Investigation of Reduced Agent and Area Treatments for *Aphis glycines* Management and its Effects on Key Predators

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Investigation of Reduced Agent and Area Treatments for *Aphis glycines* Management and its Effects on Key Predators

By

Jenny S. Enchayan

A THESIS

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The Graduate College at the University of Nebraska
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Investigation of Reduced Agent and Area Treatments for *Aphis glycines* Management and its Effects on Key Predators

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Soybeans are an abundant and valued agricultural crop in Nebraska and other parts of the world. Significant soybean aphid, *Aphis glycines* Matsumura, mortality has been observed in research plots adjacent to soybeans treated with chlorpyrifos in late July through August. The objectives of the study were to determine the efficacy of the chlorpyrifos vapor effect on *A. glycines* in adjacent untreated areas and assess the impact of the vapor effect on key biological control agents. Observation of *A. glycines* populations and sweep net samples for other arthropod species, including *Oruis insidiosus*, began weekly in mid to late July. When aphid levels reached economic thresholds, chlorpyrifos formulation Lorsban Advanced® was applied to designated spray areas. Weekly assessments continued to monitor populations both in the spray areas and at set distances from the spray areas. Results from the 2013 field season suggest an area of eight to sixteen rows may be left as untreated zones. Results from 2014 field season are inconclusive due to crop failure. Laboratory predation studies observed consumption rates of a key biological control agent, *O. insidiosus*. Studies measured life stage choice of *A. glycines* by *O. insidiosus*,
consumption rates at each life stage, and video recordings were used to quantify behavior. There was no significant difference in a preference for *A. glycines* life stage by *O. insidiosus* ($\chi^2 = 1.73, \text{df} = 2, P = 0.4204$). *Orius insidiosus* consumes increasing numbers of aphids over a 24-hour period, consuming more early stage than late stage aphids, and more late stage than adult *A. glycines*. Behavior recordings suggest *O. insidiosus* returns to previously attacked, but unconsumed *A. glycines*. 
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GRANT INFORMATION

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Introduction

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Chapter 1

Introduction and Literature Review

Soybean

Soybean (*Glycine max* (L.) Merrill) is a commonly cultivated and important crop around the world. In 2014, 83 million acres of soybeans were harvested in the United States producing 3.968 billion bushels worth $40 billion (USDA NASS 2015a, b). The projected global outlook for 2013 was 108.69 million hectares worldwide, which resulted in 267.88 million metric tons of soybeans (USDA 2013). In Nebraska, 5.3 million acres were harvested resulting in 288 million bushels of soybeans in 2014 (USDA NASS 2015a, b).

In addition to soybean’s use as a food and oil crop, soybean is used in a variety of products including biodiesel, adhesives, coatings and printing inks, lubricants, and plastics (United Soybean Board 2013).

*Aphis glycines* Biology and Ecology in North America

The soybean aphid, *Aphis glycines* Matsumara, was first described in 1917 (Matsumara 1917). It is characterized as a small light yellow or yellowish green aphid with dark cornicles. *Aphis glycines* exhibits four instars and exhibits a holocyclic and heterocious lifecycle (Wu et al. 2004).

*Aphis glycines* colonization of *Glycine max* (L.) Merrill and *Rhamnus* species distinguish it from other species (Voegtlin et al. 2004a). The host relationship is demonstrated by its migration between its primary host soybean and its overwintering *Rhamnus* hosts. *Rhamnus* hosts include the European common

The holocyclic lifecycle is demonstrated by sexual reproduction through part of its lifecycle. *Aphis glycines* overwinters as eggs on *Rhamnus* and hatch in the spring. The first generation feeds on their *Rhamnus* host developing into wingless fundatrices that produce the second wingless and primarily female generation. The third generation is primarily winged aphids that leave *Rhamnus* in search of the primary host soybean (Ragsdale et al. 2004). *Aphis glycines* has approximately 15 holocyclic generations per season on the primary host soybean and three heterocycous generations on *Rhamnus* species (Tilmon et al. 2011).

Once established on soybean, many overlapping generations of both wingless and winged individuals can occur. Winged females maybe produced because of crowding, declining host quality and reduced photoperiod and temperature (Beckendorf et al. 2008). Populations can double in as little as 1.5 to 2 days under optimal conditions with temperatures ranging from 77–86°F (25–30°C) with little to no weather events, few natural enemies, and little disease. However with weather, natural enemies, and disease factors field-doubling time averages 6 to 7 days (McCornack et al. 2004, Ragsdale et al. 2007, Tilmon et al. 2011). The optimal developmental temperature for *A. glycines* is 82°F (27.8°C) with a lower limit of 47.5°F (8.6°C) and an upper limit of 94.8°F (34.9°C). However, as temperatures increase, aphid populations tend to decrease (McCornack et al. 2004).

During early season *A. glycines* colonization of soybean, aphids are most often found on the new upper trifoliate leaves. With plant maturity and season
progression, *A. glycines* are found lower in the canopy. *Aphis glycines* most often colonize on the underside of the leaf, however they will also feed on stems and pods. (McCornack et al. 2008, Tilmon et al. 2011). *Aphis glycines* levels peak during pod filling. Population growth may be favored when plants have a potassium deficiency or an increase in nitrogen (Walter and Difonzo 2007, Noma et al. 2010).

With dropping temperatures and shortening daylength in the fall, winged *A. glycines* females called gynoparae leave soybean in search of *Rhamnus*. These gynoparae produce nymphs on *Rhamnus* called oviparae. Winged males also develop on *G. max* shortly after the gynoparae. These winged males leave *G. max* in search of the oviparae on *Rhamnus*. The males mate with the oviparae. Mated oviparae most often lay their eggs at the interface between the bud and twig (Ragsdale et al. 2004).

*Aphis glycines* Distribution

*Aphis glycines* was first detected in the United States in Wisconsin in 2000, although the consensus is it was present but undetected for some years before discovery. Its native Asian range includes China, Korea, Japan, the Philippines, Thailand, Malaysia, Indonesia, and Taiwan (Ragsdale et al. 2004, Tilmon et al. 2011). *Aphis glycines* is also a recent pest in Australia (Ragsdale et al. 2004).

In addition to the United States and its native range, *A. glycines* is found in Vietnam, Russia, and Canada (Wu et al. 2004). Following *A. glycines* discovery in Wisconsin, by the end of the summer 2000 it was found in 10 US states. As of 2009, *A. glycines* was found in 30 states and three Canadian provinces (Ragsdale et al. 2011).
Pest Status

*Aphis glycines* is considered a major pest of soybean (Ragsdale et al. 2011). Contributing to its success as a serious pest of soybean is the success of its overwintering host, *R. cathartica*, another nonnative and invasive species (Heimpel et al. 2010). Timing of colonization varies, with soybean in some areas of the United States and Canada being colonized by winged *A. glycines* as early as plant emergence. In other areas, such as Nebraska, *A. glycines* can colonize soybean during mid-reproductive stage soybean and subsequently be an economic pest of late reproductive stage soybean (Brosius et al. 2007, Hodgson et al. 2012, Prochaska et al. 2013).

Injury and Damage

*Aphis glycines* feed on soybean phloem sap with piercing-sucking mouthparts. Feeding can cause injury resulting in physical damage, such as stunting of plants, reduction in pod and seed counts, and puckering and yellowing of leaves (Diaz-Montano et al. 2007, Zhu et al. 2011). Even without visible symptoms, relatively low populations of *A. glycines* cause physiological injury to soybean (Macedo et al. 2003, Beckendorf et al. 2008).

Honeydew secretions produced by *A. glycines* encourage the growth of dark, sooty mold grow. The sooty mold layer blocks light and can inhibit light absorption needed for photosynthesis (Tilmon et al. 2011). Photosynthetic and gas exchange rates can be reduced when *A. glycines* is present at levels as low at 20 aphids per leaflet and *A. glycines* injury has the potential to compromise the ability of the plant to restore chlorophyll to a low energy, light-receptive state (Macedo et al. 2003).
Aphis glycines can cause >50% soybean yield loss. (Wang et al. 1994). Total yield and biomass can be decreased by as much as 72% to 90% (Costamagna et al. 2008). In addition to yield and biomass losses, A. glycines can also alter seed oil and protein concentrations. At high A. glycines numbers, seed oil concentrations decline linearly while protein concentrations increase linearly (Beckendorf et al. 2008).

Aphis glycines can transmit several viruses including soybean mosaic virus (SMV), alfalfa mosaic virus (AMV), tobacco ringspot virus (TRSV), peanut stunt virus (PSV), bean yellow mosaic virus (BYMV), and bean pod mottle virus (BPMV) (Clark and Perry 2002, Wang et al. 2006). In the case of SMV, A. glycines is most effective as a vector after briefly probing an infected plant before moving to a healthy plant (Wang and Ghabrial 2002).

Integrated Pest Management and Thresholds

Many pest management programs are based on the principles of integrated pest management (IPM). While there are many definitions of IPM (Bajwa and Kogan 2002), Cate and Hinkle (1994) define integrated pest management as “the judicious use and integration of various pest control tactics in the context of the associated environment of the pest in ways that complement and facilitate the biological and other natural controls of the pests to meet economic, public health, and environmental goals”.

Integrated pest management includes the important concepts of the economic injury level and economic threshold. From an entomological point of view, the economic injury level is defined as “the lowest population density that will cause economic damage” and the economic threshold is defined as “the population density
at which control measures should be initiated to prevent an increasing pest population from reaching the economic injury level” (Pedigo et al. 1986).

The practical economic threshold currently accepted for soybean is 250 *A. glycines* per soybean plant (Ragsdale et al. 2007, Hodgson et al. 2010, North Central Soybean Research Program [NCSRP] 2013) and populations are increasing. Ragsdale et al. (2007) calculated a mean economic threshold of 273±38 (95% CI) and a mean economic injury level of 674 aphids.

Methods for Managing *Aphis glycines*

There are several general methods used to manage *A. glycines*. These include host plant resistance, the conservation and utilization of natural enemies, and chemical control. Chemical control is currently the most widely used method to manage soybean aphid.

Soybean Plant Resistance and *Aphis glycines* Biotypes

Soybean varieties with resistance to *A. glycines* have been identified. Resistance was first identified in three genotypes: Dowling, Jackson, and PI71506 (Hill et al. 2004). A single dominant gene, *Rag1*, identified in the cultivar Dowling is responsible for the resistance mechanisms against *A. glycines* (Hill et al. 2006a, Li et al. 2007). In the cultivar Jackson, a single gene controls resistance. Dowling, with the *Rag1*, and Jackson have no known genetic relationship (Hill et al. 2006b). Jackson and Dowling have both been documented as expressing antibiosis (Diaz-Montano et al. 2006). Other soybeans with resistance characteristics include K169 and Pioneer 95B97, which exhibit strong antixenosis. The exact genes of resistance remain
unidentified for K169 and Pioneer 95B97 (Diaz-Montano et al. 2006). Currently
*Rag1, Rag2, Rag3*, and *Rag4* have been discovered. Two others, *Rag5* and *Rag6* are
proposed, but require further genetic studies for validation (Crossley and Hogg
2015).

While the *Rag* genes are typically associated with antibiosis and antixenosis,
the resistance mechanism tolerance has also been explored. The soybean variety
KS4204 tolerates relatively high levels of *A. glycines* without the severe impact on
yield observed for many other soybean varieties (Pierson et al. 2010, Marchi 2012,
Prochaska et al. 2013).

Some populations of *A. glycines* are able to overcome resistance genes and
are known as biotypes. As of 2012, four resistant *A. glycines* biotypes have been
identified and are listed as biotype 1, biotype 2, biotype 3 and biotype 4. Biotype 1,
also known as the Illinois biotype, and biotype 2, also known as the Ohio biotype,
are virulent on soybeans possessing the *Rag1* gene. Cultivars with the *Rag1* gene
were not released for commercial production before resistant biotypes were
discovered (Kim et al. 2008). In 2010, biotype 3 was identified as virulent to
soybean with the *Rag2* gene (Hill et al. 2010). In 2012, biotype 4 was identified as
virulent to both the *Rag1* and the *Rag2* genes, as it readily colonized plants with
these traits (Alt and Ryan-Mahmutagic 2012). *Rag* virulent soybean aphid biotypes
were discovered in the absence of large-scale *Rag G. max* plantings. This means
single-resistance gene soybean plants will be ineffective for long-term management
of *A. glycines* (Crossley and Hogg 2015).
Natural Enemies and Biological Control Agents

Predators and biological control agents are an important part of *A. glycines* suppression and a key factor for IPM (Fox et al. 2004, Rutledge et al. 2004, Desneux et al. 2006, Brosius et al. 2007, Rhainds et al. 2007, Schmidt et al. 2007, Costamagna et al. 2008). Many key biological control agents feeding on *A. glycines* in a soybean field are generalist predators. Generalist feeders have an important role for effective aphid suppression. While parasitoids are present in soybean fields, they provide only a small suppression for *A. glycines* management (Brown et al. 2003, Donaldson et al. 2007, Costamagna et al. 2008).


Donaldson et al. (2007) observed a clear density-dependent effect of predators on *A. glycines* populations in a one week period. Desneux (2006) found that in the presence of predators, *A. glycines* reached a maximum of a 2.9-fold increase versus a maximum 7.7-fold increase without predators.

In North America, there are a number of native predators and parasitoids of *A. glycines*. Among them are *Orius insidiosus* Say, the insidious flower bug, several coccinellid species (*Coccinella septempunctata* and *Harmonia axyridis*), spiders, syrphid larvae (Desneux et al. 2006), predatory midges, lacewings (*Chrysoperla*...
carnea) (Zhu and Park 2005) (Donaldson et al. 2007) and Scymnus (Pullus) louisianae J. Chapin (Brown et al. 2003). A few species of entomopathogenic fungi may also be effective A. glycines natural enemies (Nielsen and Hajek 2005).

The most abundant predators of A. glycines are H. axyridis, O. insidiosus, and Leucopis spp. (Fox et al. 2004). Orius insidiosus adults are more common than coccinellid adults with Coccinellidae reaching only a quarter the population after A. glycines levels reached high populations in late season (Yoo and O’Neil 2009).

Surrounding landscapes can impact predators, and may therefore impact A. glycines populations. Low diversity landscapes consisting of primarily corn and soybeans reduce the biological control numbers and diversity as compared to higher diversity landscapes (Gardiner et al. 2009). A more diverse landscape is associated with lower aphid populations (Noma et al. 2010).

Orius insidiosus

The insidious flower bug, Orius insidiosus Say (Hemiptera: Anthocoridae), is a generalist predator and significant predator of A. glycines in soybean field ecosystems (Fox et al. 2004, Desneux et al. 2006, Brosius et al. 2007). Orius insidiosus is the most common natural enemy in northeastern Nebraskan soybean fields. Brosius et al. (2007) used field cage plots to find a significant difference in A. glycines populations compared to predator free field cage plots. A study by Desneux (2006) found >85% in 2004 and 89.6% in 2005 of all predators surveyed were O. insidiosus. A significant negative relationship was observed between O. insidiosus and A. glycines.
The more prey provided, the more *O. insidiosus* will consume. When provided 32 *A. glycines*, one *O. insidiosus* was shown to consume a maximum of 10.23 ± 3.5 SE in a 24-hour period (Rutledge et al. 2004). When provided twice the population of *A. glycines*, at 64, *O. insidiosus* showed a Type II functional response consuming a maximum of 19.25 ± 3.38 SE in a 24-hour period. A Type II functional response has been reported for *O. insidiosus* for other prey items and is characterized by the rate of predation increasing as the rate of prey rises while eventually reaching a plateau (Holling 1959, 1965, Isenhour and Yeargan 1981, Rutledge and O'Neil 2005). Over a lifetime, an adult *O. insidiosus* consumes an average of 126 *A. glycines* (Butler and O'Neil 2008). The efficacy of *O. insidiosus* control of *A. glycines* maybe reduced as soybean plants age and become more complex (Desneux and O'Neil 2008).

*Orius* species are able to utilize the soybean plant to derive nutrients and water. When utilizing soybean, *O. insidiosus* feeds on xylem sap and mesophyll tissue (Armer et al. 1998). However, unlike some *Orius* spp, *O. insidiosus* fed only a diet of soybean leaflets do not survive to reproduction. *Orius insidiosus* is likely to survive to adulthood when it consumes a variety of prey (Kiman and Yeargan 1985, Butler and O'Neil 2007b). *Orius insidiosus* is shown to have greater fitness and may be healthier when feeding on multiple prey such as *A. glycines* and the soybean thrips, *Neohydatothrips variabilis* (Butler and O’Neil 2007a, Desneux and O’Neil 2008). Diversifying the diet of *O. insidiosus* with alternative prey such as *N. variabilis* could enhance their effectiveness as predators of *A. glycines* (Harwood et al. 2007).

*Neohydatothrips variabilis* maybe a higher quality prey item for *O. insidiosus* (Butler and O’Neil 2007a). Desneux et al. (2008) found a significant two-fold
reduction on the attack rates of *A. glycines* when *N. variabilis* were present in comparison to aphids alone. When thrips are near, *Orius* predators typically attack thrips. Thrips exhibit escape behavior when approached by *O. insidiosus. Aphis glycines*, on the other hand, move only after a nearby or direct predator attack.

*Aphis glycines* is known to excrete exudate from their cornicles as a defense mechanism against predators. Exudate has been shown to cause mortality for *O. insidiosus*, especially for early instars (Butler and O’Neil 2006). *Orius insidiosus* also significantly increase their time grooming when *A. glycines* is present (Desneux and O’Neil 2008). Other hemipteran predators frequently move their stylets to change the site of feeding position while others also inject secretions and enzymes into their prey (Cohen 1990). Aphids briefly attacked by *Orius insidiosus* have low survival, suggesting an injection of an enzyme or toxin which immobilizes or kills the aphid (Butler and O’Neil 2006).

There are 5 nymphal stages for *O. insidiosus*. The length of each instar stage varies dependent on the food supplied. In general as a higher amount and a greater diversity of prey is provided, the shorter the instar development time (Butler and O’Neil 2007a, b). Developmental times decrease as temperature increases (Isenhour and Yeargan 1981).

*Orius insidiosus* experiences seasonal life cycles increasing in population in spring and decreasing in population in fall. In Missouri soybeans, adult *Orius* are observed on V6 stage soybeans when planted May 9 and on V2 stage soybeans planted June 20 (Isenhour and Marston 1981). In East Lanning, Michigan in 2001, *O. insidious* adults began appearing in the early season with a population drop off and
nymphs appearing in the mid-season. In Indiana in 2002, adult and nymph populations began to increase in late July, with populations spiking in late September (Rutledge et al. 2004). It is suggested that *O. insidiosus* populations follow thrips populations reaching peak numbers after thrips reach their maximum densities. In northern Indiana, thrips typically appear in soybeans soon after seedling emergence in late May, reach peak populations in July or early August, and decline as plants senesce (Yoo and O’Neil 2009).

**Coccinellidae species**

Members of the family Coccinellidae are known predators of *A. glycines* (Fox et al. 2004, Rutledge et al. 2004, Brosius et al. 2007). Coccinellids, or lady beetles, exhibit a holometabolous or complete metamorphosis composed of an egg, larvae, pupa, and adult stage. Both the larval and adult stages are predatory (Koch 2003).

*Harmonia axyridis* (Pallas) is a lady beetle species native to Asia known to prey upon *A. glycines*. It was released as a biological control in the United States as early as 1916. In 1988, a naturalized population was discovered and has since spread throughout the United States. These beetles typically live 30 to 90 days but can live up to three years. They require 230 to 270 degree days to develop with a developmental threshold of 10.5°C to 11.2°C (Koch 2003, Brown et al. 2011).

While *H. axyridis* is an intraguild predator, the overall effect is a reduction of *A. glycines* populations. However, the removal of *H. axyridis* from the soybean predator guild does not improve the overall control of *A. glycines* by all predators (Gardiner and Landis 2007). *Harmonia axyridis* peak arrival and oviposition
coincides at or before the peak of aphid populations. Populations are affected by cannibalism and its intensity is inversely related to aphid populations (Koch 2003).

Predation of aphids by lady beetles varies depending on age and species. Type II functional predation response is typical and has been shown for *H. axyridis* and for *Coccinella septempunctata* (Koch 2003). For *H. axyridis* combined larval stage consumption is 90 to 370 aphids and is dependent on the aphid species. Adult females consume more than adult males at an average range of 15 to 65 aphids per day. This may be because of higher nutritional requirements of females to reach adulthood and reproductive requirements than of males (Van Den Berg et al. 1997, Koch 2003, Omkar and Srivastava 2003, Xue et al. 2009).

For *H. axyridis*, the maximum daily consumption of adult aphids is predicted to be 244 per third instar, 156 per female adult, and 73 per adult male (Xue et al. 2009). Theoretical maximum daily consumption of adult aphids by *C. septempunctata* is predicted to be 204 per third instar, 277 per female adult, and 166 per adult male. (Xue et al. 2009).

An Indonesian species of Coccinellidae, *Paederus fuscipes* consumed 37 *A. glycines* per day when populations were at high densities. *Paederus fuscipes* consumed early stage (mostly immature) *A. glycines* 88% of the time when *A. glycines* were at high densities (Van Den Berg et al. 1997).

Predators use chemical cues from plants to detect potential prey. *Coccinella septempunctata* is attracted to the volatile compound methyl salicylate. Methyl salicylate is produced when *A. glycines* colonize soybean and higher amounts are produced by early stage soybean (V1, V2, and V3). This may encourage *C. 
*sepempuncata* to prey upon *A. glycines* when present on early stage soybean (Zhu and Park 2005).

Insecticide controls

Insecticide options to control *A. glycines* include the organophosphates chlorpyrifos and dimethoate, pyrethroids, and neonicotinoids in the forms of both foliar sprays and seed treatments (Chandrasena et al. 2011). So called “reduced-risk” insecticides utilized for *A. glycines* include pyrethrins, insecticidal soaps, and narrow-range mineral oil (Kraiss and Cullen 2008a). Bioinsecticides for *A. glycines* control include spinosad (Kraiss and Cullen 2008b).

The timing of insecticide application is more important than the treatment used (Johnson et al. 2009) and can differ by region. Different areas of the United States have different recommendations for the number of effective applications. In Iowa, a single foliar application of insecticide was just as effective as multiple applications due to the ET not being exceeded twice in a single season (O’Neal et al. 2009). However in northern states like Wisconsin where *A. glycines* establishes earlier in the season, single treatments of insecticide are not effective as populations rebound to the level of untreated crops. Insecticidal treatments are required multiple times per season to decrease aphid densities. For these early infestations, an application applied at the R3 stage is the most effective at protecting yield (Myers et al. 2005).

Seed-treatments are a method currently used to manage *A. glycines*, although University entomologists do generally not recommend it. The neonicotinoid imidacloprid and thiamethoxam as seed treatments can in part reduce yield loss,
with thiamethoxam providing longer control (Magalhaes et al. 2009). When *A. glycines* are on thiamethoxam seed treated plants, they spend less time in the sieve element phase of feeding and may not be able to ingest phloem sap of the plant (Stamm et al. 2013). However, the residual activity breaks down after 35-42 days or approximately the V2 to V4 soybean stage, making their effectiveness in Nebraska soybean fields questionable because colonization typically occurs well after these stages (Magalhaes et al. 2009, Hodgson et al. 2012).

Chlorpyrifos

The chemical name of the organophosphate chlorpyrifos is O, *O*-diethyl O-3,5,6-trichloro-2-pyridinyl phosphorothioate, CPY; CAS No. 2921-88-2 (Solomon et al. 2014). Chlorpyrifos has short to moderate environmental persistence and dissipates through several mechanisms including volatilization, photolysis, abiotic hydrolysis, and microbial degradation (Solomon et al. 2014). In the first 12 hours after application, volatilization is the most common mechanism of dissipation (Mackay et al. 2014).

The mechanism of action for chlorpyrifos is to disrupt nerve impulse transmission by binding to an active site of the acetylcholinesterase molecule. For recovery, the affected organism must synthesize new acetylcholinesterase. Chlorpyrifos is considered a broad-spectrum insecticide. It is a curative product used for the control of a variety of adult and larval insect and other arthropod pests. Most commonly it is used as a foliar application, but is also effective in controlling many soil insects and as a contact control option (Solomon et al. 2014).
In the United States, chlorpyrifos was first registered in 1965. It was marketed using the names Dursban and Lorsban (The Dow Chemical Company 2014). Currently registered chlorpyrifos products by Dow AgroSciences include a granular formulation Lorsban 15G®, emulsifiable concentrates Lorsban 4E® and Lorsban Advanced®, and a water-soluble formulation Lorsban 50W®. Lorsban 4E® was replaced by the water-soluble Lorsban Advanced®. Lorsban Advanced® contains less volatile solvents and is intended to reduce air pollution and odor (AgroSciences 2012, Solomon et al. 2014).

Chlorpyrifos is no longer registered for the public in home use, except for bait containers. However it is still used in golf courses, agriculture, and for public health pests (National Pesticide Information Center 2012).

Spray drift is the unintentional movement of pesticide dust or droplets to areas other than the intended area soon after application and is concern when applying insecticides, including chlorpyrifos (EPA 2014). Drift occurs when applied pesticides drift away from the target, either by vapor drift or particle drift. When considering particle drift, applied insecticides drift away from the target, typically via wind or air currents. A volatilized insecticide involves pesticides that have evaporated into the air (Kruger et al. 2013). For chlorpyrifos, the vapor pressure is $1.73 \times 10^{-5}$ torr (Giesy and Solomon 2014).

There is a relationship between rate of volatilization, temperature, and relative humidity for chlorpyrifos. As temperature and relative humidity increase, so does volatilization (Arthur et al. 1992). Lower temperatures reduce insecticide volatilization (Uddin and Nuzhat 2006). Chlorpyrifos vaporization and penetration
are higher at 24°C than at 12°C under greenhouse conditions, indicating increased penetration and shorter residual activity in field conditions (Hill et al. 1996, Uddin and Nuzhat 2006).

Chlorpyrifos volatilization is independent of insecticide application coverage. The volatilization can be detected using silicone treated petri dish surfaces treated with a methylene chloride (CH₂CL₂) solution of 0.5% dimethyl polysiloxane (Holoman and Seymour 1983).

In another species of aphid, the Russian wheat aphid Diuraphis noxia (Mordvilko), there is a definite toxic effect due to chlorpyrifos vapor while dried chlorpyrifos residues was generally low in toxicity (Hill et al. 1995). This indicates aphids, such as A. glycines, may be affected by the chlorpyrifos vapor effect as well.

Reduced Area and Agent Treatments (RAATs)

Reduced area and agent treatments (RAATs) are documented as an effective method of managing grasshoppers in rangeland while lowering economic and environmental costs. RAATs involve intentionally leaving an area of field or rangeland untreated adjacent to a treated area. With grasshoppers, RAATs depend on the mobility of the pest and in some cases the residual of the insecticide (Lockwood and Schell 1997, Lockwood et al. 2002). RAATs are considered to be economically and environmentally superior to blanket treatments in large-scale operations (Lockwood et al. 2000).

RAATs were originally identified as Interval Swath Spacing and Direct Dosage Reduction by the Grasshopper Integrated Pest Management Program (GHIPMP) directed by the United States Department of Agriculture, Animal and
Plant Health Inspection Service, but is now most commonly termed RAATs (Larsen and Foster 1996, Cunningham and Sampson 1996-1999, Foster et al. 2000).

Experimentation for the development and assessment of RAATs with infestations of rangeland grasshoppers began in the western US. In these early experiments, the insecticides carbaryl, malathion, and fipronil were applied at recommend rates and at various reduced rates with different test sizes of alternating swaths using 16 hectare small scale plots in Wyoming. While grasshopper densities were not significantly different than densities prior to treatment, overall grasshopper densities were reduced. (Lockwood and Schell 1997). In a follow-up study, larger areas of rangeland were utilized and validated that carbaryl, malathion, and fipronil were highly efficacious when used for rangeland grasshopper management using a RAATs approach. RAATs were recommended to be adopted as the primary tool for the management of rangeland grasshoppers (Lockwood et al. 2000).

The RAATs method was utilized at an operational scale in a multi year and multi location study in South Dakota rangeland. This study also demonstrated RAATs can substantially reduce the amount of area requiring treatment and the amount of pesticide applied per area while resulting in higher economic returns (Foster et al. 2000).

RAATs may have high rates of control in untreated areas due to the mobility of the grasshopper pest into the treated areas and the preservation of biological controls in the untreated areas. Depending on the insecticide, insecticide residues also contribute to RAATs effectiveness (Lockwood and Schell 1997, Lockwood et al.
Insecticide residues are an environmental concern, however this maybe offset by applying in swaths rather than in blanket applications. The benefits and risks of RAATs are also more complicated to access and cannot be addressed using only toxicity. (Lockwood et al. 2000, Lockwood et al. 2002).

The RAATs method preserves non-target and biological control organisms (Lockwood and Schell 1997, Lockwood et al. 2000). A study even looked into the effect of RAATs and bird densities. While grasshopper densities are not a reliable predictor of bird densities, there is support for treating less area results in reduced avian impacts (Norelius and Lockwood 1999).

There are also concerns about adoption of RAATs. These involve the acceptance of using less insecticide and allowing less mortality for overall higher profits, teaching a long-term outlook approach, and realizing the RAATs method is a bit more complex than traditional blanket applications (Lockwood et al. 2000).

Another challenge for RAATs is the economic balance of insecticide cost application with adequate reduction of insect pressure. Software programs have been developed to assist in overcoming this complication. The first, in the late 1990s, was called Hopper. Hopper allowed the user to conduct analyses of precise, sophisticated and repeatable proposed treatment actions (Larsen and Foster 1996). In more recent years the advisory system CARMA, or CAse-based Rangeland grasshopper Management Advisor, has been utilized. CARMA was instrumental to the development and refinement of the RAATs method. CARMA is a sophisticated piece of software that is able to take a variety of inputs and turn them into different comparable management strategies (Hastings et al. 2009).
Thesis Objectives

Observation of high *Aphis glycines* mortality in untreated areas adjacent to areas where chlorpyrifos was applied both in insecticide efficacy trials and field applications (Hunt 2015) led to the suggestion that *Aphis glycines* management could take advantage of the chlorpyrifos vapor effect. The idea also included the possibility of using a reduced area and agent treatment (RAATs) strategy. Benefits of the strategy include reduced pesticide use, reducing impacts on non-target organisms such as natural enemies, and maintaining an integrated pest management approach.

To develop this management strategy, the efficacy of the vapor effect on *A. glycines* needed to be determined. Since reducing impacts on non-targets was an important part of the strategy, it was necessary to access the impact of the vapor effects on key biological control agents. One of these agents is the minute pirate bug, *Orius insidiosus*. To better understand the relationship between *O. insidiosus* and *A. glycines*, studies to characterize feeding behavior and predation were also conducted.

The objectives of this thesis were to:

• Determine the efficacy of the chlorpyrifos vapor effect on *Aphis glycines* in untreated areas directly adjacent to treated areas

• Assess the impact of the chlorpyrifos vapor effect on key biological control agents

• Obtain information on *Orius insidiosus* feeding behavior, and characterize its predation of the *Aphis glycines*
Chapter 2

Reduced Agent and Area Treatments (RAATs) for *Aphis glycines* Management

Introduction

Soybean (*Glycine max* (L.) Merrill) is a commonly cultivated and important food crop around the world. In 2012, 77 million acres of soybeans were planted in the United States producing 3.014 billion bushels worth $43 billion (USDA-ERS 2012). The soybean aphid, *Aphis glycines* Matsumara (Hemiptera: Aphidae), is one of soybean’s most economically damaging insect pests in the United States. As of 2009, it is found in 30 states and three Canadian provinces and has been reported to cause soybean yield losses of >50% (Wang et al. 1994, Ragsdale et al. 2011). *Aphis glycines* is a small, light yellow or yellowish green aphid with dark cornicles. It has four instars and a holocyclic and heterocious lifecycle characterized by both sexual and asexual reproduction. The colonization of *G. max* and *Rhamnus* species distinguish it from other aphid species (Voegtlin et al. 2004a, Wu et al. 2004).

After overwintering as eggs on *Rhamnus*, *A. glycines* undergoes two wingless generations, and then a winged third generation which migrates to soybean. Timing of soybean colonization varies, with soybean in some areas of the United States and Canada being colonized by *A. glycines* as early as plant emergence. In other areas, such as Nebraska, *A. glycines* typically colonizes soybean during early to mid-reproductive stage soybean and subsequently is an economic pest of late reproductive stage soybean (Brosius et al. 2007, Hodgson et al. 2012, Prochaska et al. 2013).
Predators play an important role in *Aphis glycines* suppression (Fox et al. 2004, Rutledge et al. 2004, Desneux et al. 2006, Brosius et al. 2007, Rhainds et al. 2007, Schmidt et al. 2007, Costamagna et al. 2008). The insidious flower bug, *Orius insidiosus* (Hemiptera: Anthocoridae), is a generalist predator known to prey on *A. glycines* and is one of its most significant natural enemies in soybean fields (Desneux et al. 2006, Brosius et al. 2007). Other predators include several coccinellid species (*Coccinella septempunctata* and *Harmonia axyridis*), spiders, syrphid larvae (Desneux et al. 2006), predatory midges, lacewings (*Chrysoperla carnea*) (Zhu and Park 2005) (Donaldson et al. 2007) and *Scymnus* (Pullus) *lousianae* J. Chapin (Brown et al. 2003).

The timing of insecticide application is more important than the treatment used (Johnson et al. 2009) and can differ by region. Different areas of the United States have different recommendations for the number of effective applications. In Iowa, a single foliar application of insecticide was just as effective as multiple applications due to the ET not being exceeded twice in a single season (O’Neal et al. 2009). However in northern states like Wisconsin where *A. glycines* establishes earlier in the season, single treatments of insecticide are sometimes not effective as populations rebound to the level of untreated crops, so insecticidal treatments are required multiple times per season to decrease aphid densities (Myers et al. 2005).

An organophosphate insecticide commonly used to manage *A. glycines* is chlorpyrifos. Chlorpyrifos has short to moderate environmental persistence and in the first 12 hours after application, volatilization is the most common path of dissipation (Mackay et al. 2014, Solomon et al. 2014). Lorsban Advanced® is a
chlorpyrifos formulation that contains less volatile solvents than its predecessor Lorsban 4E® and therefore reduces volatilization and consequent air pollution and odor (AgroSciences 2012, Solomon et al. 2014). Chlorpyrifos volatilization has been observed to result in *A. glycinus* mortality in areas adjacent to sprayed areas (Hunt 2015). These observations are based on significant aphid mortality in untreated plots (controls) adjacent to chlorpyrifos treated plots in insecticide efficacy trials, and likewise in untreated pivot corners next to chlorpyrifos chemigated pivot circles (Hunt 2015).

Reduced area and agent treatments (RAATs) are documented as an effective method of managing grasshoppers in rangeland while lowering economic and environmental costs. RAATs involve intentionally leaving an area of field or rangeland untreated adjacent to a treated area. With grasshoppers, RAATs depend on the mobility of the pest and the residual of the insecticide (Lockwood and Schell 1997, Lockwood et al. 2002). RAATs are considered to be economically and environmentally superior to blanket treatments in large-scale operations (Lockwood et al. 2000).

Experimentation for the development and assessment of RAATs with infestations of rangeland grasshoppers began in the western US. In these early experiments, the insecticides carbaryl, malathion, and fipronil were applied at recommended rates and at various reduced rates with different test sizes of alternating swaths (Lockwood and Schell 1997). While grasshopper densities were not significantly different than densities prior to treatment, overall grasshopper densities were reduced. (Lockwood and Schell 1997). In a follow-up study, larger
areas of rangeland were utilized and validated that carbaryl, malathion, and fipronil were highly efficacious when used for rangeland grasshopper management using a RAATs approach. RAATs have since been recommended as the primary tool for the management of rangeland grasshoppers (Lockwood et al. 2000).

The RAATs method was utilized at an operational scale in a multi-year and multi-location study in South Dakota rangeland. This study also demonstrated RAATs can substantially reduce the amount of area requiring treatment and the amount of pesticide applied per area while resulting in higher economic returns (Foster et al. 2000).

RAATs may have high rates of control in untreated areas due to the mobility of the grasshopper pest and the preservation of biological controls. Depending on the insecticide, insecticide residues can also contribute to RAATs effectiveness (Lockwood and Schell 1997, Lockwood et al. 2000, Lockwood et al. 2002). Insecticide residues are often considered an environmental concern; however, this concern maybe offset by applying in swaths rather than in blanket insecticide applications thereby reducing the total insecticide load. The benefits and risks of RAATs are complicated to assess and cannot be addressed using only toxicity (Lockwood et al. 2000, Lockwood et al. 2002). The RAATs method also preserves non-target and biological control organisms (Lockwood and Schell 1997, Lockwood et al. 2000).

There are also concerns about adoption of RAATs. These involve the acceptance of using less insecticide and allowing less mortality for overall higher profits, providing growers with a long-term outlook, and realizing the RAATs
method is a bit more complex than traditional blanket applications (Lockwood et al. 2000). Another challenge for RAATs is the economic balance of insecticide cost application for adequate reduction of insect pressure with fluctuating crop values.

Observation of lower aphid numbers in untreated areas adjacent to areas treated with chlorpyrifos both in efficacy trials and field applications led to the development of a strategy to manage *A. glycines* utilizing the chlorpyrifos vapor effect. Such an approach might constitute a reduced area and agent treatment (RAATs) strategy. Benefits of the strategy include reduced pesticide use and reduced impacts on non-target organisms. To develop this strategy, the efficacy of the vapor effect on *A. glycines* needs to be determined, and because reducing impacts on non-targets is an important part of the strategy, it was also necessary to assess the impact of the vapor effects on key biological control agents. Therefore the objectives of this study were to determine the efficacy of the chlorpyrifos vapor effect on *A. glycines* in untreated areas directly adjacent to treated areas and to assess the impact of the chlorpyrifos vapor effect on key biological control agents. The intended outcome of these objectives was to develop an *A. glyicnes* management strategy using a RAATs approach.
Materials and Methods

A preliminary study was conducted in 2012 at University of Nebraska Northeast Research and Extension Center Haskell Agricultural Laboratory (HAL) near Concord, Nebraska to determine the viability of the *A. gylcines* RAATs strategy. Based on 2012 data (see Appendix A), the experiment was refined and conducted in summer 2013.

2013 Study

In 2013, experimental plots were located at the University of Nebraska Northeast Research and Extension Center Haskell Agricultural Laboratory (HAL) near Concord, Nebraska (Latitude 42.385 and Longitude -96.9534) and at the South Central Agricultural Lab (SCAL) near Harvard, Nebraska. At Concord, the soybean variety Pioneer Hi-Bred 92Y53 was planted as no-till (soil type: Moody silty clay loam) on 5 June 2013 at 180,000 seeds per acre in 30-inch (76.2 cm) rows. Late planting was due to cool and wet weather conditions in May. Valor, 2,4-D ester and Durango herbicides were applied on 29 April 2013 and Durango and Selectmax were applied on 28 June 2013, as is standard practice in the area for weed suppression. At Harvard, the soybean variety Fontanelle 64R20 was similarly planted on 23 May 2013. Both fields used standard agricultural practices for the area and were both irrigated acres via overhead linear irrigation.

The study consisted of five replications at Concord and four replications at Harvard in a random complete block design. Each replication consisted of a spray zone of 16 rows/16 feet (4.8768 m) across, the standard width of the sprayer equipment, and was 60 feet (18.288 m) in length (Figure 2.1). There was a 20-foot
(6.096 m) buffer zone between each replication. *Aphis glycines* counts and insect sweep net samples were taken 4 rows from the spray zone, 8 rows from the spray zone, 12 rows from the spray zone, and 16 rows from the spray zone. The sample area was 30 feet (9.144 m) in length and was 15 feet (4.572 m) from the ends of the spray zone. Before sampling began, the sampling zone was marked and flagged at both ends with Tall Pennant Field Flags (Black Burn Flag Company) on ¼ inch (0.635 cm) diameter fiberglass poles 6 foot (1.829 m) in height. *Aphis glycines* counts and insect net sweeps were taken before and after insecticide spray application.

Beginning in July, arthropod populations were monitored weekly with approximately four 20-sweep samples taken at random locations of the field at both Concord and Harvard. Samples were used to estimate field population levels, chlorypifos application timings, and when to begin formal arthropod sampling. In 2013, *A. glycines* populations were slow to develop so at Concord the plot areas were artificially infested with *A. glycines* on 2 August 2013. *Aphis glycines* infested soybean plants were obtained from an outdoor *A. glycines* nursery cage located in a nearby field. The nursery was started in mid-July due to delayed natural infestation and slow natural development of *A. glycines* numbers. Soybean plants infested with *A. glycines* were cut into pieces containing approximately 10-20 *A. glycines* each and applied to the field with one infested leaf piece per 5 feet (1.524 m) in the areas to be treated and sampled.

*Aphis glycines* counts were taken weekly beginning on 20 August 2013 within the spray zone, and at 4, 8, 12, and 16 rows from outside the spray zone and
continuing for five weeks. Four soybean plants from the spray zone and each row were randomly selected and destructively sampled.

Sweep net samples were taken weekly beginning on 20 August 2013 for Concord within the spray zone, and at row 4, 8, 12, and 16 from outside the spray zone and continuing for five weeks. Twenty sweep samples were taken from each sweep sample zone. Arthropods reported include the Order Thysanoptera, families Coccinellidae, Chrysopidae, and Nabidae as well as Orius insidiosus,

Lorsban Advanced® (Dow AgroSciences) was applied with a 40-foot (12.192 m) sprayer at 32 ounces per acre (2.3385 liters per hectare) on 21 August 2013 at 11:00 AM after aphid numbers exceeded 100 aphids per plant. On the day of application, daytime high temperatures reached 88.7°F (31.5°C) and 78.1°F (25.6°C) on the day after application. While an ideal application would occur after A. glycinæ reaches the economic threshold of 250 aphids, the application was applied to mimic aphid treatment and for treatment to occur before the natural decline of A. glycinæ populations.

Harvest took place on 29 October 2013. Due to equipment constraints, two rows were harvested together instead of a single row. The entire 60 feet (18.288 m) length of each replication was harvested. Two central rows of the spray zone were randomly selected for harvest. Sample rows were combined with the inside row resulting in rows 3 and 4, 7 and 8, 11 and 12, and 15 and 16 being harvested.

At Harvard, A. glcyines populations were not present as natural populations rarely develop in south central Nebraskan soybeans. Therefore chlorpyrifos applications at this site were applied to mimic A. glcyines treatments in order to
measure the impacts on non-target arthropods in a second location. Sweep net
sweeps were taken weekly beginning on 24 July 2013 for Harvard within the spray
zone, and in row 4, 8, 12, and 16 from the spray zone and continued for five weeks.
Twenty sweep samples were taken from each sweep sample zone. Arthropods
reported were Orius, Thysanoptera, and Pentatomidae.

Lorsban Advanced® was applied at 19 ounces per acre (1.3889 liters per
hectare) on 24 July 2014 at 11:00 AM following standard practice for the area in
combating other potential pests. This application was compromised and insect
populations were significantly reduced by severe weather (29 July 2013 storm and a
1 August 2013 hail event). Lorsban Advanced® was reapplied at 32 ounces per acre
(2.3385 liters per hectare) at 10 GPA (93.5431 liters per hectare) water on 20
August 2013 at 11:00 AM.

Due to the compromises of weather, harvest data were not collected at
Harvard in 2013.

2014 Study

In 2014, experimental plots were located at the University of Nebraska
Northeast Research and Extension Center Haskell Agricultural Laboratory (HAL)
near Concord, Nebraska and at the South Central Agricultural Lab (SCAL) near
Harvard, Nebraska.

The experimental area at Concord was composed of Baltic silty clay soil on
dry land acres (Figure 2.2). The field was planted with Pioneer 92Y70 at a 140,000
seeds per acre on 30-inch (76.2 cm) rows on 19 May 2014. The herbicide Enlite was
applied at 2.8 ounces per acre (0.2046 liters per hectare) on 5 May 2014, Durango
was applied at 29 ounces per acre (1.9462 liters per hectare) and Select Max at 12 ounces per acre (0.877 liters per hectare) on 24 June 2014. Natural populations of *A. glycines* were slow to develop in 2014 and the experimental area was artificially infested in August using the same methods as in 2013.

The experimental area at Harvard was planted on 20 May 2014 with the variety Fontanelle 64R20 at 150,000 seeds per acre in 30 inch rows (76.2 cm). Plot area was 450 feet (137.16 m) by 72 row/feet (21.95 m) on irrigated acres (Figure 2.3). The herbicides RoundUp PowerMax at 32 ounces per acre (2.3385 liters per hectare) and Lockout AMS at 9 pounds (4.08233 mg) per 100-gallon (378.541 liters) water solutions were applied on 6 June 2014. Volunteer corn was rogued on 25 June 2015.

At Concord, the experimental design was a completely randomized design with four replications and altered based on 2013 results to include 16 row vapor zones (untreated) and 8 row vapor zones (untreated) between the sprayed zones at Concord. Five untreated areas not adjacent to the spray zones or vapor zones represented an untreated field. Due to space constraints at Harvard, only 8 row vapor zones were used.

The 8 row vapor zone replication design consisted of 8 rows untreated between two 16 row treated zones (Figure 2.4). The center rows of the 8 rows untreated were designated as the sampling area. The sampling area was 120 foot (36.576 m) in length with a 15-foot (4.572 m) buffer on each end and the remaining central area was divided into three equal 30-foot (9.144 m) areas. Twenty insect sweep samples and four destructive aphid samples were taken from a previously
unsampled 30-foot (9.144 m) sampling areas on each sample date. Sampling areas to be sampled were selected randomly prior to treatment. Destructive aphid samples occurred in one section first to be followed one week later by sweep samples. This was to prevent trampling of soybean plants and limit disruption of the sampling zones.

The 16 row vapor zone replication design consisted of 16 rows untreated between two 16 row treated sprayed zones (Figure 2.5). The center rows of the 16 rows untreated and 4 rows away from the spray zones were designated as the sampling areas. The sampling area was 120 foot (36.576 m) in length with a 15-foot (4.572 m) buffer on each end and the remaining central area was divided into three equal 30-foot (9.144 m) areas. Twenty insect sweep samples and four destructive aphid samples were taken from previously unsampled 30-foot (9.144 m) sampling areas at each sample date. Sampling areas to be sampled were selected randomly prior to treatment. Destructive aphid samples occurred first to be followed one week later by sweep samples. This was to prevent trampling of soybean plants and limit disruption of the sampling zone.

At Concord, aphid counts and sweeps taken on 21 August 2014 indicated insecticide treatment should be applied, but weather delayed treatment. A field average of 253.1 aphids per plant was observed on 28 August 2014, so treatment was done on 4 September 2014 with Lorsban Advanced® (Dow AgroSciences) with a 40-foot sprayer at 32 ounces per acre (2.3385 liters per hectare). Aphids were sampled on 10 September 2014. On 8 October 2014, two rows of soybeans were
harvested from the central 90-foot (27.432 m) of each sampling area in vapor zone, central two rows of treated spray zone, and central two rows of the untreated areas.

At Harvard, sweep samples began on 22 July 2014 and continued weekly for four weeks. After the second sample week, spray zone areas were treated with Lorsban Advanced® (Dow AgroScience) on 15 August 2014 at 2:30 pm at 32 ounces per acre (2.3385 liters per hectare). Harvest was conducted on 26 September 2014. Due to limited arthropod information, the possibility of deer grazing, and weather complications harvest data are not presented.

Statistical Analyses

For 2013, Fisher’s least significant differences (LSD) was used to analyze differences between the treated spray zone and the adjacent untreated vapor zones at 4, 8, 12, and 16 rows using a generalized mixed model (PROC MIXED, SAS 9.3). Significance was determined at $\alpha = 0.05$. The treatment was treated as a random variable.

For 2014, analysis included the treated spray zones, adjacent untreated vapor zones, and untreated control zones for Concord. For Harvard data analysis included treated spray zones and adjacent untreated vapor zones. Fisher’s least significant difference (LSD) test was used to analyze differences among the treated spray zone, the adjacent untreated vapor zones, and the untreated control zone using a generalized mixed model (PROC MIXED, SAS 9.3). Significance was determined at $\alpha = 0.05$. The treatment was treated as a random variable.
Results

2013 Study

In 2013 at Concord, temperatures were below ideal for chlorpyrifos vaporization when insecticide treatments were applied with the high temperature of 88.7°F (31.5°C) on the treatment day and 78.1°F (25.61°C) the day after insecticide treatment. Vaporization is higher at increased temperatures (Hill et al. 1996, Uddin and Nuzhat 2006). Measured temperatures during August 2013 were lower than average.

*Aphis glycines* levels at Row 4 away from spray zone were higher but not significantly different than the treated area for each sampling date. A significant interaction was detected between experimental treatment by date ($F = 2.51; \text{df} = 16, 65.3; P = 0.0047$) and the main effects of experimental treatments ($F = 8.60; \text{df} = 4, 15.1; P = 0.008$), and date ($F = 18.25; \text{df} = 4, 50.5; P < 0.0001$) were also significant. At each sampling date after insecticide treatment, row 4 remained significantly lower than at other distances but did not reach levels expected to cause economic damage (Figure 2.6).

For thrips levels, there was no significant interaction among experimental treatments by date ($F = 1.65; \text{df} = 16, 46.2; P = 0.0924$) and no significant main effect of experimental treatment ($F = 1.32; \text{df} = 4, 28.4; P = 0.2877$). There was a significant difference among dates ($F = 56.49; \text{df} = 4, 33.3; P < 0.0001$) as the level of thrips declined over time (Figure 2.7).

For *Orius insidiosus* levels, there was no significant interaction between experimental treatment by date ($F = 1.07; \text{df} = 16, 52.6; P = 0.4024$). There were
significant differences among experimental treatments \((F = 8.18; \text{df} = 4, 21.7; P = 0.004)\) and among dates \((F = 33.73; \text{df} = 4, 40.2; P < 0.0001)\). *Orius* levels were generally higher away from the spray zone, although some individuals were recorded in the spray zone one week after insecticide treatment (Figure 2.8).

For lady beetle levels, there was a significant interaction between experimental treatment by date \((F = 49.4; \text{df} = 16, 49.4; P = 0.0028)\) and the main effect of experimental treatment \((F = 8.27; \text{df} = 4, 31.5; P = 0.001)\) and date \((F = 17.01; \text{df} = 4, 35.8; P < 0.0001)\) were also significant \((\alpha = 0.05)\). Lady beetles were at low levels prior to and one week after insecticide treatment, but increased as aphid populations increased and followed a fluctuation pattern similar to aphids (Figure 2.9).

For lacewing levels, there was no significant interaction between experimental treatments by date \((F = 1.49; \text{df} = 16, 55.1 P = 0.1384)\). However, there were significant differences among experimental treatments \((F = 7.24; \text{df} = 4, 30.8; P = 0.003)\) and dates \((F = 19.19; \text{df} = 4, 38.9; P < 0.0001)\). Numbers were generally higher away from the spray zone. Lacewings increased in all zones throughout time and some were recorded in the spray zone one week after insecticide treatment (Figure 2.10).

For nabid levels, there was no significant interaction between experimental treatment by date \((F = 1.05; \text{df} = 16, 53.3; P = 0.4260)\). There were significant differences among experimental treatments \((F = 0.0138; \text{df} = 4, 20.6; P = 0.0138)\) and dates \((F = 13.26; \text{df} = 4, 41.3; P < 0.0001)\). Overall, nabids had relatively low levels and were generally higher away from the spray zone. Nabids increased in all
zones throughout time and some were recorded in the spray zone one week after insecticide treatment (Figure 2.11).

At Concord, there was a significant difference in yield between zones ($F = 6.06$ df = 4, 16; $P = 0.0036$) at $\alpha = 0.05$. Yield was significantly greater for the sprayed zone than rows 4, 12, and 16, but was not significantly different than row 8. Yield in rows 4, 12, and 16 were not significantly different from one another (Figure 2.12).

In Harvard, storm-damaged soybean compromised analysis, but potential pests (stink bugs, thrips) and natural enemies (Orius) were generally present in all zones at varying levels. For thrips levels at $\alpha = 0.05$, there was not a significant interactions between experimental treatment by date ($F = 0.63$; df = 16, 37.4; $P = 0.8420$) or significant differences among experimental treatments ($F = 1.65$; df = 4, 17; $P = 0.2088$). There was a significant difference among dates ($F = 36.83$; df = 4, 19.3; $P < 0.0001$) (Figure 2.13).

While numbers were low for stinkbug nymphs, there was a significant interaction between experimental treatment by date ($F = 3.93$; df = 16, 45.7; $P = 0.0001$), and significant differences among experimental treatments ($F = 4.13$; df = 4, 18.7; $P = 0.0145$), and dates ($F = 60.43$; df = 4, 40.9; $P < 0.0001$) (Figure 2.14). However for adult stinkbugs, at $\alpha = 0.05$, there was no significant interactions between experimental treatment by date ($F = 1.891$ df = 16, 17.9; $P = 0.0976$) and no significant differences were detected among experimental treatments ($F = 1.31$; df = 4, 15; $P = 0.3095$). There was, however, a significant difference among dates ($F = 17.9$; df = 16, 17.9; $P = 0.0449$) (Figure 2.15).
While numbers were also low for *Orius*, there were significant interactions among experimental treatments by date \( (F = 3.01; \text{df} = 16, 60; P = 0.0010) \), and significant differences among dates \( (F = 14.95; \text{df} = 4, 60; P < 0.0001) \). There was no significant difference among experimental treatments \( (F = 1.63; \text{df} = 4, 15; P = 0.2215) \) at \( \alpha = 0.05 \) (Figure 1.16). Numbers remained higher in the vapor zones versus the sprayed zones.

2014 Study

In 2014 at Concord, storm damage eliminated soybean aphid field populations and damaged soybean plants (e.g. extreme lodging and twisting) so the study was terminated (Figure 2.17) There were no significant differences in yield among untreated, sprayed, 8 Row vapor zones, or 16 row vapor zones \( (F = 1.48; \text{df} = 5, 18; P = 0.2452)\)(Figure 2.18).

At Harvard, potential pest species did not reach threshold values and no significant differences for thrips \( (F = 0.99; \text{df} = 3,18; P = 0.4188)\), stink bug nymphs \( (F = 3.31; \text{df} = 3, 7.64; P = 0.0809)\), stink bugs \( (F = 1.74; \text{df} = 3, 18; P = 0.1925)\), and *Orius* \( (F = 2.51; \text{df} = 3, 8.17;P = 0.131) \) were detected between vapor zones and spray zones (Figure 2.19-Figure 2.22).
Discussion

The results from the Concord site in 2013 indicate that *A. glycines* are held to levels below economic thresholds at least four 30-inch (76.2 cm) rows away from spray zone, even when temperatures were lower than optimal at the time of the spray treatment. *Aphis glycines* populations increased in late summer then peaked in mid-September before declining in late September. This is likely due to natural fluctuations that occur as the soybeans mature and begin senescence. In Row 4, *A. glycines only exceeded* the economic threshold of 250 *A. glycines* (Ragsdale et al. 2007) during the peak time on 5 September 2014, but did not exceed the economic injury level of 674 *A. glycines* (Ragsdale et al. 2007). Another potential pest of soybean, thrips, did not reach damage causing levels, was not significant between experimental treatment by date, and declined throughout the course of the experiment. This is likely due to a decline in natural populations.

Natural enemies (*Orius* and lady beetles) were preserved in these non-spray zones and were also present in the spray zone after treatment. By preserving natural enemies in non-spray zones, these arthropods are better able to combat resurgence of insect pests, such as *A. glycines*, both in the non-spray zone and in the spray zone. This result is supported by the importance of predators and biological control agents as a part of *A. glycines* suppression and a key factor for IPM as shown by multiple previous studies (Fox et al. 2004, Rutledge et al. 2004, Desneux et al. 2006, Brosius et al. 2007, Rhainds et al. 2007, Schmidt et al. 2007, Costamagna et al. 2008). Lady beetle levels also increased as *A. glycines* levels increased and seemingly peaked when *A. glycines* levels peaked. This result is supported by Koch
(2003) which states *H. axyridis* peak arrival and oviposition coincides at or before the peak of aphid populations and its intensity is inversely related to aphid populations.

Other potential pests, such as thrips, were not significantly reduced in these non-spray zones or in spray zone. This result is encouraging for the direct targeting of the pest, *A. glycines*, while reducing effects to non-targets. The retained presence of non-target pests, such as thrips, maintains a diverse and present food source for natural enemies. Diversifying the diet of predators such as *O. insidiosus*, with alternative prey such as *N. variabilis* could enhance their effectiveness as predators of *A. glycines* and *O. insidiosus* is shown to have greater fitness and may be healthier when feeding on multiple prey such as *A. glycines* and the soybean thrips, *Neohydatothrips variabilis* (Butler and O’Neil 2007a, Harwood et al. 2007, Desneux and O’Neil 2008).

At Concord in 2013, yield was significantly lower for Row 4, 12, and 16 when compared to the spray row. Row 8 was not significantly different from the spray row. Yield differences in Row 4 were attributed to unintended trampling of the sampling area and the methods were adjusted for the 2014 study. At Concord in 2014, weather events after treatment compromised data collection for *A. glycines*, non-targets, or natural enemies.

In Harvard 2013, storm damaged soybean compromised analysis, but potential pests (stink bugs, thrips) and natural enemies (*Orius*) were generally present in all zones at varying levels. In 2014, potential pests remained below the threshold levels and natural enemy values levels remained low. In 2013 and 2014
thrips were reported as not significantly different. This is likely due to naturally declining populations through the season. In 2013, significant differences were reported in nymphs and but not for adult stinkbugs. This could have been attributed to due to the differences in exoskeleton structure between nymphs and adults. However in 2014, there were no significant differences reported for either nymphs or adults. In 2013, yields were not reported. For 2014 yields were significantly different when comparing the spray zone to the adjacent vapor. However, given the various variables of low insect counts and the experimental plots locations near the field edge contributing to a deer grazing gradient, this significance could not be attributed to experimental treatments alone.

While this management strategy for *A. glycines* is termed RAATs and is similar to the RAATs strategy used for grasshoppers, there are important differences. Similarities include strip applications of pesticides, reduction of impacts to non-targets and natural enemies, minimizing costs, and lowering pest impacts (Lockwood et al. 2000, Lockwood et al. 2002). However this management method differs in the utilization of the maximum chlorpyrifos application rate (Lorsban Advanced® 32 ounces per acre, 2.3385 liters per hectare) and the emphasis on the chlorpyrifos vapor effect. The current RAATs approach for grasshoppers seeks to use lower application rates and utilizes the mobility of the insect pest for control (Lockwood and Schell 1997, Lockwood et al. 2000).

For a management strategy, results demonstrate leaving untreated strips of at least 8 rows between a 16 row spray swaths may be an effective strategy for managing *A. glycines* in areas where aphids are relatively late to colonize soybean
(mid-late July) and temperatures are relatively high during treatment (e.g. Nebraska with 90°F (32.2°C) high temperatures). Natural enemies are preserved to help manage remaining insect pests or subsequent *A. glycines* re-infestations. The implementation of this management tool for *A. glycines* will likely reduce management costs by using less insecticide (chlorpyrifos) and running down fewer soybeans with ground application equipment during insecticide application. It would be expected to use this strategy in conjunction with other management tools as part of an overall IPM strategy.
Figures

Materials and Methods

Figure 2.1 Concord, NE 2013. One replication of field study (not to scale). The arrowed lines along the rows represent the 30-foot sweep sampling and destructive aphid count sampling zones.
Figure 2.2 Concord, NE 2014 Field plot.

Figure 2.3 Field plot illustration for Harvard in 2014
Figure 2.4 Illustration of the 8-row vapor zone replication.

Figure 2.5 Illustration of the 16-row vapor zone replication.
Figure 2.6 Concord, NE 2013. Mean *Aphis glycines*. Treated with Lorsban Advanced® (Dow AgroScience) on 21 August 2013 at 11:00 AM at 32 ounces per acre (2.3385 liters per hectare). Mean ± standard error bars of *Aphis glycines* per soybean plant. Bars marked ‘A’ are not significantly different from the spray row. Bars marked ‘B’ are significantly different from the spray row at $\alpha = 0.05$. 

[Diagram showing average *Aphis glycines* per soybean plant by date and row.]

2013 Haskell Agricultural Laboratory – Concord, NE
Figure 2.7 Concord, NE 2013. Mean Thrips. Treated with Lorsban Advanced® (Dow AgroScience) on 21 August 2013 at 11:00 AM at 32 ounces per acre (2.3385 liters per hectare). Mean ± standard error bars of Thrips per twenty soybean field sweeps.
Figure 2.8 Concord, NE 2013. *Orius* species. Treated with Lorsban Advanced® (Dow AgroScience) on 21 August 2013 at 11:00 AM at 32 ounces per acre (2.3385 liters per hectare). Mean ± standard error bars of *Orius* spp. per twenty soybean field sweeps.
Figure 2.9 Concord, NE 2013. Ladybeetles. Treated with Lorsban Advanced® (Dow AgroScience) on 21 August 2013 at 11:00 AM at 32 ounces per acre (2.3385 liters per hectare). Mean ± standard error bars of Ladybeetles per twenty soybean field sweeps. Bars marked ‘A’ are not significantly different from the spray row. Bars marked ‘B’ are significantly different from the spray row.
Figure 2.10 Concord, NE 2013. Lacewings. Treated with Lorsban Advanced® (Dow AgroScience) on 21 August 2013 at 11:00 AM at 32 ounces per acre (2.3385 liters per hectare). Mean ± standard error bars of Lacewings per twenty soybean field sweeps.
Figure 2.11 Concord, NE 2013. Nabid species. Treated with Lorsban Advanced® (Dow AgroScience) on 21 August 2013 at 11:00 AM at 32 ounces per acre (2.3385 liters per hectare). Mean ± standard error bars of Nabids per twenty soybean field sweeps.
Figure 2.12 Yield data from Concord, Nebraska. Harvest on 29 October 2013. Shown as mean ± standard error for each row. At α = 0.05, there was a significant difference among treatments $P = 0.0036$. Bars marked ‘A’ are not significantly different from the spray row. Bars marked ‘B’ are significantly different from the spray row.
Figure 2.13 Harvard, NE 2013. Thrips. Retreated with Lorsban Advanced® (Dow AgroScience) on 20 August 2013 at 11:00 AM at 32 ounces per acre (2.3385 liters per hectare) at 10 GPA (93.5431 liters per hectare). Mean ± standard error bars of Thrips per twenty soybean field sweeps.
Figure 2.14 Harvard, NE 2013. Stinkbug nymphs. Retreated with Lorsban Advanced® (Dow AgroScience) on 20 August 2013 at 11:00 AM at 32 ounces per acre (2.3385 liters per hectare) at 10 GPA (93.5431 liters per hectare). Mean ± standard error bars of Stinkbug nymphs per twenty soybean field sweeps. Bars marked ‘A’ are not significantly different from the spray row. Bars marked ‘B’ are significantly different from the spray row at $\alpha = 0.05$. 
Figure 2.15 Harvard, NE 2013. Stinkbugs. Retreated with Lorsban Advanced® (Dow AgroScience) on 20 August 2013 at 11:00 AM at 32 ounces per acre (2.3385 liters per hectare) at 10 GPA (93.5431 liters per hectare). Mean ± standard error bars of Stinkbugs per twenty soybean field sweeps.
Figure 2.16 Harvard, NE 2013. *Orius* species. Retreated with Lorsban Advanced® (Dow AgroScience) on 20 August 2013 at 11:00 AM at 32 ounces per acre (2.3385 liters per hectare) at 10 GPA (93.5431 liters per hectare). Mean ± standard error bars of *Orius* spp. per twenty soybean field sweeps. Bars marked ‘A’ are not significantly different from the spray row. Bars marked ‘B’ are significantly different from the spray row at \( \alpha = 0.05 \).
Figure 2.17 Concord, NE 2014. *Aphis glycines*. Mean ± standard error bars of *Aphis glycines* per soybean plant. Treated with Lorsban advanced (Dow AgroSciences) on 4 September 2014 at 32 ounces per acre (2.3385 liters per hectare).
Figure 2.18 Yield data from Concord, Nebraska. Yield shown as mean ± standard error for each row.
Figure 2.19 Harvard, NE 2014. Thrips. Mean ± standard error bars of thrips per twenty soybean field sweeps. Treated with Lorsban Advanced® (Dow AgroScience) on 15 August 2014 at 2:30 pm (after sample) at 32 ounces per acre (2.3385 liters per hectare).
Figure 2.20 Harvard, NE 2014. Stink bug nymphs. Mean ± standard error bars of stink bug nymphs per twenty soybean field sweeps. Treated with Lorsban Advanced® (Dow AgroScience) on 15 August 2014 at 2:30 pm (after sample) at 32 ounces per acre (2.3385 liters per hectare).
Figure 2.21 Harvard, NE 2014. Stink bug. Mean ± standard error bars of stink bugs per twenty soybean field sweeps. Treated with Lorsban Advanced® (Dow AgroScience) on 15 August 2014 at 2:30 pm (after sample) at 32 ounces per acre (2.3385 liters per hectare).
Figure 2.22 Harvard, NE 2014. *Orius* species. Mean ± standard error bars of *Orius* per twenty soybean field sweeps. Treated with Lorsban Advanced® (Dow AgroScience) on 15 August 2014 at 2:30 pm (after sample) at 32 ounces per acre (2.3385 liters per hectare).
Chapter 3

Investigation of the life stage feeding preference of *Aphis glycines* and behavior of *Orius indisious*

Introduction

The soybean aphid, *Aphis glycines* Matsumara (Hemiptera: Aphidae), is described as small, light yellow or yellowish-green insect with dark cornicles. *Aphis glycines* exhibits four instars with a holocyclic and heterococious lifecycle characterized by both sexual and asexual reproduction. The aphid’s ability to colonize soybean, *Glycine max* (L) Merrill, and buckthorn species (*Rhamnus*) distinguishes it from other aphid species (Voegtlin et al. 2004a, Wu et al. 2004). As of 2009, *A. glycines* was found in 30 states and three Canadian provinces and has been reported to cause soybean yield losses of >50% (Wang et al. 1994, Ragsdale et al. 2011).

Predators and other biological control agents are an important part of *A. glycines* suppression (Fox et al. 2004, Rutledge et al. 2004, Desneux et al. 2006, Brosius et al. 2007, Rhainds et al. 2007, Schmidt et al. 2007, Costamagna et al. 2008). Many key biological control agents feeding on *A. glycines* in a soybean field are generalist predators. While parasitoids are present in soybean fields, their ability to suppress populations of *A. glycines* is limited (Brown et al. 2003, Donaldson et al. 2007, Costamagna et al. 2008).

Many parasitoids choose to attack certain sizes, ages, or life stages of their host prey (Hopper and King 1984, Lin and Ives 2003). Developmental rate, survival,
and reproduction by offspring are influenced by these preferences. Host preferences can also affect prey behavior and population growth (Wyckhuys et al. 2008).

The insidious flower bug, *Orius insidiosus* Say (Hemiptera: Anthocoridae), is one of the most important natural enemies in soybean fields in the United States. As a generalist predator, it is known to prey on *A. glycines* (Fox et al. 2004, Desneux et al. 2006, Brosius et al. 2007). Over its lifetime, an adult *O. insidiosus* can consume an average of 126 *A. glycines* (Butler and O’Neil 2008). Rutledge et al. (2004) found a maximum number of aphids consumed in a 24-hour period by *O. insidiosus* to be 10 ± 3.5 SE. However, the functional response of *O. insidiosus* to *A. glycines* when provided 64 prey items is 19.25 ± 3.38 SE in a 24-hour period (Rutledge and O’Neil 2005).

Previous studies have looked at the life history of *O. insidiosus* using *A. glycines*, however prey was limited to second, third, or fourth instar *A. glycines* (Rutledge and O’Neil 2005, Butler and O’Neil 2007a, b, Butler and O’Neil 2008).

Since other *A. glycines* biological control agents, such as parasitoids, show life stage preference of prey and life stage preference effects predator biology (Wyckhuys et al. 2008), this study looked at the life stage preference for a key natural enemy in the United States, *O. insidiosus*. A set of choice studies investigated the preference of early (first and second instar), late (third and fourth instar), and adult *A. glycines* life stage; while *A. glycines* life stage consumption by *O. insidiosus* was documented through a series of no choice studies. Video recordings provided insight into the behavior and the interactions between *O. insidiosus* and the different *A. glycines* life stages.
Materials and Methods

*Orius insidiosus*

*Orius insidiosus* nymphs were obtained from a colony provided by Syngenta Bioline (Oxnard, California). Upon arrival, nymphs were provided ad libitum all life stages of *A. glycines*. Once the nymphs reached the adult stage, they were separated daily from the nymphs and maintained on soybean aphids for four days before initiating the no choice and choice studies. Adult *O. insidiosus* used for the behavior video recording experiments were from a colony and were provided by Syngenta Bioline (Oxnard, California).

*Aphis glycines*

*Aphis glycines* were obtained from a colony initiated from a Nebraska isolate (Biotype 1) initially collected from commercial soybean near the University of Nebraska Northeast Research and Extension Center Haskell Agricultural Laboratory, Concord, NE (42° 23′ 3″ N, 96° 59′ 21″ W). The *A. glycines* colony was maintained on soybean variety KS4202 plants in a growth chamber at 21 ± 2°C and a photoperiod of 16:8 (L: D) h. The soybean variety KS4202 exhibits tolerance and as a result maintains a higher population of *A. glycines* without deleterious effects to the soybean plant for longer periods of time (Prochaska et al. 2013). Marchi-Werle (2014) utilized these rearing methods successfully.

Soybean

Soybean plants were grown in 15 cm diameter standard pots using artificial Fafard 3B mix (Hummerts International) under greenhouse conditions of 16:8 (L: D)
photoperiod, RH 60-80%, and supplemented with 400-W high-intensity lamps or LED grow lights at a temperature of 23±3°C. Soybeans were typically staged V3 to V5 when leaflets were removed for experiments, however leaves were of same approximate size and age.

Experimental Conditions

The experimental unit was a single 8-ounce (0.24 L) safe lock container (US Plastics) with a moistened cotton pad and a single soybean leaf. *Aphis glycines* were added to the experimental unit and allowed to acclimate for one hour. After one hour, a four-day-old adult *O. insidiosus* was added. Dead, killed, or missing *O. insidiosus* were replaced as is consistent with similar studies (Butler and O’Neil 2006, 2007a, b). Related species data for *Aphis gossypii* suggest approximately a day and a half per stadium (Baldin et al. 2009). To account for this rapid development, life stages were divided into groups: early life stage, late life stage, and adult aphids. Early life stages included first and second instar *A. glycines*, late life stages included third and fourth instars, and adults were female aphids of reproductive status.

Choice and No Choice Feeding Studies

Choice studies were conducted to determine a life stage preference of *A. glycines by O. insidiosus*. For the choice study, a single aphid of each life stage was placed in the experimental unit with a total of 45 experimental units. Dead and living *A. glycines* life stages were recorded. Refer to Figure 3.1 for a photograph of a choice study experimental unit.
No choice studies were conducted to document predation rates for the early, late, and adult *A. glycines* life stage. For the no choice study, ten aphids of one life stage (early, late, and adult) were placed in the experimental unit and mortality was recorded. There were a total of 60 experimental units. In this complete randomized design, controls without *O. insidiosus* served to document *A. glycines* mortality under test conditions.

Observations were recorded at an approximate doubling time frame and observation times were 30 minutes, 1, 2, 4, 8, 12, and 24 hours after the start of the experiment. This method was adapted from Carsten et al. (2008) study on chinch bug, *Blissus occiduus* Barber, on mortality to the big-eyed bug, *Geocoris uliginosus* (Say). Artificial florescent lighting remained on 24 hours and temperature was 22±2°C. A new soybean leaflet, clean cotton pad, fresh *A. glycines*, and fresh *O. insidiosus* were used for each 24-hour replication. The related aphid species *Aphis gossypii* life history data shows an instar change every 24 hours, (Baldin et al. 2009) Therefore, a 24-hour time period was used to maximize observation while minimizing the impacts of aphid development and reproduction.

**Behavior Study**

*Orius insidiosus* behaviors were recorded in relation to the consumption and preference of the *A. glycines* early, late and adult life stages. The experimental unit was a single 8-ounce (0.24 L) safe lock container (US Plastics) with a moistened cotton pad and a single soybean leaf with one early, late, and adult *A. glycines* life stage. A new soybean leaflet, clean cotton pad, fresh *A. glycines*, and fresh *O. insidiosus* were used for each recording.
Observation was conducted using a Dino-Lite Premier digital microscope AM-3011 (AnMo Electronics Corporation, Taiwan) using the program DinoCapture 2.0 Version 1.5.8. Artificial florescent lighting remained on for the entire recording process and temperature was 22±2°C. Multiple Dino-Lite Premier digital microscopes allowed up to seven recordings at a single time point for a total of 60 individual recordings. Recording times varied from 30 minutes to 15 hours, however observation of recordings was considered usable only when an interaction occurred between *O. insidiosus* and *A. glycines*. An “interaction” was considered valid when *O. insidiosus* made contact with *A. glycines*. Examples of contact include touching *A. glycines* with tarsus or insertion of stylet. Recordings were reviewed and behavior associated with interactions between *O. insidiosus* and *A. glycines* was recorded. A total of 27 recordings qualified for an interaction. A “return” behavior was recorded when *O. insidiosus* broke contact with *A. glycines*, moved away by walking, and was away more than five seconds. Figure 3.2 illustrates an interaction between *O. insidiosus* and *A. glycines*.

Statistical Analyses

For choice studies, the first life stage consumed of each experimental unit was recorded and a chi-square test of independence (PROC FREQ, SAS Institute 9.2) conducted to determine if *O. insidiosus* preferred a life stage of *A. glycines*. For no choice studies, mortality was recorded and analyzed as a repeated measure (PROC GLIMMIX, SAS Institute 9.2). All effects with P-values less than or equal to 0.05 were considered significant.
Results

Choice Study

Orius insidiosus was presented a choice of early (first and second instar), late (third and fourth instar) and adult life stage A. glycines. First choice consumption was highest of adult A. glycines at 40.0% (18/45) and lowest for the late stage at 24.4% (11/45). The early life stage A. glycines was chosen first by O. insidiosus at 35.6% (16/45)(Table 3.1). However, there was no significant effect of frequency on A. glycines life stage preference by O. insidiosus ($\chi^2 = 1.73$, df = 2, $P = 0.4204$). These values indicate no preference of A. glycines life stage by O. insidiosus.

No choice Study

Orius insidiosus consumed increasing numbers of A. glycines within a 24 hour time period. The main effect between life stage by time was not significant ($F= 0.92$; df = 12, 380; $P = 0.5261$) as was the life stage main effect ($F= 0.51$; df = 12, 380; $P = 0.6008$). The main effect of time was, as expected, significant ($F= 140.52$; df = 12, 380; $P > 0.0001$) (Figure 3.3). These results follow the functional response reported by Rutledge et al. (2004) when the number of prey provided was 10, which is approximately 4 aphids consumed in 24 hours.

Behavior Study

A total of 27 recordings (replications) contained at least one interaction between a life stage of A. glycines and O. insidiosus. Beyond attack and feeding behaviors observed for O. insidiosus, other behaviors included walking, resting, probing the soybean leaflet, and grooming. These noted behaviors are similar to
those observed and recorded between O. *insidiosus* and A. *glycines* by Desneux and O’Neil (2008).

Of those 27 recordings, 20 contained an initial interaction between O. *insidiosus* and the adult life stage for A. *glycines* or 74.07% of the interactions. Of the 20 interactions with the adult life stage, 13 (65%) of those contained a return behavior.

For the late life stage of A. *glycines* 11 of the 27 recordings contained an interaction with O. *insidiosus* (40.07%). Of those 11 interactions, 5 contained a return behavior (46.15%). For the early life stage 13 of the 27 (48.15%) recordings contained an interaction between A. *glycines* and O. *insidiosus*. Of those 13 interactions, 6 contained a return behavior (46.16%).

Within the 27 recordings, there were a total of 44 initial interactions between all life stages of A. *glycines* and O. *insidiosus*. Of those 44 initial interactions, O. *insidiosus* returned to the A. *glycines* 24 times or 54.54% of the time (Table 3.2)
Discussion

There was no significant difference in a preference for *A. glycines* life stage by *O. insidiosus*. While other enemies of *A. glycines* and other aphid species, such as parasitoids, show a prey life stage preference (Hopper and King 1984, Lin and Ives 2003), *O. insidiosus* did not in this study. This may be due to the presence of all life stages in close proximity in natural field infestations of *A. glycines*. As seen with the no choice results, *O. insidiosus* had increased consumption for smaller life stages. This may eliminate the need for a preference of life stage (or size) due to the ability to simply consume more of the smaller life stage.

This research demonstrates that *O. insidiosus* adults consume more early than adult *A. glycines* in a 24-hour time period. In a 24-hour period, *Orius insidiosus* consumed an average of 7.30 ± 0.67 early (first and second instar), 5.45 ± 0.6 late (third and fourth instar), and 4.60 ± 0.64 adult *A. glycines*. This is supported by the functional response reported by Rutledge et al. (2004) when the number of second and third instar prey provided was 10, approximately 4 aphids were consumed in 24 hours. This result is also supported by other similar life history studies (Rutledge et al. 2004, Rutledge and O’Neil 2005).

Based on recorded observations of *O. insidiosus*, the predator returns to the *A. glycines* life stage in several cases to finish feeding. *A. glycines* is known to excrete exudate from their cornicles as a defense mechanism against predators and exudate has been shown to cause mortality, especially in early instars of *O. insidiosus* (Butler and O’Neil 2006). *Orius insidiosus* also significantly increase their time grooming when *A. glycines* is present (Desneux and O’Neil 2008). Other predators of
Hemiptera frequently move their stylets to change the site of feeding position and also inject secretions and enzymes into their prey (Cohen 1990). Aphids briefly attacked by Orius insidiosus have low survival, suggesting an injection of an enzyme or toxin which immobilizes or kills the aphid (Butler and O'Neil 2006).

*Orius insidiosus* therefore may be moving away from the aphid after injecting an enzyme or toxin to allow it to process before returning to consume the aphid. Since Hemiptera frequently move their stylets *O. insidiosus* may also be searching for an ideal feeding position. Orius insidiosus may be avoiding aphid defense exudate by moving away from the aphid and by significantly increasing their grooming time. Since exudate increases mortality of *O. insidiosus* (Butler and O'Neil 2006), it may be moving away from *A. glycines* to avoid deleterious effects of exudate while waiting for the toxin or enzyme to work on the aphid.

More observations of *O. insidiosus* feeding behavior and return to prey behavior coupled with mortality observation would be necessary to determine statistical significance of this observation.

These studies demonstrate *O. insidiosus* consumes *A. glycines* indiscriminately of life stage and that *O. insidiosus* consumes increasing amounts of *A. glycines* life stages over time and consumes more early life stage than late life stage. For aphid management, this indicates life stage and life stage preference have no impact on *O. insidiosus* as a predator.
Figure 3.1 Choice Study Design. Each experimental unit contained an early (first or second instar), late (third or fourth instar), and adult *Aphis glycines*. 
Figure 3.2 *Orius insidiosus* consuming *Aphis glycines* nymph.

Table 3.1 Choice Study. Frequency of adult, early, and late *Aphis glycines* consumed first by *Orius insidiosus* for 45 total replications.

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<th>Stage</th>
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<td>Early</td>
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<td>Late</td>
<td>11</td>
<td>24.44</td>
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Figure 3.3 No choice predation. Graph shows the average number life stages early (1st and 2nd instar), late (3rd and 4th instar), and adult *Aphis glycines* consumed by *Orius insidiosus* in a 24-hour period.
Table 3.2 Stars indicate an observed interaction between *Orius insidiosus* and *Aphis glycines*. The values represent the number of subsequent interactions between the noted life stage of *A. glycines* and *O. insidiosus* after the first attack interaction.

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<th>Attacks late</th>
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References Cited


Hunt, T. E. 2015. Personal Communication. thunt2@unl.edu.


2012 Haskell Agricultural Laboratories
Concord, NE. *Aphis glycines*. Treated with Lorsban Advanced® (Dow AgroScience) on 16 August 2012 32 ounces per acre (2.3385 liters per hectare). Mean ± standard error bars of *Aphis glycines* per soybean plant. Bars marked ‘A’ are not significantly different from the spray row. Bars marked ‘B’ are significantly different from the spray row at $\alpha = 0.05$. 