management tactics do not interfere with one another.

The use of aphid management tactics can result in unintended impacts, such as natural enemy population suppression, altered behavior or delayed population growth of natural enemies (Kraiss and Cullen 2008; Ballman et al. 2012; Ghising et al. 2012; Seagraves and Lundgren 2012; Lundgren et al. 2013; Ode and Crompton 2013). When insecticides are used on soybean populations that exceed the economic threshold, they have been shown to not only lower the aphid population, but also lower the natural enemy population to a point where it is difficult to rebound (Lundgren et al. 2013). The economic threshold is the pest density at which treatment must be implemented to ensure economic return. For the soybean aphid, the density is 250 aphids per plant and that gives

a seven day window before the population reaches economic injury levels (EIL) where economic loss would occur (Ragsdale et al. 2007)

The negative impacts on natural enemies, due to aphid management options, can take two main forms. The first is through direct toxicity, resulting in either lethal or sub-lethal effects to the natural enemy (Rosenheim and Hoy 1988; Croft 1990; van de Veire 2003). The second involves lowering prey/host densities and can result in either positive or negative effects on natural enemies (Cloyd and Bethke 2010). If a natural enemy expresses positive density dependent attack rates, it experiences increased relative parasitism as host density increases. Lowering host density, which is the goal of insecticides, would thus have adverse effects on these natural enemies due to the decrease in relative parasitism at low host densities. On the other hand, natural enemies that express inverse density dependent attack rates would have an opposite effect. Since these insects experience a decrease in percent parasitism as host density increases, lowering host density would translate into higher percent parasitism. Since effective insecticide treatments generally keep soybean aphid populations low, there are implications for establishment and efficacy of biological control agents that differ in the density dependence of their attack rates.

This study investigated the influence of a neonicotinoid insecticide, thiamethoxam, on the soybean aphid biological control agent, the parasitoid *Binodoxys communis*(Gahan) (Hymenoptera: Braconidae). *Binodoxys communis* larvae develop inside *A. glycines*, feeding on the aphid hemolymph. The aphid is killed before the adult wasp emerges and the resulting husk of the aphid is termed a mummy (Wyckhuys et al.

2008). We investigated the impacts of thiamethoxam on several measures of *B. communis* fitness, including: number of mummies, proportion of mummies; proportion of emerged wasps; sex ratio of the emerged wasps; and hind tibia length of emerged wasps (a good indicator of parasitoid size and shown to correlate with female performance, specifically reproductive, in the field) (West et al. 1996; Bennett and Hoffmann 1998; Sagarra et al. 2001).

If *B. communis* exhibits inverse density dependent attack rates, it would attack a higher percentage of aphids on low versus high aphid plants (Donaldson et al 2007); creating the potential for a positive relationship with insecticide use. Furthermore, a higher aphid density on untreated plants versus seed treated plants will result in an increase in competition for resources between aphids. This increase in competition would lead to smaller, lower quality hosts which would produce smaller parasitoid larvae (Teder and Tammaru 2002; Stoepler et al. 2011) and therefore lower percent emergence of wasps from those aphids.

Sex ratio and length of hind tibiae can also be used to understand population dynamics of parasitoids in the field. Sex ratio can have a significant impact on biological control tactics since females are the sex that attacks hosts but males are needed to produce females (Heimpel and Lundgren 2000). *B. communis* possesses haplodiploid sex determination which means unfertilized eggs develop into haploid males and fertilized eggs develop into diploid females (Heimpel and de Boer 2008). Because females can choose to fertilize eggs, and because it takes a higher energy input to produce females, a large, high quality host should strongly influence sex ratio (Opp and Luck 1986; Tillman

and Cate 1993; Stoepler et al. 2011; Vacari et al. 2012). Finally, length of the hind tibia is a good indicator of parasitoid size and has been shown to correlate with female performance, specifically reproductive, in the field (Bennett and Hoffmann 1998; Sagarra et al. 2001; West et al. 1996).

The goal of this study was to determine the effect of seed treatment on *B. communis* in order to better understand the interaction between seed treatment and classical biological control. Fitness parameters of the parasitoid offspring on the two treatments were measured, including: number of aphids per plant, number of parasitized aphids (mummies), proportion of mummies, emergence rates, sex ratios, and length of hind tibiae.

Methods

Insect Colonies. All laboratory *A. glycines* and *B. communis* colonies were reared on Pioneer 91M51 variety soybean plants in early vegetative stages. Aphid colonies were obtained from soybean fields in St. Paul, MN, USA and have been in colony since 2003. The aphids were maintained on plants in the laboratory at $25 \pm 5^\circ\text{C}$, 60-80% r.h., and 16:8 L:D. The plants were potted in square three inch plastic pots. Six pots were placed on plastic trays and water was poured in the tray (bottom watering) approximately every other day. The trays were kept in square Plexiglass cages with 105 micron white nylon mesh sleeves (Questoutfitters.com; Sarasota, FL).

B. communis were obtained from a Chinese strain collected in Harbin, Heilongjiang province, China by K. Hoelmer, K. Chen, W. Meikle, and L.A. Pin and

have been in colony since 2002. The University of Minnesota colony was started from the original strain that has been held at the USDA-ARS Beneficial Insect Introduction Research Laboratory in Newark, Delaware (Wyckhuys et al. 2008). *B. communis* develop inside *A. glycines* individuals, feeding on the aphid hemolymph. The aphid is killed before the adult wasp emerges and the resulting husk of the aphid is termed a mummy (Wyckhuys et al. 2008). The colony at the University of Minnesota St. Paul campus laboratory was reared on *A. glycines*-infested soybean plants at $25 \pm 5^{\circ}\text{C}$, 60-80% r.h., and 16:8 L:D.

Field Site. The field experiment was replicated in 2010 and 2011, at the University of Minnesota Research, Outreach and Education (UMORE) Park, Rosemount, MN. An experimental field, designed by Heidel-Baker (2012) was subdivided into eight plots of 0.10 ha, measuring 24.4 x 41.1 m each. A susceptible Monsanto Roundup Ready SD01-76R variety of soybean was used and each plot was either left untreated or was treated with a thiamethoxam seed treatment, Cruiser[®] 5FS (Syngenta Crop Protection, LLC, Greensboro, NC). It was used at a rate of 0.0756 MG active ingredient per seed. The field was planted on May 19, 2010 and May 27, 2011 in 30-inch rows at 310,000 seeds per ha.

Field Experiment. Individual plants were caged with a square wire tomato cage (36cm wide and 1 m tall) buried 10 cm in the ground. The cages were covered by a fine no-see-um white mesh (Questoutfitters.com) sleeve that was also buried at the bottom and tied at the top. As a consequence of low natural infestation rates of the soybean aphid, each plant was infested with one aphid infested leaf (between 50-100 aphids)

obtained from the laboratory aphid colony 7-14 d before parasitoid release. To ensure that all released parasitoids were approximately the same age, mummies were collected from the lab colony the day prior to release and single individuals were placed in clear gel capsules (Solaray; Brick, NJ; size 0). The day of release, newly emerged (< 24-h-old) adults were sexed and five female and five male *B. communis* adults were released into each cage. The number of aphids per plant was recorded and predators removed at this time. There were two release dates in 2010, July 6 and August 19, and two in 2011, August 2 and 7. Ten days later the number of aphids and the number of mummies were recorded. Each mummy was placed individually in a clear gel capsule (Solaray size 0) and all gel caps from a single plant were placed in a 110mm Petri dish labeled with the collection date, plot number, treatment and number of aphids. The Petri dishes were stored in a cooler with a towel-covered ice pack. The Petri dishes were stored in a growth chamber at the St. Paul campus of the University of Minnesota. Each wasp that successfully emerged was sexed and slide mounted in order to measure hind tibia lengths. Hind tibiae were measured using an ocular micrometer on an Olympus BX50 compound microscope at 10x magnification.

Data Analysis. Separate one-way analyses of variance (ANOVA) were used to determine if the explanatory variable, seed treatment, affected the following response variables: aphid density at release, total mummy count, proportion of aphids that were mummified, proportion of wasps that emerged, length of hind tibiae, and sex ratio. Due to a non-normal distribution, a Wilcoxon rank sums test was used to determine if seed treatment affected the proportion of aphids that were mummified in the first release in

2010 using JMP 10.0; (SAS Institute Inc., Cary, NC, USA). The statistical analysis for aphid density at release, total mummy count, proportion of mummified aphids (for 2011 release), emergence rate, sex ratio, and male and female hind tibia length were performed using R 3.0.0; R Foundation for Statistical Computing, Vienna, Austria.

Results

Seed treatment did not significantly affect the number of aphids per plant in either release in 2010 (Table 2-1). There was also no significant difference in number of mummies per plant between seed treated and untreated plots (Table 2-1). Similarly, the proportion of aphids that were mummified as well as the proportion of the wasps that emerged did not significantly differ between treatments (Table 2-2). Sex ratio and length of female and male hind tibiae also did not significantly differ between treatments (Table 2-1).

Due to missing data for number of aphids at release from the first release in 2011, analyses for seed treatment effects on aphids per plants as well as proportion of aphids that were mummified, were not performed (Table 2-2). Treatment did not significantly affect number of mummies per plant, proportion of wasps that emerged or the sex ratio of the wasps (Table 2-2). Female wasps had significantly longer hind tibiae in untreated versus treated plots but there was no significant difference in male hind tibia lengths (Table 2-2).

Although the second release in 2011 did not result in a significant difference in number of aphids between treatments, there were significantly more mummies on

untreated versus treated plants (Table 2-2). Treatment did not significantly affect the proportion of aphids that were mummified per plant (Table 2-2). There was a significantly higher proportion of emerged wasps in untreated versus treated plots (Table 2-2). Sex ratio and length of female and male hind tibiae did not significantly differ between treatments (Table 2-2).

Discussion

As demonstrated in this study, neonicotinoid seed treatments had a slight effect on the aphid parasitoid *Binodoxys communis*. There was no significant effect of seed treatment on number of aphids, number of mummies, proportions of aphids that were mummified, emergence rate, sex ratio, or hind tibia length in 2010. The 2011 results were similar with three exceptions. The females in the first release in 2011 had longer hind tibiae in the untreated versus seed treated plots. Similarly, the untreated plots in the second release in 2011 yielded more mummies and a higher proportion of emergence than the seed treated plots. Although this suggests that there might be a slight toxicity effect, the lack of significance for all of the other parameters in both 2010 and 2011 suggests that the results may not be biologically significant.

One explanation for the lack of effect of neonicotinoid seed treatment could be due to decreased concentration levels in the plant as it develops. In 2010, the releases for *B. communis* occurred 48 and 92 days post-planting and in 2011, 67 and 72 days post-planting. Seed treatments are most effective within the first 49 days after planting (McCornack and Ragsdale 2006; McCornack 2007; Magalhaes et al. 2009). It is possible

that if the study was conducted earlier in the year, there may have been a more significant impact due to higher concentrations of seed treatment. The effect of neonicotinoid seed treatment remains an important topic that requires further investigation, for classical biological control agents.

Table 2-1. Results from 2010. ^a Anova, ^b Wilcoxon rank sums test. Standard errors are left out due to sample size of one.

Release Number	Response Variable	Treatment	Mean \pm SE	Transformation	Df or n _a , n _b	F value	P value
1	Aphids Per Plant ^a	Untreated	37.75 \pm 8.94	None	1, 6	0.079	0.789
1	Aphids Per Plant ^a	Seed Treated	34 \pm 9.96				
1	Mummies Per Plant ^a	Untreated	2.625 \pm 1.75	Log _e Transformed (Y+1)	1, 6	2.667	0.154
1	Mummies Per Plant ^a	Seed Treated	9.875 \pm 8.57				
1	Proportion Mummification ^b	Untreated	0.04 \pm 0.04	None	4, 4		0.166
1	Proportion Mummification ^b	Seed Treated	0.34 \pm 0.17				
1	Proportion Emergence ^a	Untreated	0.71	None	1, 2	2.041	0.289
1	Proportion Emergence ^a	Seed Treated	0.19				
1	Sex Ratio ^a	Untreated	0.143	None	1, 2	0.216	0.688
1	Sex Ratio ^a	Seed Treated	0.07 \pm 0.07				
1	Female Hind Tibia Length ^a	Untreated	37	None	1, 7	2.25	0.177
1	Female Hind Tibia Length ^a	Seed Treated	41.44 \pm 0.986				
1	Male Hind Tibia Length ^a	Untreated	36.34 \pm 2.60	None	1,14	0.086	0.773
1	Male Hind Tibia Length ^a	Seed Treated	37.34 \pm 1.76				
2	Aphids Per Plant ^a	Untreated	46.75 \pm 7.87	None	1, 6	0.943	0.369
2	Aphids Per Plant ^a	Seed Treated	113.5 \pm 68.27				
2	Mummies Per Plant ^a	Untreated	3.5 \pm 1.76	None	1, 6	0.000	1.000
2	Mummies Per Plant ^a	Seed Treated	3.5 \pm 1.85				
2	Proportion Mummification ^a	Untreated	0.07 \pm 0.04	None	1, 6	0.388	0.556
2	Proportion Mummification ^a	Seed Treated	0.04 \pm 0.03				
2	Proportion Emergence ^a	Untreated	0.90 \pm 0.05	None	1, 4	0.041	0.850
2	Proportion Emergence ^a	Seed Treated	0.92 \pm 0.08				
2	Sex Ratio ^a	Untreated	0.59 \pm 0.21	None	1, 4	0.191	0.685
2	Sex Ratio ^a	Seed Treated	0.70 \pm 0.15				
2	Female Hind Tibia Length ^a	Untreated	34.86 \pm 1.01	None	1, 7	1.178	0.314
2	Female Hind Tibia Length ^a	Seed Treated	37.51 \pm 2.46				
2	Male Hind Tibia Length ^a	Untreated	36.26 \pm 1.27	None	1, 13	0.131	0.724
2	Male Hind Tibia Length ^a	Seed Treated	35.53 \pm 1.53				

Table 2-2. Results from 2011. Number of aphids at release and proportion mummification were left blank due to missing data.

Release Number	Response Variable	Treatment	Mean \pm SE	Transformation	Df or n_a, n_b	F value	P value
1	Aphids Per Plant	Untreated	NA	NA	NA	NA	NA
1	Aphids Per Plant	Seed Treated	NA				
1	Mummies Per Plant	Untreated	29.50 \pm 9.56	None	1, 6	0.145	0.716
1	Mummies Per Plant	Seed Treated	40.75 \pm 27.95				
1	Proportion Mummification	Untreated	NA	NA	NA	NA	NA
1	Proportion Mummification	Seed Treated	NA				
1	Proportion Emergence	Untreated	0.69 \pm 0.10	None	1, 6	0.242	0.640
1	Proportion Emergence	Seed Treated	0.74 \pm 0.04				
1	Sex Ratio	Untreated	0.44 \pm 0.15	None	1, 6	0.562	0.482
1	Sex Ratio	Seed Treated	0.57 \pm 0.06				
1	Female Hind Tibia Length	Untreated	3.77 \pm 0.06	None	1, 130	9.69	0.002
1	Female Hind Tibia Length	Seed Treated	3.55 \pm 0.04				
1	Male Hind Tibia Length	Untreated	3.44 \pm 0.07	None	1, 71	0.369	0.545
1	Male Hind Tibia Length	Seed Treated	3.38 \pm 0.06				
2	Aphids Per Plant	Untreated	487.50 \pm 71.22	None	1, 6	0.763	0.416
2	Aphids Per Plant	Seed Treated	398.75 \pm 72.45				
2	Mummies Per Plant	Untreated	48.50 \pm 11.26	None	1, 6	8.131	0.029
2	Mummies Per Plant	Seed Treated	13.50 \pm 4.87				
2	Proportion Mummification	Untreated	0.11 \pm 0.04	None	1, 6	3.309	0.119
2	Proportion Mummification	Seed Treated	0.04 \pm 0.01				
2	Proportion Emergence	Untreated	0.86 \pm 0.16	None	1, 6	6.612	0.042
2	Proportion Emergence	Seed Treated	0.63 \pm 0.08				
2	Sex Ratio	Untreated	0.43 \pm 0.23	None	1, 6	0.005	0.945
2	Sex Ratio	Seed Treated	0.42 \pm 0.14				
2	Female Hind Tibia Length	Untreated	3.82 \pm 0.04	None	1, 127	0.285	0.595
2	Female Hind Tibia Length	Seed Treated	3.87 \pm 0.07				
2	Male Hind Tibia Length	Untreated	3.95 \pm 0.06	None	1, 65	1.311	0.256
2	Male Hind Tibia Length	Seed Treated	3.71 \pm 0.13				

Chapter III: Type IV functional response in the soybean aphid parasitoid *Binodoxys communis*

Introduction

The soybean aphid, *Aphis glycines* Matsumura, has had a substantial economic impact on the soybean crop in the United States. Since its discovery in 2000, the need to secure a sustainable control method has intensified (Ragsdale et al. 2011; Hodgson et al. 2012). Biological control in Asia, the soybean aphid's native range, plays an important role in suppressing aphid populations, and in North America is a promising management option that can provide valuable aphid control services as well (Van Den Berg et al. 1997; Heimpel et al. 2004; Miao et al. 2007; Ragsdale et al. 2011; Zhang and Swinton 2012). Classical biological control is a subset of biological control that aims to establish populations of natural enemies of the pest species imported from its native range (Murdoch et al. 1985). In Eastern Asia, the soybean aphid's native range, populations are regulated, in part, by natural enemies such as the braconid parasitoid wasp *Binodoxys communis* Gahan (Wu et al. 2004; Miao et al. 2007; Wyckhuys et al. 2007a). *Binodoxys communis* larvae develop inside *A. glycines*, feeding on the aphid hemolymph. The aphid is killed before the adult wasp emerges and the resulting husk of the aphid is termed a mummy (Wyckhuys et al. 2008). However, in North America, where *A. glycines* populations can reach levels that cause economic injury to soybean, the establishment of *B. communis* as a classical biological control agent has been unsuccessful to date (Asplen et al. 2011; Ragsdale et al. 2011).

Since *A. glycines* populations are much higher in North America than in Eastern Asia, the response of *B. communis* to high aphid densities may explain the wasp's performance in the introduced range. The functional response of an insect represents how the rate of feeding or parasitism of the insect varies with prey or host density (Solomon 1949; Holling 1959). Useful information can be attained from the functional response of a biological control agent and used when assessing the impact it has on a host population (Houck and Strauss 1985; Tully et al. 2005). For example, if the predation/parasitism rate is higher at high versus low prey/host densities, it may be more effective to release the agent into a host population during peak population densities.

The different limiting factors to predation/parasitism rate determine the resulting functional response. If handling time is the major limiting factor for parasitism rate it results in a type II functional response (Holling 1959). At low prey densities a predator/parasitoid possessing a type II functional response spends most of its time searching and at high prey densities they spend most of their time on prey handling. If a predator/parasitoid must create a search image, it would be a limiting factor in the predation/parasitism rate that would, in turn, lead to a type III functional response. A type III response accelerates at low host densities and decelerates at high host densities, creating a sigmoidal response curve (Holling 1959). In terms of parasitoids, handling time refers to how long it takes a parasitoid to subdue and oviposit in a host. Factors such as group defense of hosts may lead to a domed type IV predation/parasitism rate (Liznarova and Pekar 2013). This was shown to be the case with a pentatomid bug *Podisus modestus* and their jack pine sawfly prey (Tostowaryk 1972) as well as ant-

eating spiders, *Zodarion rubidum*, and their ant prey *Tetramorium caespitum* (Liznarova and Pekar 2013). A type IV response increases up to a critical threshold and then decreases in number of hosts parasitized per parasitoid as host number increase past a critical period (Holling 1961; Mori and Chant 1966; Tostowaryk 1972).

If *B. communis* exhibits a domed functional response, it would attack fewer aphids at high versus medium, and potentially low, aphid densities. Furthermore, higher aphid densities translate to an increase in competition for resources between aphids which should lead to smaller hosts and lower host quality. Lower host quality should, in turn, produce smaller parasitoid larvae (Teder and Tammaru 2002; Stoepler et al. 2011) and theoretically lower percent emergence of wasps from those aphids. Wyckhuys et al. (2008) found that although emergence did not vary based on age of aphids, *B. communis* was successful at attacking a higher proportion of younger versus older *A. glycines*. This could be due to a high proportion of older aphids expressing effective defensive behaviors (Wyckhuys et al. 2008).

Sex ratio and hind tibia length are also used to understand the response of *B. communis* to varying host densities. Sex ratio can have a significant impact on biological control tactics since females are the sex that attacks hosts (Heimpel and Lundgren 2000). Parasitoid wasps like *B. communis* possess haplodiploid sex determination, which means unfertilized eggs develop into males and fertilized eggs develop into females. Because females can choose to fertilize eggs, and because it takes a higher energy input to produce females, a large, high quality host should strongly influence sex ratio (Opp and Luck 1986; Tillman and Cate 1993; Stoepler et al. 2011; Vacari et al. 2012). Finally, length of

the hind tibia is a good indicator of parasitoid size and has been shown to correlate with female performance, specifically reproductive, in the field (West et al. 1996; Bennett and Hoffmann 1998; Sagarra et al. 2001).

The goal of this study was to determine the functional response of *B. communis* in order to better understand the impact it has on *A. glycines*, as a classical biological control agent.

Methods

Insect Colonies. All laboratory *A. glycines* and *B. communis* colonies were reared on Pioneer 91M51 variety soybean plants in early vegetative stages. Aphid colonies were obtained from soybean fields in St. Paul, MN, USA and have been in colony since 2003. The aphids were maintained on plants in the laboratory at $25\pm 5^{\circ}\text{C}$, 60-80% r.h., and 16:8 L:D. The plants were potted in square three inch plastic pots. Six pots were placed on 25 cm by 35 cm plastic trays and water was poured in the tray (bottom watered) approximately every other day. The trays were kept in square Plexiglass cages with 105 micron white nylon mesh sleeves (Questoutfitters.com; Sarasota, FL).

The parasitoids were obtained from a Chinese strain of *B. communis* in Harbin, Heilongjiang province, China by K. Hoelmer, K. Chen, W. Meikle, and L.A. Pin and have been in colony since 2002. The University of Minnesota colony was started from the original strain that has been held at the USDA-ARS Beneficial Insect Introduction Research Laboratory in Newark, Delaware (Wyckhuys et al. 2008). The colony at the

University of Minnesota St. Paul campus laboratory was reared on *A. glycines*-infested soybean plants at $25\pm 5^{\circ}\text{C}$, 60-80% r.h., and 16:8 L:D.

Functional Response Experiment. The functional response of *B. communis* on *A. glycines* was measured at six aphid densities, ranging from 10 to 1000 per soybean plant. Fitness parameters of *Binodoxys communis* offspring were measured, including: number of parasitized aphids (mummies), emergence rates, sex ratios, and hind tibia length. Between 7 and 13 days prior to the start of each experiment two male and three female wasps were placed on three aphid-infested soybean plants that were potted together in a 15-cm diameter plastic pot. To ensure that the wasps would stay on the plant and parasitize aphids, we covered each pot with a clear plastic cylinder (21 cm in height x 9.7 cm in diameter) that had eight mesh covered holes. A seal was made by placing a foam strip between the pot and the base of the cylinder, based on Dieckoff and Heimpel (2010). Each single experimental plant was also potted in 15-cm diameter plastic pot and a clear plastic cylinder (21 cm in height x 9.7 cm in diameter) with eight mesh-covered holes. The treatments were 10, 50, 100, 250, 500, and 1000 aphids per plant and each was replicated 13 times. Individual 1st and 2nd instar *A. glycines* were placed on the plant via paintbrush, two d prior to parasitoid release, to allow aphid settling. To ensure that all experimental wasp adults were approximately the same age, mummies were collected from the respective colony tubes, started one week prior, and newly emerged (< 24-h-old) adults set aside and sexed. To ensure mating, one female and one male adult parasitoid were placed together in a 0.65ml microcentrifuge tube for four h prior to release. One mated female parasitoid was then placed on each experimental plant and left for 24 h.

After 10 d, all mummies were collected, placed in gel capsules (Solaray; Brick, NJ; size 0), and allowed to emerge in growth chambers. Once emerged, each wasp was sexed to calculate sex ratios and was then slide-mounted to measure hind tibia lengths. Hind tibiae were measured using an ocular micrometer on an Olympus BX50 compound microscope at 10x magnification, in order to estimate size of the emerged adults from each treatment.

Data Analysis. The model used for the type II response was taken from Holling (1959)(Model 1):

$$N_a = \frac{aTN}{1 + aT_h N}$$

N_a is the number of host attacked with attack rate/searching efficiency, a , per time period, T , at given prey/host density, N , and T_h is handling time. The model for Type III was taken from Murdoch et al. (2003)(Model 2):

$$N_a = \frac{aN^2}{N^2 + z}$$

where z is a constant. There are four different models that have been used to describe a type IV functional response (Tostowaryk 1972; Fujii et al. 1986; Hassell et al. 1977; Liznarova and Pekar 2013). Tostowaryk (1972) proposed (Model 3):

$$N_a = \frac{aTN}{1 + aT_h N + acN^3}$$

where c is the rate of cooperative defense, sometimes termed the inhibition of prey parameter. Hassell et al. (1977) proposed (Model 4):

$$N_a = \frac{uTN^2}{1 + uT_hN^2 + vN}$$

With this model, u and v are both unknown parameters. The model was designed to be used for type III but can be used as a type IV response if the value for parameter v is negative. This will create the slight domed curve of a type IV response. Fujii et al. (1986) proposed (Model 5):

$$N_a = \frac{TaNe^{cN}}{1 + T_haNe^{cN}}$$

This model was meant to capture all four different functional responses. If parameter, c , is more than 0, the resulting curve will be slightly domed. Liznarova and Pekar (2013) proposed (Model 6):

$$N_a = \frac{aTN}{1 + aT_hN + acN^2}$$

This model was designed specifically for a domed type IV response.

For the functional response of *Binodoxys communis* on *Aphis glycines*, each of the four type IV models were fit to the entire data and parameters, a , T_h , and c , were estimated and fit analyzed using SigmaPlot® version 11.2 (Systat Software, Inc., San Jose California USA). For this study T was always one d, and N represented the various aphid densities. SigmaPlot® was also used to fit a quadratic polynomial to the length of male and female hind tibia on aphid density. A simple linear regression was used to determine if aphid density affected total mummy count, proportion of wasps that emerged, and sex ratio. The statistical analysis for total mummy count, proportion of

wasps that emerged, sex ratio, and male and female hind tibia length were performed using JMP 10.0 (SAS Institute Inc., Cary, North Carolina, USA).

Results

The average number of mummies per plant increased with the number of aphids per plant up to 500 aphids with fewer mummies per plant at 1000 aphids ($F_{2,75}=20.0017$, $P<0.0001$). The number of mummies at various aphid densities was fit to a type II (Model 1) ($F_{2,76}=30.7989$, $P<0.0001$), type III (Model 2) ($F_{1,76}=32.1918$, $P<0.0001$), and the best fitting type IV (Model 6) ($F_{2,75}=18.3197$, $P<0.0001$) (Fig. 3-1; Table 3-1). It was also fit to the other three type IV functional response models: Model 3 ($F_{2,75}=1.1824$, $P=0.3122$); Model 4 ($F_{2,75}=15.8960$, $P<0.0001$); and Model 5 ($F_{2,75}=15.9280$, $P=0.3122$) (Table 3-1).

The type II response begins to reach its asymptote at around 200 aphids and approximately 50 mummies (Fig. 3-1). The response increases slightly as aphid numbers increase, with around 60 mummies at 1000 aphids. The type III response looks almost identical but has a slightly higher R^2 value at 0.2975 (Table 3-1). The type IV response with the best fit, model 6, had the highest R^2 at 0.3282 (Table 3-1). Model 6 reaches its maximum of 70 mummies at approximately 400 aphids. After 400 aphids, as the aphid numbers increases, the response decreases with approximately 50 mummies at 1000 aphids (Fig. 3-1).

Although there was no significant difference in the proportion of wasps that emerged across the aphid densities, the general trend was a decrease in proportion emergence as aphid densities increased ($F_{1,76}=3.6011$, $P=0.0615$). Aphid density did not

have a significant effect on sex ratio ($F_{1,76}=0.0926$, $P=0.7617$). The length of the male hind tibiae were fit to a quadratic polynomial and decreased at a decreasing rate with increasing aphid density ($F_{2,72}=5.7061$, $R^2=0.1368$, $P=0.0320$) (Fig. 3-2a). Similarly, the length of the female hind tibia decreased at a decreasing rate with increasing aphid density ($F_{2,59}=10.1346$, $R^2=0.2557$, $P=0.0012$) (Fig. 3-2b).

Discussion

This study demonstrates that the aphid parasitoid, *Binodoxys communis*, possesses a type IV functional response. Model 6 is the best fitting type IV model based on parameter significance and higher R^2 compared to the other three type IV models. Based on the better overall fit of type IV over type II and III, it can be concluded that *B. communis* possesses a type IV, domed functional response. Even though the increase in aphid density caused a decrease in the number of mummies, it did not significantly affect parasitoid emergence or sex ratio. It did, however, affect both male and female average hind tibia length, by decreasing length of hind tibiae at a decreasing rate as aphid numbers increased, for both sexes. This indicates that as aphid densities increase they yield smaller, less fit parasitoid offspring compared to offspring reared on aphids at a low density (West et al. 1996). Though it is unclear why a non linear fit best described the data for hind tibiae length, it is possible that an increase in aphid numbers may induce plant defenses that are more noticeable at high aphid densities, as is the case with *Myzus persicae* on *Arabidopsis thaliana* (Arabidopsis) (de Vos et al. 2007). These plant

defenses may not be induced until high aphid densities are reached which could decrease the quality of the aphids and thus decrease size of parasitoids.

There are several reasons that may explain the better fit of the Liznarova and Pekar (2013) model for a type IV response. The model designed by Tostowarky (1972) was designed to fit host densities of approximately 40 individuals with most of the variation experienced between 5-10. This concentration on low host densities, in conjunction with the relatively few low aphid densities as well as the higher aphid densities represented in this study, could explain the poor fit Tostowaryk's model had to this data. The model described by Hassell et al. (1977) was designed to be a general fit for all functional responses and the model described by Fujii et al. (1986) was designed to explain a type III or IV functional response. The best fitting model, Liznarova and Pekar (2013), was designed specifically for a type IV, domed functional response. The non-specificity to a domed functional response in the models described by Hassell et al. (1977) and Fujii et al. (1986), compared to Liznarova and Pekar (2013) may explain the slightly higher R^2 for the latter.

It is difficult to explain exactly why *B. communis* possess a type IV functional response. Liznarova and Pekar (2013) suggest that host defense is a limiting factor for predation rate in a type IV response. As aphid numbers increase, the potential for group defense increases (Wyckhuys et al. 2008). When under attack by parasitoids, aphids have been shown to produce cornicle secretions, kick, raise, and rotate their body (Wyckhuys et al. 2008; Desneux et al. 2009). The dome shape of the parasitism rate could be exaggerated by aphid honeydew accumulation with increased host densities that could

create obstacles for parasitoids, as is the case with the whitefly parasitoid, *Encarsia formosa* (Hymenoptera: Aphelinidae) (Hoddle et al. 1998). Host crowding could also hinder parasitism rates since crowded aphids are physically close together which would make oviposition difficult. Since parasitoids require physical contact with hosts in order to oviposit, obstacles like sticky honeydew and host crowding, could lead to lower numbers of mummies at high host densities. In the field, response of *B. communis* is also affected by higher intraguild predation in areas with high aphid densities (Chacon et al. 2012), possibly explained by predator aggregation to aphid density (Chacon and Heimpel 2010). A type IV functional response reflects the wasp's positive response to lower aphid densities as a consequence of less group defense, honeydew, crowding, and intraguild predation.

This study may help to explain why *Binodoxys communis* has had difficulty as a classical biological control agent of *Aphis glycines* in the north central soybean growing region of the United States. The more that is known about a natural enemy's functional response the easier it will be to predict its efficacy as a biological control agent. At high host densities, an agent with a type II functional response should be producing the same number of mummies per wasp as it would be at medium host densities (Holling 1959). This translates into a maximum number of wasps being produced for the next generation. This is not the case for an agent with a type IV response. The type IV functional response results in fewer mummies being produced per wasp at higher host density as compared to medium host densities (Holling 1961; Mori and Chant 1966; Tostowaryk 1972). Similar results were found for the parasitoid *Aphidius ervi* and its host, the pea aphid

Acyrtosiphon pisum where the parasitoid went extinct even though it was exposed to high pea aphid densities (Ives and Settle 1996). Past the host threshold, there are fewer than maximum number of wasps to produce the next generation and in the field, higher risk for intraguild predation (Chacon et al. 2010 and 2012). Theoretically, these biological control agents may be more effective at keeping host populations low early in the season before an outbreak occurs.

This study emphasizes the importance of the functional response of classical biological control agents. When an agent is released into high host densities, a type IV functional response may explain not only establishment of the agent, but suppression of the host as well. It also has implications for a potential parasitoid release, both in selecting the time of the season, and a location based on when and where host outbreaks occur. Due to these implications for successful biological control, when investigating functional response it is crucial to include a wide range of host densities. If an agent possesses a type IV response and the study does not reach peak densities, the dome of the type IV response may not be reached. This will yield an overestimation of the agent's ability to control host populations. The more that is understood about the response of a biological control agent to varying host densities, the easier it will be to predict the efficacy it will have in an introduced range.

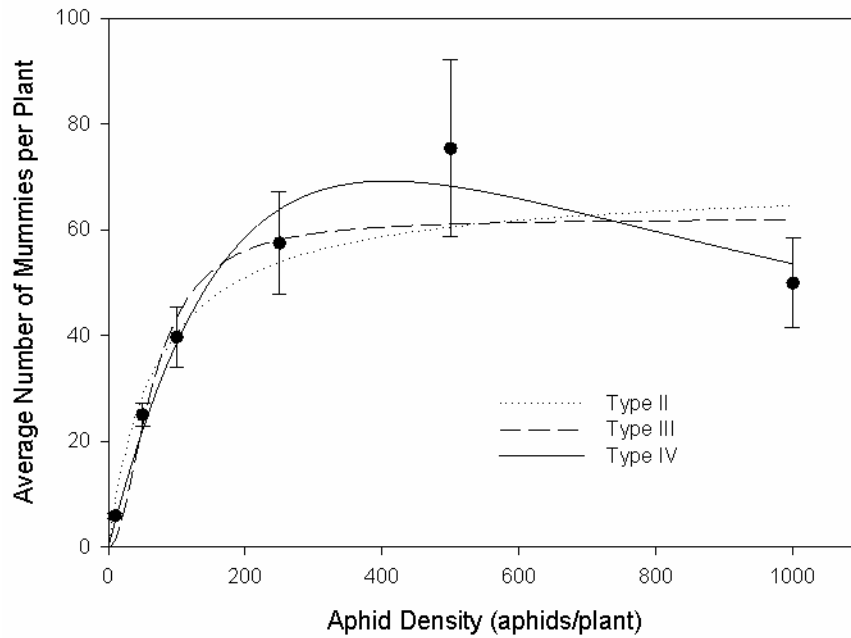


Figure 3-1. Average number of *B. communis* mummies per one parasitoid per 24 hour period as a function of densities of *A. glycines*. Treatment means fitted to a type II

functional response (Model 1) $y = \frac{(0.976)x}{1 + (0.976)(0.015)x}$, type III (Model 2)

$y = \frac{(62.109)x^2}{x^2 + 4325.09}$, as well as the best fitting type IV functional response (Model 6)

$y = \frac{(0.497)(x)}{1 + (0.497)(0.005)(x) + (0.497)(1.2E - 5)(x^2)}$. Error bars represent standard errors of

the means.

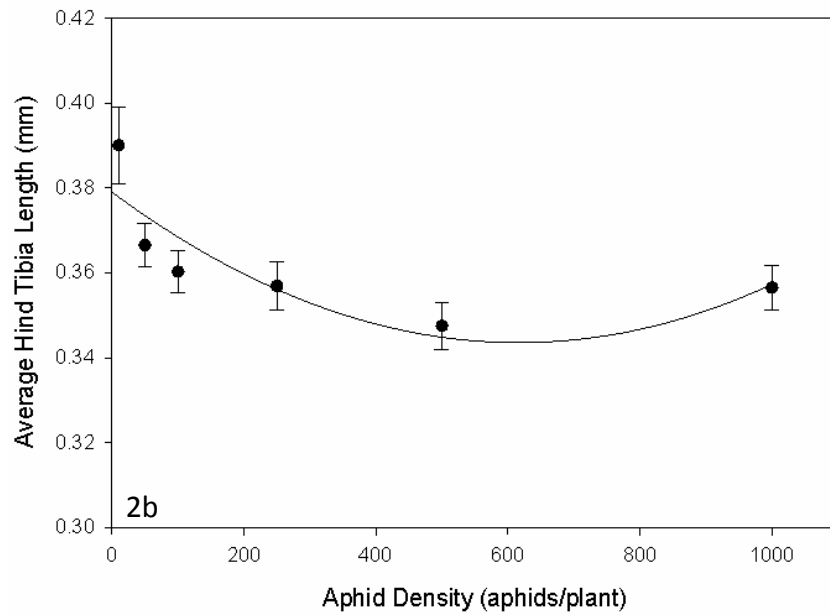
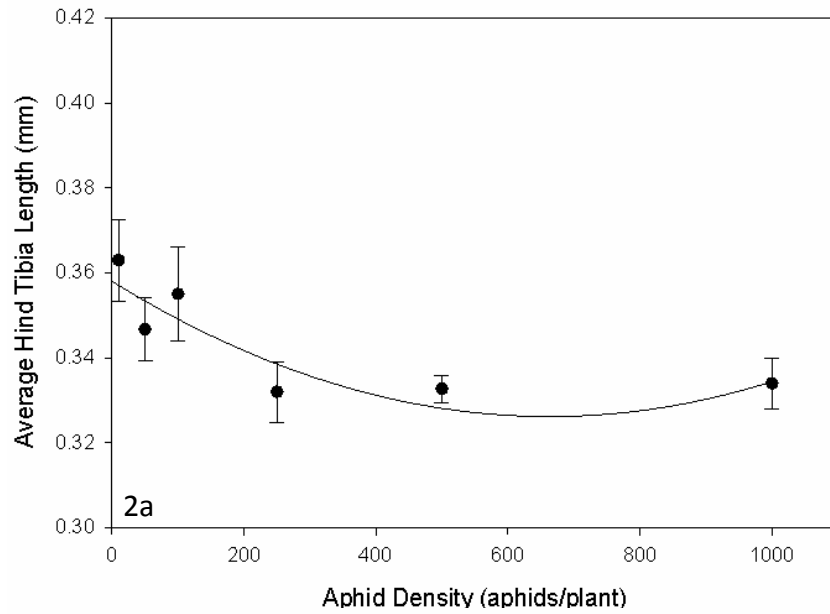


Figure 3-2. Average *B. communis* hind tibia lengths (mm) as it relates to densities of *A. glycines* (number/plant). Error bars represent standard errors of the means. Fig 2a male hind tibia length. Fig 2b female hind tibia length.

Table 3-1. Comparison of the different models used to describe type II, III, and IV functional responses for *Binodoxys communis*. * = $p < 0.05$; ** = $p < 0.01$.

Functional Response	R ²	$a \pm \text{s.e.}$	$t_h \pm \text{s.e.}$	$c \pm \text{s.e.}$	$z \pm \text{s.e.}$
(1)Type II	0.2884	0.09762 ± 0.040	$0.015 \pm 0.002^{**}$	-	-
(2)Type III	0.2975	$62.1096 \pm 5.689^{**}$	-	-	4325.09 ± 2297.14
(3)Type IV	0.0306	$-2.99E7 \pm 7.41E13$	$0.025 \pm 0.001^{**}$	$-7.58E-9 \pm 5.43E-9$	-
(4)Type IV	0.2977	$\frac{(0.01*N)}{(1+(-0.002)*N)}$	$0.016 \pm 0.003^{**}$	-	-
(5)Type IV	0.0298	10.9706 ± 9.244	$0.016 \pm 0.001^{**}$	$0.0053 \pm 0.003^*$	-
(6)Type IV	0.3282	$0.4973 \pm 0.19^{**}$	0.0046 ± 0.005	$1.20E-5 \pm 6.59E-6$	-

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