

Compatibility of soybean aphid integrated pest management strategies

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Dedication

To Aunt Kathy Heidel and my dad, David Heidel

My family who taught me to think independently, question everything, and always,
always, always remember where I came from.

Abstract

The soybean aphid, *Aphis glycines*, is a major economic pest of Midwest soybean. Since its discovery in the United States in 2000, significant strides have been made in developing an integrated pest management (IPM) program for this pest. The primary method of controlling soybean aphid outbreaks is through the use of foliar insecticides, but alternative management strategies such as host plant resistance varieties, reduced-risk insecticides and neonicotinoid seed treatments are also available. In addition, natural control from soybean aphid natural enemies can contribute towards aphid suppression. Conserving these natural enemies is an important component of soybean aphid IPM. The focus of this dissertation is to better understand the interactions between the management strategies currently available for soybean aphid management and whether the strategies are 1) effective at managing soybean aphid populations and 2) can successfully be utilized in combination with natural enemies to improve on soybean aphid IPM. To investigate these questions, both laboratory and field studies were conducted over three field seasons. Field studies demonstrated varying impacts of reduced-risk insecticides on the soybean aphid and its natural enemies. Minimal effects on soybean aphid and their natural enemies were demonstrated from the use of aphid resistant soybean and seed-treated soybean. Laboratory studies showed minimal non-target risks to natural enemies from exposure to seed treatments and host plant resistance. A field study to enhance aphid biological control with a buckwheat intercrop demonstrated minimal effects on either aphid or natural enemy populations. In summary, alternative soybean aphid management strategies exist that successfully suppress aphid population while also

minimizing risks to natural enemies. Results of this research will help improve the integration of management strategies in soybean aphid IPM.

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Chapter 1: An overview of the soybean aphid and management in North America

Introduction

In 2000, a new invasive insect species the soybean aphid (*Aphis glycines* Matsumura; Hemiptera: Aphididae) was discovered in the United States (Ragsdale et al. 2004). This pest, first discovered in Wisconsin (Wedberg et al. 2001), quickly established itself as the most significant economic pest of soybean in the midwestern United States and continues to be so today (Tilmon 2011, Hodgson et al. 2012). The occurrence of this invasive pest has led to significant changes to the management of soybean overall and the development of new control strategies to minimize the economic damage incurred from soybean aphid pest outbreaks throughout the soybean production areas of North America (Ragsdale et al. 2011).

Soybean is a crop valued at over \$27 billion annually (Ragsdale et al. 2011), and the majority of production in the United States occurs in the north central states, including Minnesota. Total soybean acreage planted for 2011 in the United States was estimated at over 75 million acres, of which 7.1 million acres (~10% of overall acreage) are in Minnesota (2012). Prior to the aphid's discovery, soybean was a minimally managed crop in regards to arthropod pests. Insect pest outbreaks were occasional, and soybean generally lacked severe perennial insect pests (Hodgson et al. 2012), making it a profitable crop to grow since it required minimal inputs regarding insect pest suppression. With the introduction of the soybean aphid came the need to change many aspects of soybean management to minimize economic losses. Significant changes included the development of sampling protocols (Onstad et al. 2005, Hodgson et al. 2007, McCornack et al. 2008), identification of effective control strategies (Li et al. 2004, Rutledge et al.

2004, Myers et al. 2005, Desneux et al. 2006), determination of an economic threshold (ET) and economic injury level (EIL) (Ragsdale et al. 2007) as well as identification of the pest itself (Voegtlin et al. 2004b, Hill et al. 2010).

The purpose of this chapter is to review and summarize the published literature on the soybean aphid, the management options available for this invasive pest, and the current status of soybean aphid integrated pest management. This information will provide the knowledge base for the following chapters of this dissertation that address the effectiveness and utilization of multiple control options used concurrently in a soybean aphid integrated pest management program.

The Soybean Aphid

The soybean aphid is an invasive pest originally from Asia. Its introduction into North America is likely to have occurred prior to 2000, although it was not discovered until that year (Venette and Ragsdale 2004). Previous studies conducted in China on the soybean aphid provided an excellent knowledge base (Hirano 1996, Wu et al. 2004) on which we have been able to build our understanding of the life cycle and population dynamics of this pest species in North America.

Biology

Knowledge of the basic biology and life cycle of the soybean aphid is necessary to understand why this is such a significant economic pest in North America and how to best approach pest management. For example, biological characteristics of this insect such as asexual reproduction and telescoping generations within asexual female aphids

allow for extremely rapid aphid population growth in soybean, adding to this pest's capability to become a significant economic pest in a very short time period (Ragsdale et al. 2004).

Soybean aphid has a heteroecious holocyclic life cycle meaning it requires two different host plants to complete the asexual and sexual stages of its life cycle (Ragsdale et al. 2004). Soybean aphid completes the asexual stage of its life cycle on soybean (*Glycine max*) and the sexual stage on buckthorn (*Rhamnus spp.*). While the soybean aphid is able to use several species of *Rhamnus* present in North America, including *R. alnifolia*, *R. lanceolata*, and *Frangula alnus* (also called *Rhamnus frangula*) (Voegtlin et al. 2004a, Voegtlin et al. 2005, Yoo et al. 2005, Hill et al. 2010), *R. cathartica* or common buckthorn is the principal overwintering host in North America due in large part to its wide distribution and high abundance throughout much of the north central United States (Knight et al. 2007).

Economic damage on soybean from the soybean aphid occurs during its asexual reproductive phase. Both wingless and winged female morphs reproduce parthenogenetically on soybean during summer months when they give birth to live young (Ragsdale et al. 2004). The asexual winged females allow for aphid migration between plants and across larger geographic scales such as between fields or even between states (Hodgson et al. 2005). Two environmental triggers for the development of the winged female morphotype are high aphid densities and changing photoperiod (Ragsdale et al. 2004, Hodgson et al. 2005). The multiple overlapping generations, birth

of live young, and development of winged morphotypes explain why soybean aphid populations are capable of increasing very rapidly in soybean crops.

Sexual reproduction of the soybean aphid occurs on buckthorn, but to reach the overwintering egg stage that results from sexual reproduction, the aphid population must first pass through a complex series of different male and female morphotypes. In late summer, winged female aphids called gynoparae are produced in soybean in response to changing photoperiod and temperature. These aphids migrate to buckthorn and reproduce asexually to produce oviparae. These oviparous females begin the sexual phase of the soybean aphid's life cycle on buckthorn (Ragsdale et al. 2004). The oviparae await the arrival of male aphids to buckthorn; these males also form on soybean late in the season. The oviparae and males mate, and the oviparae lay eggs on buckthorn. The eggs hatch in spring around the time of buckthorn bud break (Bahlai et al. 2007), and the eggs produce a fundatrix female. The fundatrices reproduce asexually, and in late spring after several generations on buckthorn, winged asexual females develop that migrate to the summer host plant of soybean (Ragsdale et al. 2004).

Numerous factors can affect the population dynamics of the soybean aphid. In addition to host plant quality effects, plant viruses contained in the host plants can also affect soybean aphid populations. Donaldson and Gratton (2007) demonstrated that the presence of plant viruses in soybean can decrease population growth of the soybean aphid. Rhainds et al. (2010a) determined that the use of different maturity groupings for soybean may have little effect on soybean aphid abundance. Environmental factors such as temperature can also significantly affect soybean aphid population dynamics.

Temperature in particular can significantly affect the aphid growth rate, and McCornack et al. (2004) demonstrated that at 25°C, growth rates are highest and populations can double in as little as 1.5 days.

The ability to predict soybean aphid outbreaks spatially or temporally would allow implementation of effective management plans. Understanding the population dynamics of this pest may aid in the ability to predict soybean aphid outbreaks. In 2005 an extensive aphid suction trap network was set up throughout the Midwest to determine whether migration patterns of the soybean aphid could aid in outbreak prediction (Schmidt et al. 2012). The suction trap network provided a better understanding of the seasonal phenology of aphid populations (peaking mid to late season) as well as a positive correlation of aphids with increasing latitude that also corresponds to an increasing population of the overwintering host *R. cathartica*. However, this regional suction trap network did not aid in prediction of early-season or local outbreaks of the soybean aphid. Another example of studying population dynamics to predict pest outbreaks was the observation of a two-year oscillation cycle of soybean aphids and their natural enemies during an eight-year sampling period in Indiana (Rhainds et al. 2010b). These temporal patterns implied the impact that natural enemies and late-season predation can have on aphids the following year. However, this cyclical pattern was not observed during the last two years of that study and has not been demonstrated in soybean aphid outbreaks in other states. Further research to elucidate the mechanisms behind this cycle would provide indications of the primary driving factors causing

soybean aphid outbreaks. Utilizing weather and temperature extremes to predict soybean aphid outbreaks has also been explored (McCornack et al. 2005, Bahlai et al. 2007).

Damage

The soybean aphid is the most damaging economic pest of soybean in the midwestern United States (Tilmon 2011). Feeding on soybean during outbreaks of this pest can result in up to 40% yield losses (Ragsdale et al. 2007). As a piercing-sucking insect, the soybean aphid is a phloem feeder. Damage from the soybean aphid is incurred by feeding of large numbers of aphids on a single plant. Numerous factors can affect the degree of injury including aphid density, within-plant distribution of aphids (McCornack et al. 2008), nutritional quality of the plant, and plant growth stage (Tilmon 2011), however, study results are not in agreement regarding the impact of plant growth stage on soybean aphid since two studies (Rutledge and O'Neil 2006, Rhainds et al. 2010a) demonstrated a lack of effect from growth stage and maturity group on aphid abundance. Direct injury from high levels of aphid feeding can cause economic losses in several different ways. Aphid feeding can reduce vegetative growth, reduce flower and pod development, decrease seed set, and decrease seed quality (Ragsdale et al. 2011, Tilmon 2011), and economic losses can be incurred from reduced seed yield as well as reduced seed quality (Beckendorf et al. 2008).

Aphid feeding can also cause indirect crop damage to both soybean and other crops. Honeydew is a sugary waste product excreted by aphids, and it provides an excellent substrate for the growth of the sooty mold fungus. Extensive sooty mold growth on soybean inhibits photosynthesis, causing reduced growth and stunting of the plant

(Tilmon 2011). Another indirect effect of aphid feeding is the vectoring of plant diseases. Aphids are well known vectors of plant pathogens such as viruses (Nault 1997, Davis et al. 2009), and soybean aphid has been shown to vector diseases to other crops such as snap beans (Mueller et al. 2010) and potatoes (Davis and Radcliffe 2008). However, vectoring plant diseases can also directly impact the soybean aphid by increasing mortality rates and reducing aphid growth rate (Donaldson and Gratton 2007).

Invasive species

Invasive species are an increasing concern to human health, biodiversity, and the environment (Andow 2003) and can lead to environmental damage costs estimated to be nearly \$120 billion annually (Pimentel et al. 2005). In an increasingly globalized world, the risk of new invasive species grows. These invasive species can permanently alter ecosystems, making environments unfavorable for native species but favorable for themselves (Knight et al. 2007, Nuzzo et al. 2009). A lot of research is now being conducted to reduce the risk of new invasive species occurring (Sheppard and Raghu 2005), manage invasive species already here (Hoddle 2004), and predict potential future invasive species (Koch and Galvan 2008, Hlasny 2011).

The soybean aphid is an excellent case study for the extensive impact invasive species can have in a new geographic region. Not only is the soybean aphid an invasive species, but *R. cathartica*, its obligate overwintering host plant, is itself a noxious weed throughout much of the Midwest United States (Knight et al. 2007). While not necessarily considered an invasive species, soybean is a non-native plant that also originated in Asia. If either *R. cathartica* or soybean were not so prolific throughout

North America, the soybean aphid would likely not have been able to reproduce, thrive, and become such a significant economic pest. Heimpel et al. (2010) describe the soybean aphid/buckthorn scenario in the context of a broader invasional meltdown of eleven different invasive species in North America. They also address management challenges such as controlling multiple invasive species simultaneously that such a complex invasive species situation raises.

Integrated Pest Management and the Soybean Aphid

Integrated pest management (IPM) is a pest management strategy that focuses on the suppression of a pest population by taking into consideration the biology and ecology of the pest and applying one or more control tactics in a way that is both environmentally friendly and economically favorable (Radcliffe et al. 2009). The definition and major components of IPM are continually changing as society and human values change. In the past, IPM was primarily focused on crop economics and efficacy of control practices (Lynch et al. 1996). Today, reducing environmental and human health risks have become an important component of IPM programs (Thomas 1999, Andow and Zwahlen 2006, Pedigo and Rice 2006), and numerous studies have been conducted to address these concerns in IPM research (Teng and Savary 1992, Andow and Zwahlen 2006, Kraiss and Cullen 2008b, Lundgren et al. 2009a, Bahlai et al. 2010).

Since the discovery of the soybean aphid in the United States, extensive research has been conducted to identify effective prevention and suppression methods for outbreaks of this pest species (Hodgson et al. 2012). Curative rather than preventative

pest strategies have been the primary focus of management, and much of the current IPM management strategy is reliant on the use of broad spectrum foliar insecticides (Ragsdale et al. 2011). While this management tactic has proven very effective at rapidly suppressing aphid outbreaks, concerns arise regarding the development of resistance to pesticides from such widespread use as well as non-target effects on other insect communities such as beneficial insects. Song and Swinton (2009) demonstrated a significant economic gain could be attained from the adoption of IPM in soybean aphid management. In particular, they showed that the use of the economic threshold determined for the soybean aphid by Ragsdale et al. (2007) could yield an economic benefit of \$1.3 billion nationally over a 15-year period. Previous studies conducted in the aphid's native range of Asia provided some insights into approaching pest management for this new pest, however, since it is only a sporadic pest in Asia (Wu et al. 2004), control recommendations applicable to North America were relatively limited.

Creating a successful IPM program can be an extensive process. Development of an effective IPM program requires knowledge about the pest species itself including its life cycle, population dynamics, host plant preferences, and the development of sampling protocols and effective management options. The following sections will further describe major components and control strategies of IPM and discuss their importance and application to soybean aphid IPM.

Economic thresholds

The development and determination of economic thresholds (ET) and economic injury levels (EIL) are important components of IPM. Economic thresholds or action

thresholds are defined as a pest density at which a control measure should be utilized. An ET is designed to prevent a pest population from surpassing the EIL, the pest density at which an economic loss would be incurred (Pedigo and Rice 2006). A cornerstone of soybean aphid IPM was the determination of the ET and the EIL (Ragsdale et al. 2007). Based on a three-year study conducted in six different states across the Midwest, the soybean aphid ET was estimated at 273 aphids per plant, and the EIL was estimated at 674 aphids per plant. Based on the dataset used to develop these values, these estimates are valid through the R5 growth stage of soybean, and the ET provides a 7-day action period in which insecticide treatment can be applied before aphid populations would surpass the EIL. Biological characteristics such as population growth rates and doubling time of the pest were used and incorporated into the development of the soybean aphid ET (Ragsdale et al. 2007). Limitations exist to the utilization of an ET and EIL. For example, adopting an ET assumes the pest population would surpass the EIL if no control measure is taken within the action period, but populations that surpass the ET do not always end up exceeding the EIL (Hodgson et al. 2012). In such a situation, utilizing the ET would be counterproductive and costly since the EIL was not exceeded and no yield losses occurred.

Chemical control

Chemical control for pest management is a very proven effective control option (Pedigo and Rice 2006), but overreliance and overuse of this measure can lead to serious issues such as resistance development and increased economic costs. Utilizing pesticides in an IPM program can be beneficial as it may lead to reduced application rates and

reduced dependency on a single control option, ultimately resulting in reduced costs and environmental and human risks (Song and Swinton 2009). Chemical control of insect pests is typically used as a curative rather than a preventative management tactic, however, there are cases where insecticides have also been utilized for prevention, such as in a seed treatment. In the usage of seed treatments, the decision to use this seed is made prior to knowing whether a pest outbreak will ever occur.

The extensive use of insecticide applications on soybean in the North Central United States growing region is a relatively new phenomenon, beginning only in the past 12 years. This increased dependence is due solely to the discovery of the soybean aphid in the U.S. (Ragsdale et al. 2011, Hodgson et al. 2012). Today, the application of insecticides is the primary control tactic for soybean aphid management. Because of this, insecticide use in Midwest soybean has increased 130-fold due to the presence of soybean aphid (Ragsdale et al. 2011), causing both increases in production costs as well as the risk of non-target effects to beneficial insects and to the environment. The choice of insecticide utilized for soybean aphid management can have significant impacts on the degree of non-target effects. The broad spectrum insecticides commonly used in soybean aphid management can cause severe negative impacts on natural enemy communities (Hodgson et al. 2012). Establishing an ET and EIL to reduce the number of sprays and to target pest populations at critical densities can aid in reducing or eliminating these non-target environmental impacts (Pedigo and Rice 2006). Utilizing reduced-risk insecticides that target a particular pest taxon is another method to reduce non-target effects from insecticides. In other cropping systems like cotton in the southwestern U.S., the adoption

of reduced-risk insecticides has increased control of cotton insect pests while reducing negative impacts on the natural enemy community (Naranjo et al. 2003, Naranjo et al. 2004).

Cultural control

Cultural control is the manipulation or modification of a cropping environment in a way that makes the crop unfavorable for the pest and minimizes pest damage.

Ecological pest management is a broader term that incorporates cultural control and reflects the need to understand the pest's ecology in developing management practices (Pedigo and Rice 2006). Because of this holistic ecological approach, cultural control is often used as a preventative rather than curative pest control strategy. Some examples of common cultural control practices include sanitation, tillage, altered planting date, water management, host plant resistance, and diversified cropping including trap cropping, intercropping, and crop rotation (Teng and Savary 1992, Thomas and Waage 1996, Way and van Emden 2000, Pedigo and Rice 2006, Radcliffe et al. 2009). In this dissertation, the focus will be on the use of host plant resistance and diversified cropping practices in soybean aphid management.

Host plant resistance can be a very effective cultural control management tool in an insect IPM program. Host plant resistance is defined as the possession of heritable characteristics by a plant that allow it to minimize damage from insect pest feeding (Pedigo and Rice 2006). Use of this management tool can be advantageous in IPM because it is specific to a particular pest species, effective over a long period of time, easily used and adoptable by farmers, and is relatively environmentally risk-free (Panda

and Khush 1995). Knowing the mechanism(s) of resistance informs our understanding of the insect's response to the plant and how to best incorporate pest resistant varieties into IPM. The three mechanisms of genetic host plant insect resistance are antixenosis, antibiosis, and tolerance. Antixenosis deters insects from colonization on the plant and causes a behavioral change in the insect. Antibiosis causes physiological changes in the pest from plant feeding, resulting in reduced growth, reproductive, or survival rates. Antibiosis is typically the most common and desired type of resistance (Pedigo and Rice 2006). Tolerance is the ability of a plant to withstand insect feeding or damage with reduced economic loss. Unlike the first two mechanisms that cause changes in the pest, only tolerance elicits a response by the plant. More than one of these mechanisms may be used in the development of a host plant resistant variety (Harris and Frederiksen 1984).

The development of aphid-resistant soybean varieties is a significant cultural control method developed for soybean aphid management. The *Rag1* gene was the first source of resistance discovered (Hill et al. 2006), but other sources of resistance have since been found (Hesler and Dashiell 2008, Kim et al. 2008, Hill et al. 2010, Hesler et al. 2012). Currently only varieties containing the *Rag1* gene are commercially available for soybean producers, however additional *Rag* genes and stacked resistance events will likely become commercially available in the near future (Hodgson et al. 2012). The discovery of soybean aphid biotypes poses a challenge to the future use of aphid-resistant soybean varieties (Kim et al. 2008, Hill et al. 2010, Hesler et al. 2012) in the Midwest. Until the range and biology of these different biotypes are better understood, the use of aphid-resistant varieties may be limited (Kim et al. 2008, Hesler et al. 2012).

Diversified cropping practices involve planting multiple crops that either vary temporally or spatially for the objective of pest management. Crop rotation is the best example of effective temporal crop diversification. An excellent case study of its effectiveness has been the control of corn rootworms in North American corn crops utilizing an annual corn/non-corn crop rotation (Meinke et al. 2009). Spatial diversification of crops includes practices such as trap cropping and intercropping. The objective of these practices is to protect a crop by drawing pests away to a more favorable crop or plant where the pest is then either controlled or left to develop (Pedigo and Rice 2006). Trap cropping typically uses a greater spatial separation from the economic crop of interest whereas intercropping uses two different crops in the same area. Diversified cropping practices such as these have also been used in conjunction with aphid biological control enhancement, which will be discussed further in the next section.

Biological control

Natural enemies can provide important regulation of a pest population and in turn can be a very effective pest control measure when used in IPM. Several different types of biological control exist including classical, conservation, and augmentation biological control (Van Driesche et al. 2008, Radcliffe et al. 2009). Classical biological control is the introduction of a new natural enemy to an area and relies on the establishment of the natural enemy for pest suppression. Oftentimes, these natural enemies are from the pest's geographic area of origin and host specificity to the pest species is a desired characteristic. Conservation biological control uses natural enemies already present in a cropping environment and relies on enhancing and conserving those natural enemy

populations. Augmentation biological control is most like the use of insecticides where the release of natural enemies is used for short-term pest suppression and establishment is not a goal (Van Driesche et al. 2008). In this dissertation, the focus will be on conservation and classical biological control, as these are most applicable to soybean aphid management.

Biological control is an important component of soybean aphid management in both Asia and the United States, and the ecosystem services of aphid control provided by these natural enemies has been estimated at \$84 million dollars (Zhang and Swinton 2012). A large community of natural enemies contribute towards soybean aphid suppression, and important natural enemy groups in the Midwest include ladybeetles such as *Harmonia axyridis* and *Coleomegilla sextempunctata* (Fox et al. 2004, Rutledge et al. 2004), as well as the insidious flower bug *Orius insidiosus* (Rutledge et al. 2004, Rutledge and O'Neil 2005, Desneux et al. 2006), aphidophagous dipteran larvae (Fox et al. 2004, Costamagna et al. 2007), and fungal pathogens such as *Pandora neoaphidis* (Koch 2011). In Asia, parasitoid wasps also provide significant soybean aphid biological control (Han 1997, Wu et al. 2004), however this natural enemy group has not been a significant consistent biological control agent of North American soybean aphid.

Conservation biological control is currently the primary method of biological control being used in soybean aphid management. Numerous studies have been conducted documenting the impact of predators and parasitoids already present in North American soybean on the soybean aphid (Fox et al. 2004, Rutledge et al. 2004, Fox et al. 2005, Desneux et al. 2006, Costamagna et al. 2007, Gardiner et al. 2009b, Gardiner et al.

2009a, Xue et al. 2009, Frewin et al. 2010, Rhainds et al. 2010b). Several studies demonstrated that the effects of different natural enemies may vary throughout the season (Rutledge et al. 2004, Rutledge and O'Neil 2005) which may in part be due to a density response preference of different natural enemy groups. In addition, agricultural practices that increase diversity of the cropping habitat (Schmidt et al. 2007, Woltz et al. 2012) and the broader landscape diversity (Gardiner et al. 2009b, Gardiner et al. 2009a, Schmidt et al. 2011) can enhance conservation biological control for this pest.

An extensive classical biological control research program is ongoing for the soybean aphid (Heimpel et al. 2004). While classical biological control of the soybean aphid has not yet been established in the United States, research is ongoing to pursue this control option. Numerous factors point to the potential success of classical biological control of this pest. These include the absence of large pest outbreaks of this pest in its native range of Asia due to natural enemy suppression and the presence of high numbers of parasitoid wasps that target the soybean aphid (Hirano 1996, Han 1997, Wu et al. 2004). Parasitoids of the soybean aphid are a natural enemy guild largely lacking in North America, and introducing them has been the focus of soybean aphid classical biological control in the United States. Currently, *Binodoxys communis* is the only soybean aphid parasitoid approved for release, however, since its first open environmental release in 2007, no evidence of establishment in the U.S. has been found (Ragsdale et al. 2011). Other parasitoids specific to the soybean aphid are now being pursued for classical biological control. Most recently, the non-native generalist parasitoid *Aphelinus certus* was discovered in Minnesota. While this parasitoid species

was rejected for classical biological control due to its broad host range, it has demonstrated effectiveness at suppressing soybean aphid populations in Ontario, Canada (Frewin et al. 2010).

Other considerations

The agricultural system used for crop management can be an important consideration in developing an ideal pest management program. One popular example of this is the comparison between conventional and organic agricultural systems (Zehnder et al. 2007, Chavas et al. 2009, Garratt et al. 2011). Organic agriculture is an environmentally based farming system that strives for long-term sustainability with reduced inputs. In U.S. soybean production, the majority of acreage is managed under conventional production practices, but Minnesota is also one of the leading organic soybean producing states in the country. In fact, in 2007, Minnesota was the leading producer of organic soybeans in the United States with nearly 17,000 acres planted to organic soybeans (2007). While this was a small percentage of the total soybean acreage in Minnesota, addressing the needs of organic soybean producers is important because they approach pest management in a different manner than conventional producers.

Organic pest management focuses on prevention of pest issues such as insects, diseases, and weeds, and only when prevention fails are curative control measures such as organically approved pesticides allowed. This approach to pest management fits well into the overall views of IPM in general. The challenge is that many organically approved insecticides, particularly in the case of the soybean aphid, have either limited efficacy or may not be as reduced-risk to beneficial organisms as perceived. For example, pyrethrum

can be effective for soybean aphid control can also break down rapidly when exposed to UV light, resulting in a very short to nonexistent residual period (Isman 2006).

Compatibility of Soybean Aphid Management Options

Key components to developing a successful IPM program are understanding the compatibility potential of different management options as well as effective implementation of them into IPM. The assumption that the different management options are fully capable of being utilized simultaneously without prior careful assessment can be a major limitation to creating a functional IPM program (Thomas 1999), and extensive research should be conducted to further develop and fully integrate IPM control options (Way and van Emden 2000).

Host plant resistance is often assumed to have little to no non-target effects on natural enemies, but without adequate studies, this assumption may not be valid (van Emden 1990). In soybean aphid IPM, several studies have been conducted to date describing the potential negative impacts of host plant resistance on natural enemies. In one of the studies conducted to date on this topic, Lundgren et al. (2009b) demonstrated in laboratory that direct exposure to aphid resistant soybean may increase the longevity of the ladybeetle *H. axyridis* although another important soybean aphid natural enemy, *Orius insidiosus*, did not experience any direct effects from host plant resistance. Field studies by Chacón et al. (2012) demonstrated potential indirect effects of host plant resistance on the aphid parasitoid *Binodoxys communis*. However, Tinsley et al. (2012), demonstrated a lack of indirect effects from both host plant resistance and seed treatment

on the natural enemy community. Such conflicting study results demonstrate the need for additional research to explore these potential non-target impacts of host plant resistance on the soybean aphid and determine how such impacts can affect soybean aphid IPM.

Insecticides can provide exceptional control on a target pest species, but numerous concerns also arise with overuse and overdependence of this control option. Such concerns include resistance development, pest resurgence, and environmental non-target risks, particularly on beneficial insects such as natural enemies (Pedigo and Rice 2006, Gentz et al. 2010), and negative non-target impacts of foliar insecticides on natural enemies are well documented (Al-Deeb et al. 2001, Elzen 2001, Walker et al. 2007, Ohnesorg et al. 2009) These environmental concerns raise questions as to the feasibility of utilizing insecticides with other control options such as biological control.

In soybean aphid IPM, broad-spectrum insecticides are no longer the only chemical control options available. Numerous reduced-risk insecticides have been explored for potential use in soybean aphid management (Kraiss and Cullen 2008a, Kraiss and Cullen 2008b, Ohnesorg et al. 2009, Bahlai et al. 2010), with several insecticides demonstrating effective soybean aphid suppression. In addition, the use of neonicotinoid seed treatments for soybean aphid management provides a preventative application method of soybean aphid insecticides. Seed treatments have demonstrated negative effects on soybean aphid natural enemies in other cropping systems (Al-Deeb et al. 2001, Moser and Obrycki 2009). Minimal effects of seed treatment on soybean aphid natural enemies were demonstrated in several studies (Koch and Ragsdale 2011, Tinsley et al. 2012), while other studies found a significant effect of seed treatments (Magalhaes

et al. 2008, Seagraves and Lundgren 2012). These varied results demonstrate the need for further research to elucidate the impacts of seed treatments on a case-by-case basis for use in soybean aphid IPM.

Conclusion

The focus of this dissertation is to better understand whether different management options available for soybean aphid management can be utilized in combination to improve upon the existing soybean aphid IPM program. The following chapters investigate the efficacy and feasibility of concurrently utilizing conservation biological control with other available control strategies for soybean aphid management. In Chapter 2 my objective was to explore the use of reduced-risk foliar insecticides and aphid resistant soybean to both suppress soybean aphid populations and reduce non-target risks to soybean aphid natural enemies. In Chapters 3 and 4 my objectives were to elucidate the impacts of aphid-resistant soybean and insecticide seed treatments on the aphid natural enemy community. More specifically, Chapter 3 investigates the population-level effects on a field scale of these management tactics while Chapter 4 investigates survival and longevity effects of individual natural enemies from exposure to these management tactics. In Chapter 5 my objective was to explore enhancement of conservation biological control in an organically managed soybean production system through the use of a flowering intercrop. Overall the results of these chapters aim to provide knowledge on the interactions occurring between these management tactics so that we can further develop a truly integrated soybean aphid IPM program.

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**Chapter 2. Effects of reduced-risk insecticides and *Rag1* resistance on soybean
aphid and natural enemies**

Introduction

Integrated pest management (IPM) often relies on the use of multiple control options to manage a pest population, and understanding how different management options interact with one another is necessary for developing a successful IPM program. IPM is a decision-making process that requires knowledge about the pest's biology, control options, and environmental and economic risks of each control option to make informed management decisions (Radcliffe et al. 2009). IPM often involves the use of economic thresholds in combination with different management options such as chemical control, cultural control, biological control, or resistant plants (Pedigo and Rice 2006). Chemical control through foliar application of insecticides can be an effective method of pest management, particularly when used in combination with economic thresholds, but this management tactic also carries the risk of non-target environmental effects. Reduced risk insecticides are insecticides that target a particular pest species while minimizing adverse effects on non-target organisms (such as natural enemies) and the environment (Stansly et al. 1996). Utilizing a selective pesticide that targets a particular pest group can aid in reducing non-target effects. Biological control can be very effective at reducing pest populations, but it can be extremely sensitive to non-target effects exerted by other management options. When biological control is a component of an IPM program, conserving natural enemies becomes an important consideration. Reduced-risk insecticides can be particularly useful in situations where biological control is important in pest management and therefore conservation of natural enemies is necessary. Resistant

plants can also effectively suppress insect populations, but non-target effects on other organisms such as natural enemies are poorly understood.

Since its discovery in 2000, the soybean aphid, *Aphis glycines*, has been a significant economic pest of soybean in North America, and management of this insect may be necessary to prevent economic losses of up to 40% (Ragsdale et al. 2011). Foliar insecticides and host plant resistance to aphids are two management options currently used for management of the soybean aphid. Broad-spectrum foliar insecticides are the primary reactive management option available, and while this option can be a very effective tool in suppressing soybean aphid populations, this management option can nearly eliminate beneficial insects such that natural control is adversely affected which may contribute towards pest population resurgence. Aphid resistant soybean varieties are a more recent management option for soybean aphid, primarily as *Rag1* resistant varieties. Soybeans containing the *Rag1* gene suppress aphid populations primarily through antibiosis (Diaz-Montano 2006). The effect of *Rag1* resistance on the natural enemy complex is not fully understood.

Conservation biological control involves the manipulation and maintenance of the environment to make it unfavorable for the pest or reduce pest damage (Pedigo and Rice 2006). Natural enemies can effectively suppress soybean aphid populations in soybean (Fox et al. 2004, Rutledge and O'Neil 2005, Costamagna et al. 2007). Key predators of the soybean aphid in the Midwest include *Harmonia axyridis* (Coleoptera: Coccinellidae) and *Orius insidiosus* (Hemiptera: Anthocoridae) (Costamagna et al. 2007). Natural enemies are not always able to suppress aphid population below economically damaging

levels, and in this situation, additional management strategies may be necessary. The effects of management choices on suppressing or enhancing biological control was demonstrated in cotton insect pest IPM in the southwestern United States (Naranjo 2001). By utilizing reduced-risk insecticides to control cotton pests, non-target impacts on cotton natural enemies were reduced, allowing for increased pest suppression with fewer necessary insecticide applications (Naranjo et al. 2003)

The choice of insecticide is a critical and important decision for managing a pest insect in an IPM program. Spirotetramat and pyriproxyfen are two reduced-risk insecticides that are toxic to piercing-sucking herbivorous insects, and previous work in cotton showed a reduced effect from the use of such insecticides on natural enemy populations (Naranjo et al. 2004, Brück et al. 2009). While neither of these insecticides is currently approved for use on soybean, they are approved to control other aphid pests such as the cotton aphid, *Aphis gossypii* Glover, and the potato aphid, *Macrosiphum euphorbiae* (Thomas) in other cropping systems (2008b, 2008a). Spirotetramat is a systemic insecticide effective against piercing-sucking insects and functions as a lipid biosynthesis inhibitor (Nauen et al. 2008), and it is most effective against immature stages of pest insects (Brück et al. 2009). Several studies (Johnson and O'Neal 2007, Bahlai et al. 2010) have demonstrated successful efficacy of spirotetramat against the soybean aphid. Pyriproxyfen is an insect growth regulator (IGR) specifically targeted towards piercing-sucking insect pests. It functions as a juvenile growth hormone analog (Liu 2003). Laboratory studies of pyriproxyfen on the soybean aphid demonstrated

sublethal effects on aphid nymphs (Richardson and Lagos 2007), indicating a strong potential for field-level control of this aphid.

The purpose of this chapter was to investigate how two new management options, foliar insecticides and host plant resistance, could be used along with conservation biological control in soybean aphid IPM. The objectives of this study were to determine the efficacy of these control measures 1) for soybean aphid management and 2) in combination with biological control to determine whether these control options are compatible with one another and could be incorporated into an IPM program.

Materials and Methods

Field location and study design

The field experiment was conducted in 2009 at the University of Minnesota Outreach, Research and Education (UMore) Park near Rosemount, MN in Dakota County (N44° 43' 25", W93° 4' 2"). Soybean plots were planted using no-till production practices on 26 May 2009. Plots were either planted with the aphid resistant *Rag1* cultivar LD05-16060 or the near-isoline aphid susceptible cultivar SD01-76R depending on the assigned treatment. Soybean was planted using 76-cm row spacing and a seeding rate of 371,000 seeds per hectare. The experimental field was surrounded by soybean on two sides and a road and driveway on the other two sides.

The experimental design was a randomized complete block design with five experimental treatments (three foliar insecticide treatments, one *Rag1*-resistant soybean line treatment with non foliar insecticide application, and an untreated susceptible control) replicated within four blocks, and each plot within a block was randomly assigned a treatment. The insecticide treatments included two reduced-risk insecticides (spirotetramat and pyriproxyfen) and one broad-spectrum insecticide (lambda-cyhalothrin). All plots consisted of 12 rows 18.3m in length (total plot: 9.1 by 18.3 m). Blocks were separated by a 7.6 m fallow alley, and within blocks, a 2.4 m fallow alley separated plots. Foliar insecticides were applied to treatment plots when the average aphid density per treatment reached the economic threshold of 250 soybean aphids per plant. Since spirotetramat and pyriproxyfen were not registered for soybean use at the time of this study, application rates were based on information provided from their commercial sources. Treatments and applicable insecticide application rates can be found in Table 2-1.

Aphid and natural enemy sampling

To determine treatment effects on soybean aphid, aphids were sampled weekly throughout the growing season. Estimates of aphid density were based on whole plant counts of aphids. Sampling intensity per plot varied depending on the percent infestation rate of individual plants for that particular treatment. When less than 50% of plants were infested with soybean aphid, 20 plants/plot were counted. This number decreased to 10 plants/plot as the infestation rate increased to 80%. At an infestation rate of 80-100%, 5 plants/plot were sampled per plot (Ragsdale et

al. 2007). Cumulative aphid-days (CAD) (1 CAD = 1 aphid/plant/day) were calculated using weekly average aphid densities to provide a measure of seasonal aphid pressure.

To determine treatment effects on aphid natural enemies, plots were sampled weekly to estimate natural enemy populations. Natural enemy abundance was sampled using two methods: direct observation and sweep net sampling. These sampling methods were used to maximize sampling of both mobile and stationary natural enemies (Schmidt et al. 2008). Direct observation of natural enemies was conducted by recording all natural enemies observed on plants within a 1-m length of row within one plot. Three observations were made per plot. Sweep net sampling consisted of 20 sweeps per plot in a single row, and rows for sweep net sampling was varied per week to minimize damage to the plants. Within a single sampling week, direct observations and sweep net sampling were conducted in different rows from one another.

Yield

To obtain yield estimates from each treatment, two yield rows were designated per plot at the beginning of the season. Designated yield rows were not used for seasonal aphid and natural enemy sampling to reduce sampling effects on yield. Harvest data were collected on 3 November 2009, utilizing a 2-row plot combine. Samples per plot were collected, weighed, and measured for moisture. Yields were corrected to 13% moisture and reported as tons per hectare.

Data analysis

To determine treatment effects on soybean aphid abundance, mean aphids per treatment, CAD, and yield results were compared between treatments using repeated measures analysis of variance (ANOVA). Fixed effects were treatment, date, and the interaction of these variables with date as the repeated variable (nlme package, R, 2.10.0, R Development Core Team 2010). Data were log (x+1) transformed when necessary to meet the normality assumptions of ANOVA. Means were separated using Tukey's HSD test, and the significance level was 0.05 for all statistical tests.

To determine treatment effects on natural enemy abundance, natural enemy counts were analyzed with repeated measures ANOVA where fixed effects included treatment, date, and the interaction of these variables with date used as the repeated measure. To assess treatment effects on specific natural enemy groups, treatment comparisons were also conducted on two important soybean aphid natural enemies, *Harmonia axyridis* and *Orius insidiosus*. Direct observations and sweep net sampling counts of natural enemies were combined to obtain natural enemy counts per plot for analysis. Data were log (x+1) transformed when necessary to meet assumptions of ANOVA. Means were separated using Tukey's HSD test. A significance level of 0.05 was used for all statistical tests. While interactions of factors were included in the analysis, only significant interactions terms will be addressed in the results.

Results

Effects on soybean aphid

To ensure foliar insecticide applications coincided with the soybean aphid ET of 250 aphids/plant, foliar applications for all insecticide treatments were applied to the appropriate treatments on 23 July 2009. Prior to insecticide application, aphids averaged 231 ± 106 aphids per plant in the susceptible plots on 22 July, and by 28 July the ET was surpassed in the untreated control (579 ± 305 aphids/plant) indicating timing of spraying coincided with reaching the ET in the susceptible plots. Soybean aphid populations in the *Rag1* treatment did not surpass the ET until after 28 July and were not included in calculating the application date for insecticide treatments.

Abundance of soybean aphid varied greatly between treatments and throughout the season. There was a significant effect of treatment on soybean aphid abundance ($F = 26.9$, $df = 4, 12$; $P < 0.0001$) and between sampling dates ($F = 31.7$, $df = 11, 44$; $P < 0.0001$). All treatments were significantly different from the untreated control, and the lambda-cyhalothrin, spirotetramat, and *Rag1* resistance treatments were not significantly different from one another (Figure 2-1a). Highest aphid abundance (4521 ± 755 aphids/plant) across all treatments was observed in the untreated control plot on 10 August.

To reveal changes in aphid population growth rate changes during the season, aphid abundance was plotted on a log-based scale (Figure 2-1b). This demonstrated that all treatments experienced similar positive population growth until around mid August. After this point, negative growth occurred until sampling was discontinued in September (Figure 2-1b). Two exceptions in the positive growth rate trend were observed shortly

after insecticide applications of lambda-cyhalothrin and spirotetramat, but aphid growth resumed again at a rate similar to pre-application of insecticide.

Plant exposure to aphids measured in CAD also showed significant variation between treatments. Between the five treatments, the lambda-cyhalothrin treatment experienced the lowest exposure to soybean aphid (10,504 CAD) followed by spirotetramat (11,135 CAD) and the *Rag1* resistant treatment (22,893 CAD) (Figure 2-1c). On individual sampling dates, significant treatment differences were found between all treatments from 30 June until 1 Sept.

Effects on natural enemies

A total of 9,544 natural enemies were observed in all plots throughout the season. Of this overall total, ladybeetles (family Coccinellidae) comprised the largest group with 4,944 individuals (51.8%) followed by parasitoid wasps (2,248 parasitized aphids or 23.6%) and *O. insidiosus* (1166 individuals or 12.2%). Other natural enemy groups observed included hoverfly larvae (family Syrphidae) or *Aphidoletes aphidimyza* larvae (5%), lacewings (2.2%), *Nabis spp.* (<1%), and *Podisus maculiventris* (<1%). Ladybeetle populations primarily consisted of *Harmonia axyridis* (36.3% of all ladybeetles) followed by *Coccinella septempunctata* (11.7%). Other ladybeetle species included *Hippodamia variegata*, *Hippodamia convergens*, and *Coleomegilla maculata*. The entomopathogenic fungus *Pandora neoaphidis* was also observed infecting soybean aphid, however, infection rates were not quantified for this study.

Natural enemies were observed in all treatments throughout the season, but the number of natural enemies observed varied significantly between treatments ($F = 6.4$, $df=4, 12$; $P=0.005$) and sampling date ($F=210.3$, $df=11, 44$; $P<0.0001$) (Figure 2-2a). Natural enemy abundance in all treatments was significantly different from the untreated control except pyriproxyfen. Natural enemy abundance in the lambda-cyhalothrin, spirotetramat, and *Rag1* resistant treatments were not significantly different from one another. Throughout the season, the untreated control contained the greatest number of natural enemies observed with $3,085 \pm 41$ natural enemies. The lowest number of natural enemies occurred in the spirotetramat treatment with 884 ± 11 natural enemies. The greatest number of natural enemies observed on any one sampling date was 206.0 ± 35 natural enemies on 18 Aug in the untreated control plots. Natural enemy growth rates varied between treatments (Figure 2-2b) but in general increased in all treatments until mid-August where growth rates began decreasing. Two exceptions to this trend were observed late July in the lambda-cyhalothrin, spirotetramat, and untreated control treatments where growth rates decreased for a time before rebounding.

Treatment effects on ladybeetles and *O. insidiosus* varied depending on the natural enemy group. Ladybeetle populations demonstrated significant effects on soybean aphid abundance between treatments ($F=24.9$, $df = 4,12$, $P<0.0001$) and sampling dates ($F=251.9$, $df = 11,44$; $P<0.0001$) (Figure 2-3a). Ladybeetle abundance in all treatments was significantly different from the untreated control except for pyriproxyfen. Ladybeetle abundance differed significantly between lambda-cyhalothrin

and *Rag1* resistance treatments, but there was no significant difference between spirotetramat and either lambda-cyhalothrin or *Rag1* resistant treatments. *Orius insidiosus* did not demonstrate a significant response to treatments ($F=1.0$, $df=4,12$; $P=0.47$) but did have a significant effect from sampling date ($F=37.6$, $df = 11,44$; $P<0.0001$) (Figure 2-3b).

Yield

Yield varied from 43.0 ± 3.0 bu/acre (*Rag1* resistant) to 55.5 ± 5.4 bu/acre (lambda-cyhalothrin). Yield analysis showed a significant treatment difference ($F=12.7$, $df=4,12$; $P=0.0003$), however, a mean separation test showed only the *Rag1* resistant treatment to be significantly different from all other treatments.

Discussion

Results from this study confirmed that *Rag1* resistant soybean does suppress Minnesota soybean aphid populations in comparison to untreated SD01-76R, the susceptible control. While suppression was observed, *Rag1* resistance did not prevent aphid populations from exceeding both the economic threshold (ET) of 273 aphids per plant and the economic injury level (EIL) of 674 aphids per plant. *Rag1* resistance did succeed in delaying the date that aphid populations exceeded the ET and EIL by nearly 7 days. This delay can have significant consequences when growth stage of soybean is considered. The ET and EIL are robust up to the R6 reproductive stage of soybean (Ragsdale et al. 2007), but beyond this growth stage, research is lacking to demonstrate

that the thresholds are appropriate. Other factors such as extreme environmental conditions and changing plant quality may cause unknown effects on aphid population growth. Mid-season aphid decreases due to changes in plant quality have been observed in other aphid-plant systems (Karley et al. 2004). Delaying time to the ET may therefore provide enough time to reach these critical growth periods where aphid suppression may be enhanced due to changes in plant physiology.

Results demonstrated that reduced-risk insecticides can significantly lower aphid populations to the level of the conventional broad-spectrum control, however, choosing the appropriate reduced-risk insecticide is critical. Both insecticides included in this study specifically targeted piercing-sucking insect pests, but only one demonstrated significant efficacy for controlling the soybean aphid. In this study, spirotetramat was effective at suppressing soybean aphid while pyriproxyfen was not. This evidence of efficacy by spirotetramat is supported by similar conclusions in other studies (Johnson and O'Neal 2007, Bahlai et al. 2010). Pyriproxyfen did not demonstrate activity against the soybean aphid, and this result was unexpected. Previous laboratory studies by Richardson and Lagos (2007) indicated a likelihood of activity against this pest, yet the results demonstrated no effect in the field, and a study by demonstrated by Kearns and Stewart (2000) demonstrated both laboratory and field efficacy of pyriproxyfen against another aphid pest, the cotton aphid (*Aphis gossypii*). One explanation for the lack of efficacy in this study may be the rate of application used was not appropriate for the soybean aphid. As stated previously, this compound is not currently labeled for use on soybean and therefore rates were estimated from other crops and aphid pests labeled for pyriproxyfen.

Overall, the study demonstrated that reduced-risk insecticides can be effective in a soybean aphid IPM program, but potential reduced-risk insecticides must be studied on a case-by-case basis to determine whether efficacy against the targeted pest species is possible. If only considering soybean aphid control and not any potential effects on non-target organisms, spirotetramat demonstrated itself as a strong candidate as a reactive pest management option for the soybean aphid.

Other effects considered in this study were potential non-target effects from soybean aphid management options on the natural enemy community present within soybean. *Rag1* resistant soybeans did reduce abundance of soybean aphid natural enemies, and this reduction was consistent in the two major natural enemy groups, the ladybeetle and *O. insidiosus* populations. This indicates that *Rag1* resistance may be causing non-target effects. Similar reduction effects as *Rag1* were observed in the spirotetramat treatment, but pyriproxyfen demonstrated no effect on the natural enemy community. Both *Rag1* resistance and spirotetramat may therefore be causing non-target effect on soybean aphid natural enemies, but whether this reduction is due to direct or indirect effects is not clear. There is evidence that this reduction in abundance could be due to direct effects on natural enemies. Previous studies indicated that *Rag1* varieties may have direct effects on these natural enemies (Lundgren et al. 2009, Chacón et al. 2012). In addition, omnivorous predators such as *O. insidiosus* are at a greater risk of direct non-target effects from systemic reduced-risk insecticides such as spirotetramat due to their plant feeding behavior (Armer et al. 1998, Kaplan and Thaler 2011). However, the natural enemy abundance reduction could also be due to indirect effects

such as responding to changing prey densities in soybean. Several studies have demonstrated the relationship between aphid density and natural enemies in soybean (Rutledge and O'Neil 2005, Donaldson et al. 2007). In this study, aphid populations varied between treatments but growth rates did not, and natural enemy populations followed similar trends. For example, aphid populations peaked around 11 Aug in most treatments, and natural enemy populations also peaked from 11 Aug to 18 Aug before declining as aphid populations did.

Yield analysis from this study was inconclusive. While yield in the *Rag1* treatment was lower than all other treatments, this difference was due likely to the *Rag1* LD05-16060 variety being an inappropriate maturity group for the study location; at the study location, the appropriate maturity group is 1.9 for Rosemount, but LD05-16060, the only *Rag1* variety with sufficient seed supply for the size of plots used, was a maturity group 3. At the time of harvest (3 Nov), pods in the resistant plots were still green and high in moisture, indicating the incompatibility of the study location and maturity group. The maturity group for the SD01-76R variety used in other treatments was appropriate for the study location. A sufficient seed supply of a non-*Rag1* bearing isoline to LD05-16060 was not available.

These results show that while both *Rag1* host plant resistance and reduced-risk insecticides can be effective at reducing soybean aphid populations, each option must be assessed individually to determine both its efficacy on the target pest as well as compatibility with natural enemies. As both management options have demonstrated efficacy against the soybean aphid, there is potential to use them in a soybean aphid IPM

program. However, their impacts on the natural enemy community bring into question their compatibility with biological control. Further studies are necessary to determine the specific mechanisms responsible for impacting these natural enemy populations.

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Table 2-1. Treatments and application rates used in the reduced-risk insecticide and host plant resistance study during 2009 at Rosemount, MN.

| Active Ingredient | Soybean cultivar | Susceptible/ Resistant | Application Rate ¹ |
|----------------------------------|------------------|---------------------------|----------------------------------|
| Lambda-cyhalothrin | SD01-76R | Susceptible | 2.5 fl. oz. |
| Spirotetramat | SD01-76R | Susceptible | 4.5 fl. oz. |
| Pyriproxyfen | SD01-76R | Susceptible | 9 fl. oz. |
| Untreated/ <i>Rag1</i> resistant | LD05-16060 | Resistant | NA |
| Untreated/susceptible | SD01-76R | Susceptible | NA |

¹Foliar product rates are given as product per acre.

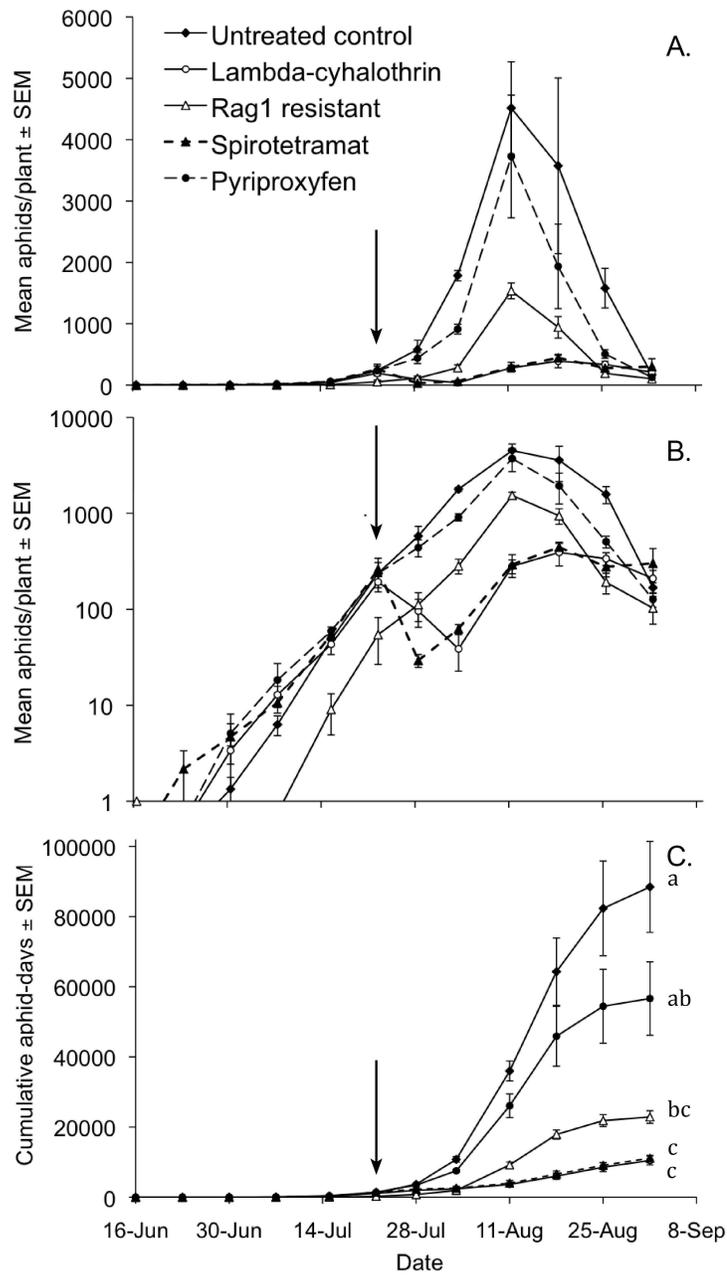


Figure 2-1. Seasonal soybean aphid populations for the study at Rosemount, MN in 2009. Aphid abundance is expressed as aphids per plant (A), aphid per plant on a log-10 scale (B), and cumulative aphid days (CAD) (C) over the 2009 season. Within (C), different letters represent a significant difference in CAD at the end of the season. Arrows indicate date of insecticide application for all treatments.

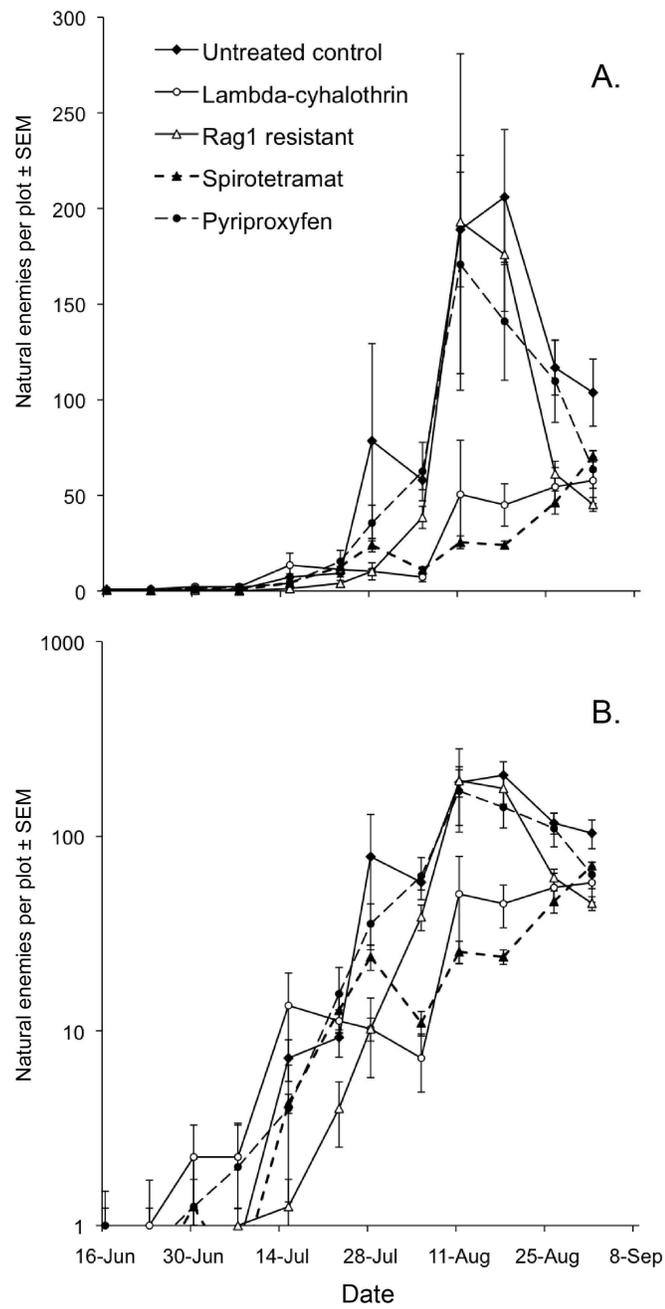


Figure 2-2. Populations of natural enemies observed in soybean in 2009 at Rosemount, MN. Natural enemy abundance is shown as the total natural enemies sampled per plot (A) and on a log₁₀-based scale (B).

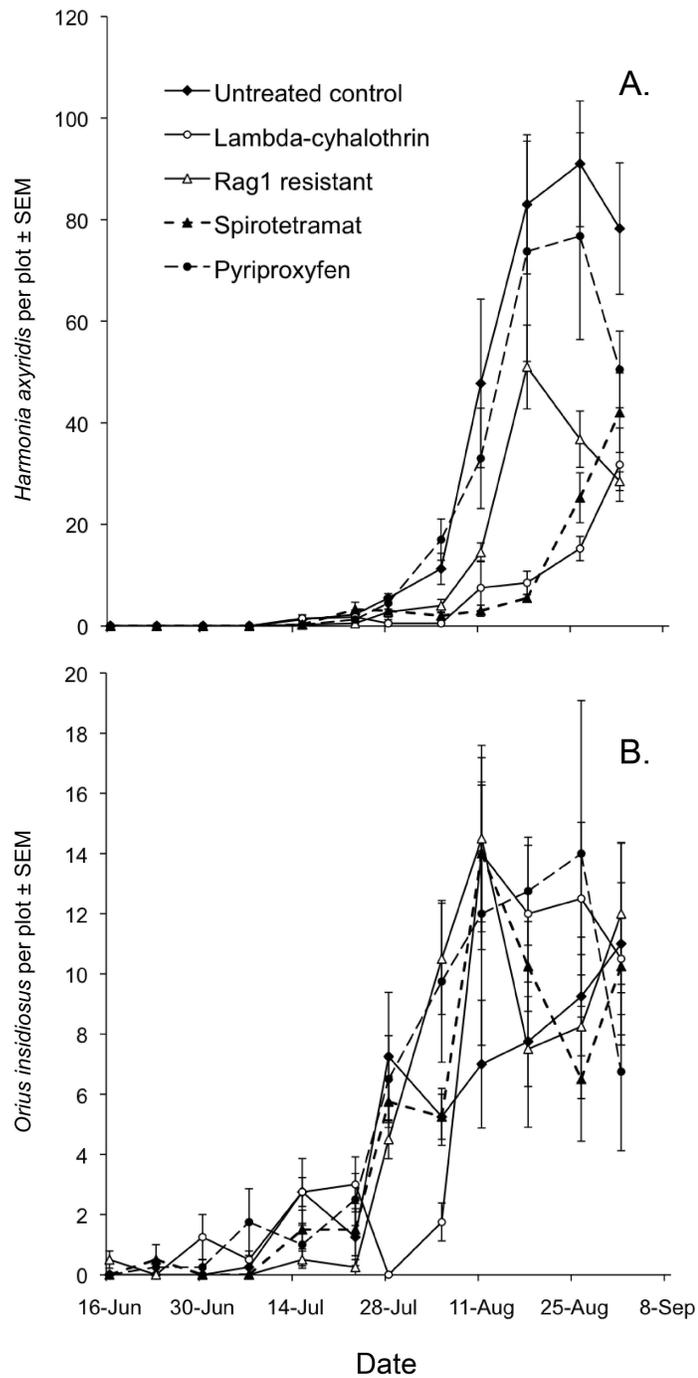


Figure 2-3. Populations of the aphid natural enemies *Harmonia axyridis* (A) and *Orius insidiosus* (B) observed in soybean in 2009 at Rosemount, MN. Numbers are expressed as the total number of natural enemies sampled per plot.

Chapter 3: Interactions of host plant resistance and neonicotinoid seed treatment on soybean aphid and its associated natural enemies

Introduction

The soybean aphid, *Aphis glycines* Matsumura, is a significant economic pest of soybean throughout the North Central United States (Ragsdale et al. 2004). This invasive pest was first discovered in the United States in 2000 and has since become a consistent economic pest species in soybean (Ragsdale et al. 2011, Schmidt et al. 2012). Feeding damage from this pest can affect seed yield, seed quality, and photosynthetic processes of the plant (Beckendorf et al. 2008).

Current management for this pest primarily relies on consistent sampling, use of the economic threshold (ET) established for the soybean aphid (Ragsdale et al. 2007), and reactive control options, primarily in the form of foliar applied insecticides. However, preventative management options have become available in recent years for soybean aphid management, including the development of aphid resistant varieties and insecticide treated soybean seed. Several sources of aphid resistant soybeans have been found for soybean aphid management (Hill et al. 2006, Hesler and Dashiell 2008, Kang et al. 2008, Mian et al. 2008), but soybean containing the *Rag1* gene was the first identified and was the only resistance gene currently commercially available in 2011. The mechanism for *Rag1* aphid resistance is primarily through antibiosis, affecting fecundity and life span of the aphid (Li et al. 2004, Hill et al. 2006). Seed treatments for soybean aphid management utilize neonicotinoid insecticides such as thiamethoxam and imidacloprid (Schulz et al. 2009).

Natural enemies can have a significant impact on soybean aphid regulation (Fox et al. 2004, Desneux et al. 2006, Costamagna et al. 2007). Natural enemies such as the

lady beetles *Harmonia axyridis* and *Coccinella septempunctata* and the insidious flower bug *Orius insidiosus* are present in soybean fields and are significant predators, particularly early in the season, that help regulate soybean aphid populations. The effect these natural enemies have on soybean aphid population dynamics was taken into consideration in the development of the 250 aphids/plant ET. However, effects of management options such as *Rag1* host plant resistance and neonicotinoid seed treatment on the natural enemy community in soybean are not well understood. The interactions that occur between insect pest management options are necessary for developing a successful integrated pest management (IPM) program. Previous studies (Lundgren et al. 2009c, Seagraves and Lundgren 2012) have demonstrated the risks that these management options may pose to soybean aphid biological control, but further studies are needed to confirm these effects, especially under field conditions.

The purpose of this chapter was to 1) evaluate the effect of two relatively new soybean aphid management options, *Rag1* host plant resistance and neonicotinoid seed treatment, against the soybean aphid and 2) evaluate the effect of these two management options on the soybean aphid natural enemy community to determine compatibility of these management options for use in soybean aphid IPM in large field plots where predator and prey populations are not manipulated.

Materials and Methods

Field Studies

Field plots. Field sampling was conducted in experimental soybean plots at the University of Minnesota Outreach, Research and Education (UMORE) Park near Rosemount, MN in Dakota County (N44° 41' 48.6486", W93° 3' 39.1428") during the 2010 and 2011 growing seasons. Soybean plots were planted on 19 May in 2010 and 27 May in 2011 and were maintained under no-till production practices. Plots were planted with either an aphid resistant (LD05-16121) or susceptible (SD01-76R) seed variety depending on the assigned treatment using a 76-cm row spacing and seeding rate of 310,000 seeds per ha. Each experimental plot was 0.10 ha in size and measured 24.4m x 41.1m. A 3.0m and 7.6m fallow border separated all plots in 2010 and 2011 respectively. In both years, commercial soybean fields surrounded the experimental soybean plots.

Experimental design. To evaluate the effects of host plant resistance and seed treatment on soybean aphid and its natural enemies, a 2x2 factorial randomized complete block design was used where the first factor was soybean variety (susceptible or resistant) and second factor was seed treatment (untreated or treated), resulting in four treatment plot combinations. Treatments were replicated four times in blocks, and the susceptible untreated treatment served as a control. Insecticide-treated seed was treated with the neonicotinoid thiamethoxam (Cruiser® 5FS, Syngenta Crop Protection, LLC, Greensboro, NC) at a rate of 0.0756 mg a.i. per seed.

Aphid sampling. To determine treatment effects soybean aphids were sampled weekly during the growing season in 2010 and 2011 utilizing a whole-plant destructive sampling method. Aphid sampling was initiated on 7 June in 2010 and 24 June in 2011 and continued until 31 Aug and 14 Sept in 2010 and 2011 respectively when aphid

densities declined. Sampling intensity per plot depended on the percent infestation rate of individual plants within that treatment. When less than 50% of plants were infested with soybean aphid, 20 plants/plot were sampled. During 50-80% infestation, 10 plants/plot were sampled, and at 80-100% infestation, 5 plants/plot were sampled (Ragsdale et al. 2007). To compare aphid abundance per treatment, average aphid densities (aphids/plant) and cumulative aphid-days (CAD) were calculated per plot. Cumulative aphid days (1 CAD = 1 aphid/plant/day) were used to measure the seasonal exposure of soybean plants to soybean aphid and were calculated using weekly mean aphid densities (Ragsdale et al. 2007, Ohnesorg et al. 2009).

Natural enemy sampling. To determine treatment effects on soybean aphids, natural enemies, plots were sampled weekly to estimate natural enemy populations. Natural enemy abundance was sampled using two methods: direct observation and sweep net sampling. These sampling methods were used to maximize sampling of both mobile and stationary aphid natural enemies (Schmidt et al. 2008). Direct observation of natural enemies was conducted by recording all natural enemies observed on plants within a 1-m length of row in a single plot, and these observations were repeated three times per plot. Sweep net sampling consisted of 20 sweeps per plot in a single row, and rows for sweep net sampling was varied per week to minimize plant damage. Within a single sampling week, direct observations and sweep net sampling were conducted in different rows from one another. Direct observations were initiated on 28 June 2010 and 29 June 2011, and sweep net

sampling was initiated on 6 July for both 2010 and 2011. Natural enemy sampling continued weekly until aphid sampling was terminated.

Identification of natural enemies occurred in field, and the level of identification depended on the natural enemy collected. Ladybeetle (Coleoptera: Coccinellidae) adults were identified to species and larvae to either species level when larvae were large enough or just to family when early instars were collected. The insidious flower bug *Orius insidiosus* (Hemiptera: Anthocoridae) was identified to species, and the damsel bugs (Hemiptera: Nabidae) were identified to genus. All other natural enemies were identified to family.

Yield. In both years, yield was estimated from each plot to determine treatment effects on yield. At the beginning of each season, two rows per plot were designated as yield rows and were not used for aphid or natural enemy sampling to reduce sampling effects on yield. Yield rows were harvested 6 October and 5 October in 2010 and 2011 respectively utilizing a 2-row plot combine. Plot yield samples were weighed and corrected to 13% moisture. All yield are reported as bushels per acre.

Statistical analysis

To determine treatment effects on soybean aphid mean densities and CAD, a two-way repeated measures analysis of variance (ANOVA) was used with factors being variety (*Rag1* resistant variety vs. susceptible) and seed treatment (treated vs. untreated). The statistical models included variety, seed treatment, blocking, sampling date, and all interactions as factors. Sampling date was considered the repeated variable and plot represented the experimental unit (nlme package, R, 2.14.2, R Development Core Team

2012). To meet the assumptions of the model, data were $\log(x+1)$ transformed when necessary. Mean separation tests were conducted using Tukey's HSD test, and a significance level of $\alpha = 0.05$ was used for all tests. Data were analyzed separately for each year.

To determine treatment effects on soybean aphid natural enemies, data were analyzed using mean natural enemy abundance data and predator-prey ratios. Calculating the predator-prey ratios provide a way to directly compare natural enemy abundance relative to the pest species' abundance and more easily monitor effects of management tactics on the predator-prey community in a cropping environment (Van Driesche et al. 2008). Predator-prey ratios per plot were calculated by dividing the mean aphids per plot by the mean natural enemies per plot. While natural enemy eggs were counted, only the predaceous stages of larvae and adult natural enemies were included in the analysis for the overall and individual natural enemy groups. Both response variables were analyzed utilizing two-way repeated measures ANOVA where fixed effects included variety, seed treatment, date, and interaction of these variables with date as the repeated measure. Natural enemy counts from direct observations and sweep net samples were combined to obtain the total natural enemy count per plot used in analysis, and data were analyzed separately for each year. To assess effects on different natural enemy groups, treatment comparisons were made on overall natural enemies as well as for the specific natural enemy groups ladybeetles and *O. insidiosus*.

Yield analysis was conducted using averaged yield estimates for each treatment adjusted to 13% moisture. Yield data were analyzed using a two-way ANOVA (nlme

package, R, 2.14.2, R Development Core Team 2012) and Tukey's HSD for mean separation.

Results

Soybean aphid

Seasonal phenology of soybean aphid was similar in both 2010 and 2011. Aphids were first detected in the experimental plots the weeks of 16 June in 2010 and 24 June in 2011. The highest soybean aphid populations were found in the untreated susceptible control during both years, and aphid abundance in this treatment peaked on 9 Aug in 2010 at 439.5 ± 25.6 aphids per plant and 22 August in 2011 at 437.8 ± 195.4 aphids per plant, coinciding with the R5 reproductive stage of soybean for both years. Aphid population decline was observed in all treatments by 16 Aug in 2010 and 7 Sept in 2011.

Both seed treatment and variety selection significantly affected soybean aphid density during 2010 and 2011 (Figure 3-1 A&B). Aphid suppression was observed in all treatments compared to the untreated/susceptible control. *Rag1* resistance significantly affected aphid abundance in 2010 ($F=24.62$, $df=1,12$, $P=0.0003$) and 2011 ($F=19.25$, $df=1,12$, $P=0.01$). Seed treatment also significantly affected aphid abundance in both 2010 ($F=14.78$, $df=1,12$, $P=0.002$) and 2011 ($F=27.20$, $df=1,12$, $P=0.0004$). Effects of variety and seed treatment on aphid abundance were additive as there was no interaction between the two factors (2010: $F=0.741$, $df=1,12$, $P=0.406$; 2011: $F=1.593$, $df=1,12$, $P=0.231$). Mean separation tests showed that all treatments were significantly different from the untreated/susceptible control during both years, and all treatments were

significantly different from one another except for the *RagI* resistant: untreated vs. seed treated treatments in 2010 and the *RagI* resistant/seed treated vs. susceptible/seed treated treatments in 2011.

Season-long soybean aphid pressure was measured using CAD. We determined if CAD was significantly affected by soybean variety, seed treatment, or a combination of both variety and seed treatment factors in both 2010 and 2011 (Figure 3-1C&D). All plots containing the *RagI* resistant variety or seed treatment accumulated fewer aphid days than the susceptible/untreated control. The highest aphid CAD was observed in the untreated/susceptible control for both years (2010: 6515 ± 354 CAD; 2011: 9572 ± 2104 CAD). Lowest aphid exposure was observed in the *RagI* resistant/seed treated treatment in both years (2010: 2537 ± 228 CAD; 2011: 1339 ± 268 CAD). *RagI* resistance alone (untreated seed) significantly affected aphid abundance in 2010 ($F=58.75$, $df=1,12$, $P<0.0001$) and 2011 ($F=51.27$, $df=1,12$, $P<0.0001$). Seed treatment alone also significantly affected aphid abundance in 2010 ($F=20.78$, $df=1,12$, $P=0.0007$) and 2011 ($F=83.71$, $df=1,12$, $P<0.0001$). There was no significant interaction between variety and seed treatment in either year (2010: $F=1.929$, $df=1,12$, $P=0.406$; 2011: $F=0.008$, $df=1,12$, $P=0.93$). All treatments were significantly different from one another in 2010 (Figure 3-1B). In 2011, all treatments were significantly different from each other except the *RagI* resistant/untreated vs. the susceptible/seed treated treatment.

To determine whether soybean variety or seed treatment affects aphid population growth rates, aphid densities throughout the season were plotted on a \log_{10} -based scale to visualize changes in population growth rates occurring between treatments (Figure 3-2).

Aphid growth rates were calculated for two different time intervals in 2010 (Interval I from 12 July to 9 Aug; Interval II from 9 Aug to 31 Aug) and 2011 (Interval I from 22 July to 22 Aug; Interval II from 30 Aug to 14 Sept). These time intervals coincided with the primary periods of positive aphid growth (Interval I) and negative aphid growth (Interval II) that corresponds roughly with vegetative growth to early reproductive stages (R1-R4) and late reproductive stages (R5). In 2010, treatment lines were almost parallel, indicating a similar growth rate between treatments during peak population increase (before 9 Aug) and population decrease (Figure 3-2A). Statistical analysis indicated no significant differences between growth rates in 2010 for either interval (I: $F=2.92$, $df=3,9$, $P=0.093$; II: $F=2.05$, $df=3,9$, $P=0.18$). In 2011, aphid growth rates were not as similar and parallel as 2010, but analysis showed only a significant treatment difference during positive aphid growth (I: $F=20.20$, $df=3,9$, $P=0.00025$; II: $F=3.86$, $df=3,9$, $P=0.050$). Mean separation tests showed all treatments during this first interval were significantly different from the *Rag1* resistant/untreated treatment.

Yield analysis showed there were no significant effects on yields from either variety or seed treatment in 2010 (variety: $F=0.096$, $df=1,9$, $P=0.764$; seed treatment: $F=2.091$, $df=1,9$, $P=0.182$) or in 2011 (variety: $F=0.111$, $df=1,9$, $P=0.745$; seed treatment: $F=2.423$, $df=1,9$, $P=0.148$). Yield measurement varied from 45.3 ± 2.6 bu/acre (resistant/untreated control) to 48.1 ± 2.9 bu/acre (resistant/seed treated) in 2010 and from 34.3 ± 0.6 bu/acre (resistant/untreated) to 36.2 ± 2.4 bu/acre (resistant/seed treated) in 2011. There were no significant interactions between factors.

Natural enemies

During this study, a diverse community of natural enemies was observed in the experimental soybean plots over both years (Table 3-1). Lady beetles comprised the majority of natural enemies observed in terms of species abundance with six species observed in soybean (*H. axyridis*, *C. septempunctata* L., *Hippodamia convergens* Guérin-Méneville, *Cycloneda munda* (Say), *Hippodamia variegata* (Goeze), and *Coleomegilla maculata* De Geer). The most prevalent lady beetle species was *H. axyridis*. Other major aphid natural enemy groups observed included *O. insidiosus*, *Nabis* spp., hover fly larvae (Family Syrphidae), green lacewing larvae (*Chrysoperla* spp.), brown lacewing larvae (Family Hemerobiidae), and predatory midge larvae (*Aphidoletes aphidimyza* Rondani). Overall natural enemies peaked 29 July 2010 at 31 ± 16 natural enemies/plot and 18 Aug 2011 at 28.25 ± 10 natural enemies/plot.

The effect of *Rag1* resistance and seed treatment on the natural enemy community was not consistent across the sampling years. In 2010, no significant effects from either variety or seed treatment were observed on overall natural enemy abundance (variety: $F=0.496$, $df=1,9$, $P=0.499$; seed treatment: $F=3.236$, $df=1,9$, $P=0.106$). This trend did not continue into 2011. Both variety and seed treatment significantly affected overall natural enemy abundance (variety: $F=6.831$, $df=1,9$, $P=0.0241$; seed treatment: $F=14.49$, $df=1,9$, $P=0.00291$) in 2011, and all treatments were significantly different from the untreated/susceptible control. Within the lady beetle community, lady beetle abundance demonstrated a significant effect to *Rag1* resistance in both years (2010: $F=12.01$, $df=1,9$, $P=0.007$; 2011: $F=14.32$, $df=1,9$, $P=0.003$), but only a significant effect to seed treatment in 2011 (2010: $F=4.66$, $df=1,9$, $P=0.0592$; 2011: $F=12.62$, $df=1,9$, $P=0.0045$).

The piercing-sucking predator *O. insidiosus* was not affected by either variety or seed treatment in 2010 (variety: $F=2.19$, $df=1,9$, $P=0.173$; seed treatment: $F=0.17$, $df=1,9$, $P=0.69$), but their abundance was affected by the seed treatment in 2011 (variety: $F=3.135$, $df=1,9$, $P=0.104$; seed treatment: $F=4.933$, $df=1,9$, $P=0.0483$). There was no interaction between variety and seed treatment in any of the natural enemy groups analyzed.

Analysis of the predator-prey ratios revealed no effect from using the management options of *Rag1* resistance or thiamethoxam seed treatment in soybean aphid management. Predator-prey ratios using overall natural enemy counts were consistently not affected by either *Rag1* resistance (2010: $F=0.689$, $df=1,9$, $P=0.428$; 2011: $F=3.572$, $df=1,9$, $P=0.0854$) or seed treatment (2010: $F=0.242$, $df=1,9$, $P=0.428$; 2011: $F=3.39$, $df=1,9$, $P=0.0927$). Similar results were found when predator-prey ratios were calculate using only lady beetle or *O. insidiosus* abundance. There was also no evidence of any interactions between variety and seed treatment for either sampling year.

Discussion

These results confirm that *Rag1* resistant soybean does reduce soybean aphid densities. This pest suppression was consistent across both sampling years and is easily observed when comparing mean aphid densities between treatments on individual sampling date from mid-July to mid-August (Figure 3-1). This suppression is also apparent when comparing season-long aphid exposure in CAD (Figure 3-1 C & D). These results are consistent with previous studies demonstrating the suppressive effect of

Rag1 host plant resistance on soybean aphid (Hill et al. 2006, Chacón et al. 2012, Hesler et al. 2012, Wiarda et al. 2012). In addition to significantly reducing soybean aphid populations, the *Rag1* resistant treatments also temporally delayed colonization by this pest by at least a week. This delayed colonization occurred in both 2010 and 2011 and is particularly apparent in 2010 (Figure 3-2A) where from early July until aphid decline in mid-August, *Rag1* resistance treatments cause a one-week delay for aphid densities to reach populations in susceptible plots. The 2011 aphid populations experienced an even longer time delay for aphid populations to increase above 50 aphids/plant (Figure 3-2b). Delaying colonization by soybean aphid has several management benefits. First, this delay can temporally push back the need for insecticide applications, providing time for biotic factors such as natural enemies or abiotic factors (rainfall, temperature, etc.) to affect aphid populations. Secondly, this time delay allows soybeans to further mature, possibly to later reproductive stages that may be less affected by aphid feeding or when aphid decline begins to occur from decreasing photoperiod that triggers the production of migrant sexual aphids (McCornack et al. 2004, McCornack et al. 2005). Finally, this temporal delay may also allow for changes in environmental conditions such as cooler temperatures and high humidity that favor development of entomopathogenic fungi and increased fungal disease in soybean aphid (Koch 2011).

These results demonstrate that thiamethoxam seed treatments can significantly reduce soybean aphid densities. This suppression of aphid densities from seed treatment was observed in both years throughout each season, however, this season-long suppression of aphids due to seed treatment was unexpected. Based on previous studies

(McCornack and Ragsdale 2006, McCornack 2007, Magalhaes et al. 2009), neonicotinoid-treated soybean seed loses its bioactivity against aphids between 23-49 days. In this study bioactivity from the seed treatment should have lasted until July 12, 2010 and July 15, 2011 (49 days post-emergence). Aphid populations coinciding with these dates were extremely low and never exceeded an average of 10 aphids/plant for any treatment (Figure 3-1), yet on these dates, treatments containing the seed treatment continued to see reduced aphid abundance and lower CAD. Numerous other studies have demonstrated similar results where seed treatment successfully suppresses soybean pest populations during the growing season but does not result in a significant yield gain (Reisig et al. 2012, Seagraves and Lundgren 2012, Tinsley et al. 2012). However, these results are not consistent since Magalhaes et al. (2009) observed a yield gain in soybean from seed treatment when aphid populations exceeded the ET. This study and other previous studies indicate inconsistent benefits to utilizing thiamethoxam seed treatments for soybean aphid management. Although there may be additional benefits to using a neonicotinoid seed treatment that includes a fungicide (Bradshaw et al. 2008, Esker and Conley 2012) or for increased drought tolerance (Cataneo et al. 2010), the lack of a consistent yield gain observed from using seed treatments indicates that adopting this practice may not be justified as an added economic benefit to soybean producers for soybean aphid control.

The use of *Rag1* resistance and seed treatment along with their combined treatment all succeeded in reducing aphid populations, and aphid densities were consistently maintained below the economic threshold of 250 aphids/plant in nearly all

treatments (except one date in 2010). However, it is worth noting that aphid populations never reached the economic injury level (EIL) of 674 aphids per plant (Ragsdale et al. 2007) during the two years of this study in any treatment including the susceptible control without seed treatment. Failure to reach the EIL should result in no measureable yield loss. Therefore, this study confirms that under low aphid populations no treatment whether applied as a foliar spray (reactive IPM) or as a preventative IPM program (seed treatment or aphid-resistant variety) was needed to prevent loss due to soybean aphid alone in 2010 and 2011 in Rosemount, MN.

Soybean aphid natural enemy populations were largely unaffected by the use of either *Rag1* resistance or neonicotinoid seed treatments. This lack of effect was shown in the overall natural enemy community as well as in ladybeetle populations where previous work has shown that these aphid specialists are indeed an important top-down control factor (Costamagna et al. 2007, Fox et al. 2004). The insidious flower bug *O. insidiosus* was also an important aphid predator of soybean aphid in this study confirming work by Desneux et al. (2006) and Rutledge et al. (2005). Lack of treatment differences in both natural enemy density (Figure 3-3) and predator-prey ratios during both sampling years confirm this conclusion and indicate there is little evidence on a field level of negative impacts from either management tactic on the aphid natural enemy community. Similar conclusions were made by Tinsley et al. (2012) in their study, however, they did not combine the two management tactics into a single treatment as was done in this study. Lundgren et al. (2009c) demonstrated potential direct effects from *Rag1* resistance on *H. axyridis* and *O. insidiosus* in laboratory studies, but here on a field scale, these effects do

not appear to affect aphid biological control. Koch and Ragsdale (2011) concluded that neither *Rag1* resistance nor seed treatments had any effect on another natural enemy group, the entomopathogenic fungus *Pandora neoaphidis*.

Altogether, this study demonstrates that preventative IPM management tactics do not adversely affect natural enemies of soybean aphid. However, our two-year study showed no significant benefit to these preventative tactics when aphid abundance was generally low. Here we conclude that both *Rag1* resistant soybean and a thiamethoxam seed treatment are compatible with natural enemies and cause no measureable differences in species diversity, abundance or predator prey ratios and thus are compatible within the context of an overall IPM program that combines preventative and reactive control tactics.

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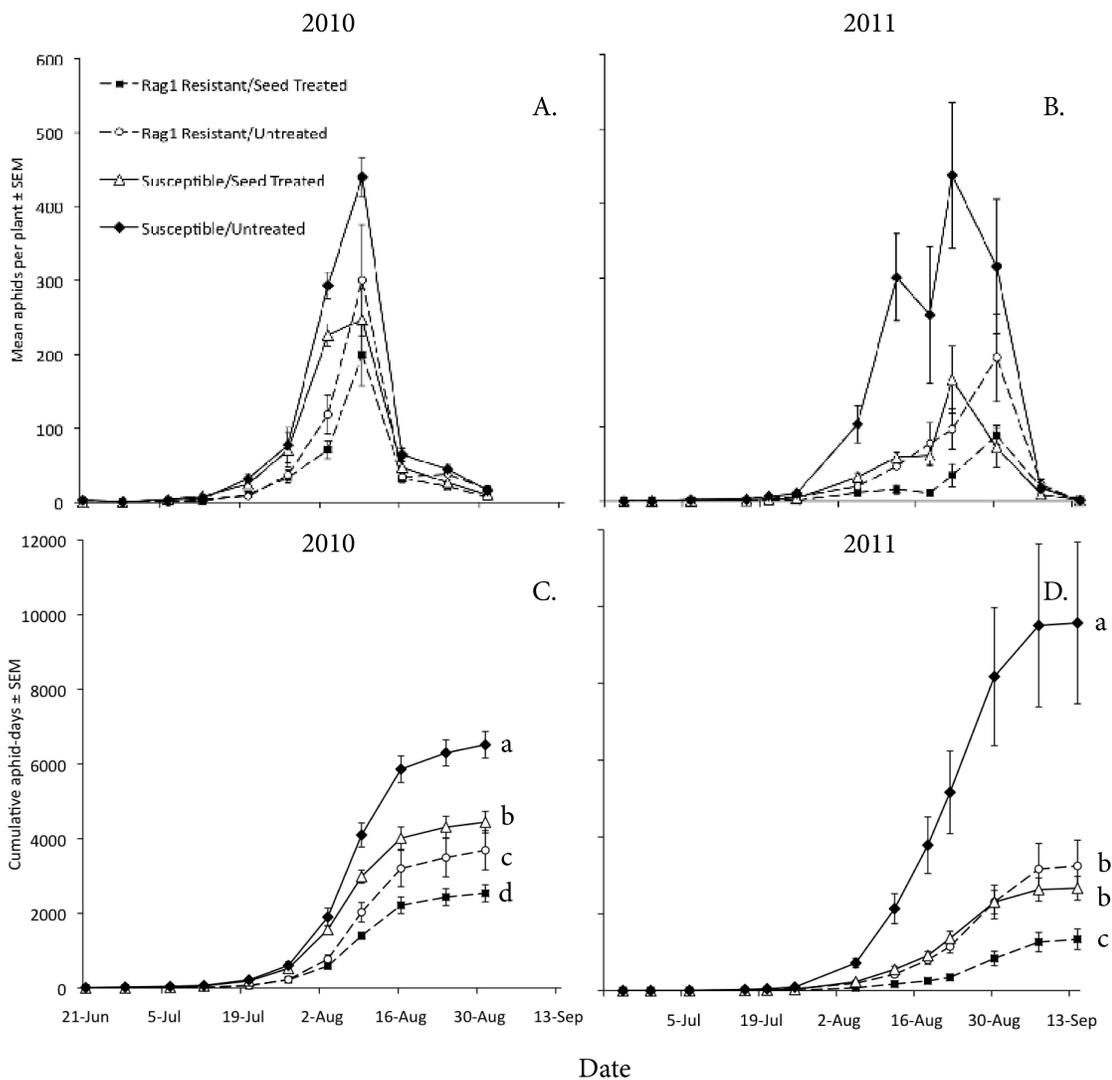


Figure 3-1. Seasonal abundance of soybean aphid populations expressed as average aphid density per plant (Panels A and B) and cumulative aphid days (CAD) (Panels C and D) in soybean collected in 2010 and 2011. Error bars indicate standard error of the mean.

Differing letters in Panels C and D indicate significant differences between treatments (Tukey's HSD test, $\alpha = 0.05$).

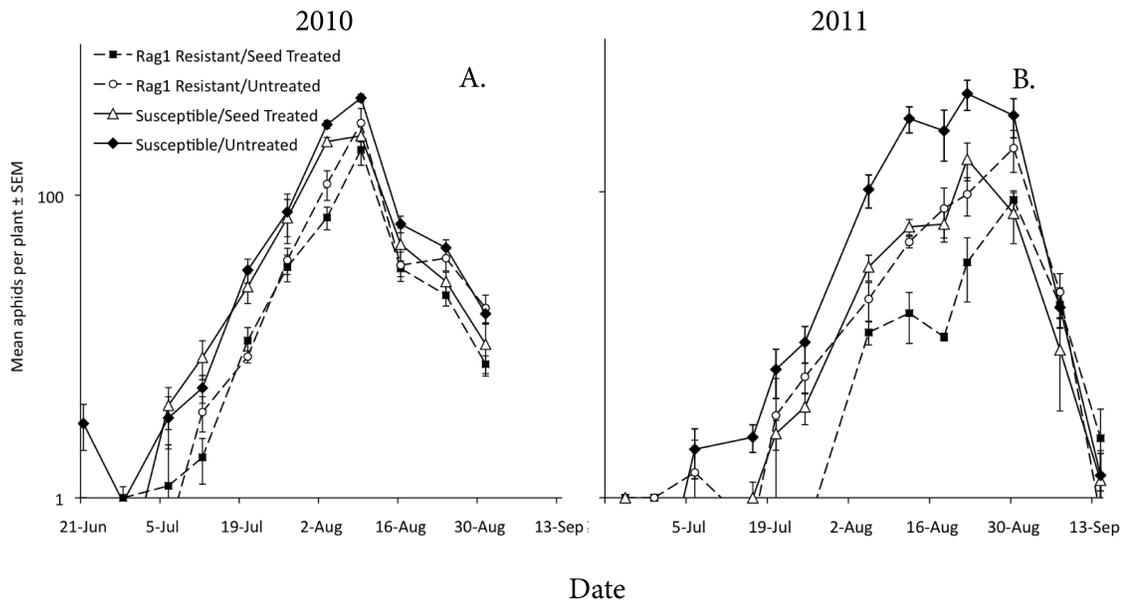


Figure 3-2. Average soybean aphid densities on a log₁₀-based scale for 2010 (A) and 2011 (B) to demonstrate aphid population growth between treatments during the growing season. Error bars indicate standard error of the mean.

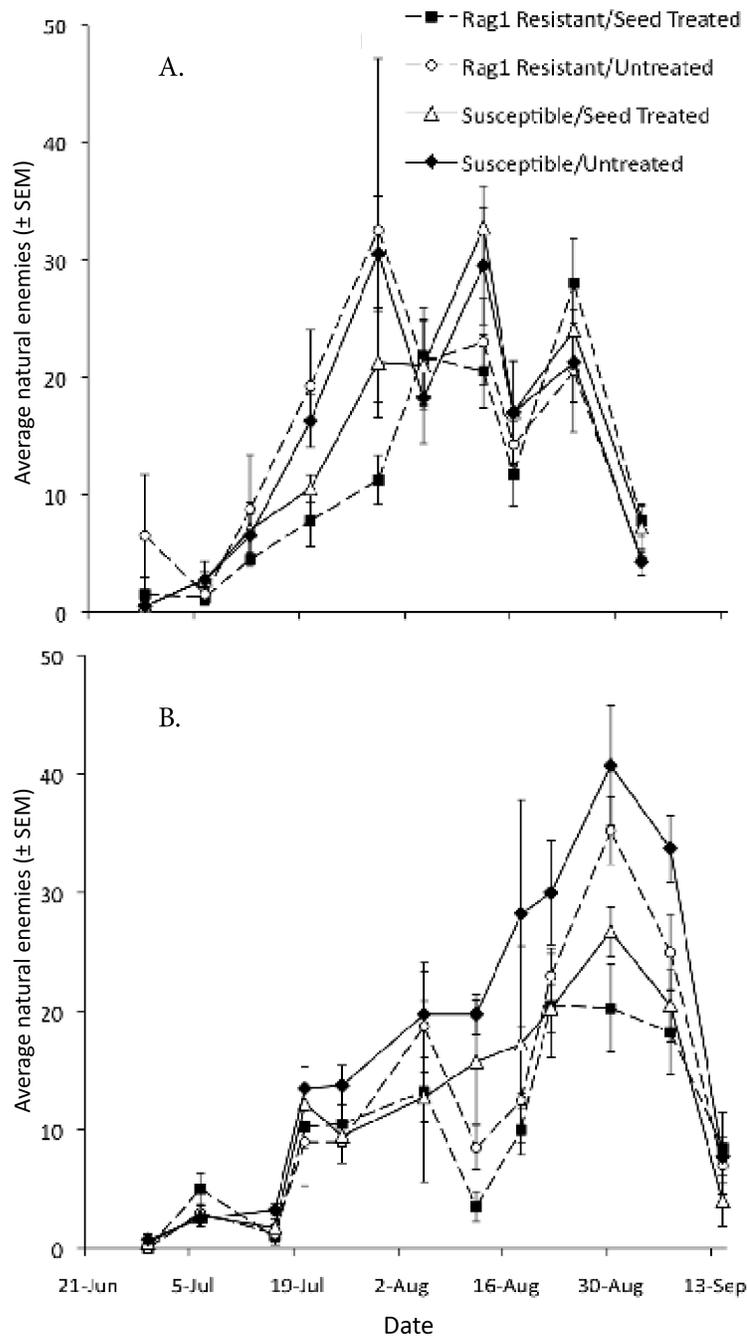


Figure 3-3. Seasonal changes in the natural enemy community of soybean in 2010 (A) and 2011 (B). Error bars indicate standard error of the mean.

Table 3-1. Natural enemy community composition in soybean fields in Rosemount, MN during 2010-11.

| Order | Family | Species |
|--------------|---------------|----------------------------------|
| Coleoptera | Coccinellidae | <i>Harmonia axyridis</i> |
| | | <i>Coccinella septempunctata</i> |
| | | <i>Hippodamia convergens</i> |
| | | <i>Cycloneda munda</i> |
| | | <i>Hippodamia variegata</i> |
| | | <i>Coleomegilla maculata</i> |
| | | Unidentified ^a |
| Hemiptera | Anthocoridae | <i>Orius insidiosus</i> |
| | Nabidae | <i>Nabis</i> spp. |
| Neuroptera | Chrysopidae | <i>Chrysoperla</i> spp. |
| | Hemerobiidae | |
| Diptera | Cecidomyiidae | <i>Aphidoletes aphidimyza</i> |
| | Syrphidae | |
| Hymenoptera | Aphelinidae | |
| | Braconidae | |

^a Early instar lady beetle larvae were not identified to species.

Table 3-2. Seasonal averages (\pm SEM) of natural enemies per plot across *Rag1* resistant and neonicotinoid seed-treated treatment combinations in 2010 and 2011. Treatment means within a row followed by the same letter are not significantly different (Tukey's HSD test, $\alpha = 0.05$).

| | Treatment | | | |
|-------------------------|---------------------------|------------------------------|-------------------------------------|--|
| | Susceptible/ Untreated | Susceptible/ Seed Treated | <i>Rag1</i> Resistant/ Untreated | <i>Rag1</i> Resistant/ Seed Treated |
| 2010 | | | | |
| Total Natural Enemies | 14.7 \pm 2.86 a | 14.4 \pm 2.66 a | 15.2 \pm 4.52 a | 11.6 \pm 2.20 a |
| Lady Beetles | 5.1 \pm 1.36 a | 4.5 \pm 1.15 ab | 3.2 \pm 1.11 bc | 2.4 \pm 0.88 c |
| <i>Orius insidiosus</i> | 3.00 \pm 1.04 a | 3.83 \pm 1.16 a | 3.15 \pm 1.14 a | 3.65 \pm 1.00 a |
| 2011 | | | | |
| Total Natural Enemies | 17.8 \pm 2.76 a | 12.0 \pm 2.44 b | 12.7 \pm 2.14 b | 10.1 \pm 2.59 b |
| Lady Beetles | 4.7 \pm 0.83 a | 2.1 \pm 0.63 b | 2.4 \pm 0.69 b | 1.2 \pm 0.60 b |
| <i>Orius insidiosus</i> | 8.0 \pm 1.43 a | 5.9 \pm 1.24 b | 6.4 \pm 1.42 ab | 5.6 \pm 1.33 b |

**Chapter 4: Survival analysis of soybean aphid natural enemies on neonicotinoid
seed treated and host plant resistant soybean**

Introduction

The soybean aphid, *Aphis glycines*, is a major economic pest of Midwest soybean, and since its discovery in the United States, significant strides have been made in developing management options for this pest (Ragsdale et al. 2004, Ragsdale et al. 2011, Hodgson et al. 2012). The primary method of controlling soybean aphid outbreaks is through the use of foliar insecticides. However, to improve the efficiency, effectiveness, and economic feasibility of these applications, an integrated pest management (IPM) program has been developed that incorporates economic thresholds (ET) and economic injury levels (EIL) into insecticide treatment recommendations (Rutledge and O'Neil 2005, Ragsdale et al. 2007, Hodgson et al. 2012), improvements in sampling and scouting methods (Hodgson et al. 2007), and the impacts of natural enemies (Ragsdale et al. 2007).

Natural enemies of the soybean aphid have been shown to significantly impact soybean aphid populations and can provide important population regulation (Fox et al. 2004, Fox et al. 2005, Costamagna et al. 2007, Desneux and O'Neil 2008, Koch 2011). Important natural enemies include the ladybeetles, particularly the multicolored Asian ladybird beetle, *Harmonia axyridis* (Pallas) (Koch 2003, Rutledge et al. 2004, Costamagna et al. 2007) and piercing-sucking true bugs such as *Orius insidiosus* (Say) (Rutledge and O'Neil 2005, Desneux et al. 2006). Because these natural enemies provide additional aphid suppression, it is important to conserve these natural enemies in soybean aphid management and understand how natural enemies may interact with other management options utilized in an IPM program.

Two additional management options, aphid resistant soybean varieties and neonicotinoid seed treatments, are more recent tools made available for soybean aphid management. Both management options are preventative, designed to prevent or reduce aphid outbreaks compared to an untreated, unmanaged field. Unlike reactive foliar insecticide applications, these management options must be incorporated into the management plan at planting time to be effective. Several genes conferring soybean aphid resistance have been identified from conventional plant breeding (Hill et al. 2006, Kang et al. 2008, Wiarda et al. 2012), however the only commercially available soybean aphid resistant varieties at this time contain the *Rag1* resistance gene. Numerous studies have demonstrated the effectiveness of *Rag1* varieties in suppressing soybean aphid populations (Kim and Diers 2009, Hesler et al. 2012, Wiarda et al. 2012). In seed treatment for soybean aphid control, neonicotinoid insecticides are utilized. These insecticides are taken up systemically and translocated in the plant via the xylem, making them extremely effective against piercing-sucking insects (Tomizawa and Casida 2005), but despite the potential for success, efficacy against the soybean aphid has shown mixed results with some studies reporting significant soybean aphid suppression in some years and locations (Magalhaes et al. 2009, Schulz et al. 2009) and others reporting no yield benefit to utilizing seed treatment for soybean aphid control (Seagraves and Lundgren 2012, Tinsley et al. 2012).

The effects of host plant resistance and seed treatment can extend beyond the target pest. As mentioned previously, biological control via natural enemies can provide significant soybean aphid regulation, and therefore, it is important to understand the

impacts these management tactics can have on the soybean aphid's natural enemies. Several studies have been conducted to investigate the effects of either host plant resistance (Lundgren et al. 2009c, Chacón et al. 2012) or neonicotinoid seed treatment (Seagraves and Lundgren 2012) on soybean aphid natural enemies, but only two known studies (Koch and Ragsdale 2011, Tinsley et al. 2012) investigate the interactions of both insecticide seed-treatment and host plant resistance on soybean aphid natural enemies. In general, more studies are needed to fully understand the effects of these management options on soybean aphid natural enemies.

In this chapter, the objective was to further investigate the potential effects of seed treatment and aphid host plant resistance on soybean aphid natural enemies. This chapter is complementary to Chapter 3 where we investigated field-level effects of these management options on aphid and natural enemy abundance. Here we elucidate whether exposure of natural enemies to these management options could affect particular life history traits, and we investigated the effects of this exposure on longevity and survival rates of three different natural enemies in the laboratory. *Harmonia axyridis*, *O. insidiosus*, and *Nabis americanoferus* were the three natural enemy species included in this study. These species were chosen to represent two major soybean aphid predators (*H. axyridis* and *O. insidiosus*) and two piercing-sucking predators (*O. insidiosus* and *N. americanoferus*) that could potentially feed directly on plant tissue and therefore increase their risk of non-target impacts from a systemic seed treatment. By assessing differences in longevity and survival rates, we could determine if exposure alone to these management options is causing any negative non-target impacts on the natural enemy

community. The broader implications of these studies would allow us to further elucidate whether biological control is indeed compatible with the use of seed treatments and host plant resistance in soybean aphid management.

Materials and Methods

Colony maintenance

All natural enemies used in this study were maintained under controlled conditions, and natural enemy colonies were initiated from field-collected adults from either soybean or alfalfa fields in Rosemount, MN. The *O. insidiosus* colony was initiated in May 2010, and the colony was reared in growth chambers at 23°C and 16:8 L:D conditions. The colony was maintained in large petri dishes (14-cm diameter) half filled with plastic craft beads to reduce the incidence of cannibalism. *Orius insidiosus* were fed frozen *Ephestia kuehniella* eggs and provided green beans as an oviposition substrate. The *H. axyridis* colony was initiated summer 2010, and the colony was maintained in greenhouse cages with soybean plants and fed soybean aphids ad libitum. To obtain individuals for experimental assays, egg masses were isolated from the greenhouse colony and maintained in 9-mm petri dishes in growth chambers at 23°C and 16:8 L:D until eclosion. Upon eclosion, larvae were isolated and maintained on a diet of frozen *E. kuehniella* eggs until needed for studies. The *N. americanoferus* colony was initiated in June 2011 and reared in growth chambers at 23°C and 16:8 L:D conditions. The colony was maintained in large petri dishes (14-cm diameter) and fed frozen *Heliothis virescens* eggs. Green beans were provided as an

oviposition substrate. For all natural enemies, water was provided ad libitum via moistened floral foam cubes.

Soybean aphids used for feeding natural enemies were reared under controlled conditions (25°C and 16:8 L:D). The soybean aphid colony was established from field-collected apterous adults from Rosemount, MN in 2009 and subsequently maintained in growth chambers in the Plant Growth Facilities on the University of Minnesota St. Paul campus. The colony was supplemented with field-collected aphids in 2010. Aphids were reared on soybean plant variety S19R5.

Experimental assay and data collection

To determine the effects of the neonicotinoid thiamethoxam as a seed treatment and *Rag1* host plant resistance on natural enemy survival, natural enemies were exposed to field-collected soybean leaves from one of four treatments that included the two different factors (insecticide seed treatment and host plant resistance). This study was conducted mid-season to capture any survival effects of the seed-treatment and host plant resistant management options when soybean aphid pressure historically begins to significantly increase in the field (Ragsdale et al. 2007).

All experiments performed in this study were conducted in growth chambers at 23°C and 16:8 L:D conditions on the U of M St. Paul campus. Natural enemies were maintained individually in 9-cm Petri dishes and provided water and laboratory-reared soybean aphids as food. Individuals were checked daily until death or termination of the study. Protocols for each natural enemy studied varied slightly. The *O. insidiosus* survival study was initiated on 16 July 2010. *O. insidiosus* adults, 1-2 days old, were used for the study and fed 20 soybean aphids every other day (Butler and O'Neil 2007). A total

of 160 *O. insidiosus* adults were used (40 in each treatment, 20 per leaf age per treatment). The *O. insidiosus* survival study was terminated after three weeks. The *H. axyridis* and *N. americanoferus* survival studies were initiated on 26 July 2011. Eighty newly emerged third-instar *H. axyridis* larvae were used for this study (20 per treatment), and individuals were monitored until adults were 28 days old. *H. axyridis* individuals were fed 40 soybean aphids per day until the 4th larval stage and 100 aphids per day afterwards. Eighty-eight fourth instar *N. americanoferus* nymphs were used for this study (22 per treatment), and individuals were monitored until adults were 42 days old. *Nabis americanoferus* individuals were fed 20 soybean aphids every day, and *H. axyridis* and *N. americanoferus* individuals were maintained on healthy soybean trifoliate from the 5th-8th nodes from the base of the plant. The sex of *H. axyridis* and *N. americanoferus* adults was recorded to determine whether sex could affect survival rate.

In addition to the seed treatment and host plant resistance factors, leaf age was an additional factor in the *O. insidiosus* survival analysis. Adults were reared on either a young trifoliolate (from 9th or higher node from base of plant) or old trifoliolate (3rd-4th node from base of the plant). Trifoliate leaves collected were designated as either young or old leaves to identify which part of the plant it was collected from and to determine whether exposure to a particular leaf age could influence effects of the seed treatment and resistance factors.

Soybean trifoliolate leaves used in the study were collected from the same soybean plots used in Chapter 3. These plots were located at the Minnesota Agricultural Experiment Station (MAES) in Rosemount, MN. Treatment combinations were the same

as those used in Chapter 3: susceptible/untreated, susceptible/seed treated, *Rag1* resistant/untreated, and *Rag1*/seed treated. The soybean plots were planted on 19 May in 2010 and 27 May in 2011 and were planted with either an aphid resistant (LD05-16121) or susceptible (SD01-76R) seed variety. Excised soybean leaves used in the studies were inserted into wet floral foam to provide moisture and were replaced every three days with a new field-collected trifoliolate.

Statistical analysis

To test whether seed treatment or *Rag1* host plant resistance affected survival time of soybean aphid natural enemies, the Kaplan-Meier estimates of the survival function were used to create survivor curves (Esker et al. 2006, Benitez-Parejo et al. 2011). The Kaplan-Meier method is a non-parametric procedure that estimates the survival function, and the survival function measures the probability that an individual survives up to a particular time point (Guo 2010). Survival times were calculated as the number of days from the experimental start date to death. Individuals that escaped during the study period or remained alive at the termination of the study constituted the censored events included in the analysis.

To assess the influence of seed treatment and *Rag1* host plant resistance on natural enemy survival, Cox proportional hazards modeling was used. For *O. insidiosus*, the effect of leaf age on adult survivorship was assessed as an additional covariate, and for the damsel bugs and *H. axyridis*, sex was included as an additional covariate. The Cox proportional hazards model investigates the influence of covariates on the hazard function where the hazard function is the instantaneous probability of death of an

individual (Guo 2010). Testing for significant interactions between variables was conducted in all survival analyses, but only significant interactions were addressed. All statistical analyses were conducted using the *survival* package in R (2.14.2, The R Foundation, 2012) (Crawley 2007, Guo 2010).

Results

Survival analysis

Harmonia axyridis survival analysis. Results of the *H. axyridis* survival study indicated that neither seed treatment ($\chi^2 = 0.46$, $df = 1$, $p=0.65$) nor host plant resistance ($\chi^2 = 0.49$, $df = 1$, $p=0.96$) significantly affect survival times of *H. axyridis* individuals (Figure 4-1A). When sex of the individuals was included as an additional covariate in the Cox proportional hazards model, the model was not significantly improved by the addition of any factor ($\chi^2 = 1.68$, $df = 3$, $p=0.64$) (Figure 4-1B), indicating that none of these covariates significantly affected the survival times of *H. axyridis*.

Of the 80 *H. axyridis* individuals included at the initiation of the study, a total of 77 individuals were included in the analysis, however, 61 (79%) of these individuals were censored in the analysis (escaped or were alive at termination of study) and only 16 individuals (21%) died during the duration of the study. This resulted in low mortality rates per treatment (21-29%) during the study duration (Table 4-1).

Development time of *H. axyridis* to different life history stages was also considered and summarized. The length of time for the larvae to reach adulthood did not vary much between treatments (20.6 ± 0.5 days in susceptible/untreated to 21.8 ± 0.2

days in resistant/ untreated), but adult longevity differed ranging from 9.7 days (resistant/seed-treated) to 34.3 days (resistant/untreated) (Table 4-1). Sex ratios between treatments differed from $27.8 \pm 11.4\%$ females (resistant/untreated) to $83.3 \pm 7.9\%$ females (susceptible/untreated) (Figure 4-5A).

Orius insidiosus survival analysis. Results of the *O. insidiosus* survival study showed that seed treatment ($\chi^2 = 0.60$, $df = 1$, $p=0.55$) and host plant resistance ($\chi^2 = 0.61$, $df = 1$, $p=0.54$) did not significantly affect survival times of *O. insidiosus* adults (Figure 4-2A). Adding leaf age (older or younger leaves of the plant) into the model did not significantly improve the model ($\chi^2 = 0.83$, $df = 3$, $p=0.84$) (Figure 4-2B, Figure 4-4), even though generally, survival times were longer on older leaves. On old leaves, survival times ranged from 9.0 ± 1.3 days (resistant/untreated) to 11.8 ± 1.4 days (susceptible/untreated). On younger leaves, survival times ranged from 3.7 ± 0.9 days (susceptible/untreated) to 9.1 ± 0.9 days (resistant/seed treated) (Table 4-2).

Of the initial 160 *O. insidiosus* individuals included at the start of the study, 159 individuals were included in the analysis. Of these individuals, 147 individuals (92%) died during the duration of the study, and only 12 were censored individuals. This resulted in high mortality rates per treatment (92-97%) during the study duration (Table 4-1). Since only adult *O. insidiosus* adults were included in the survival analysis, only effects on the adult stage were assessed. Mean adult longevity of *O. insidiosus* differed among treatments ranging from 9.6 ± 0.9 days (susceptible/untreated) to 11.0 ± 0.8 days (susceptible/seed-treated) (Table 4-1).

Nabis americoferus survival analysis. Results of the *N. americoferus* survival study also showed that seed treatment ($\chi^2 = 0.85$, $df = 1$, $p=0.39$) and host plant resistance ($\chi^2 = 0.65$, $df = 1$, $p=0.52$) did not significantly affect survival times of this natural enemy (Figure 4-3A). Once again, including these factors and sex of individuals did not significantly improve the model ($\chi^2 = 5.97$, $df = 3$, $p=0.11$) (Figure 4-3B, Figure 4-5B). Overall, there was a significant difference between the death probabilities between the sexes ($\chi^2 = 2.43$, $df = 1$, $p=0.02$), but there was no significant interaction of sex with either seed treatment or host plant resistance.

Of the 88 *N. americoferus* individuals included at the start of the study, all were included in the analysis, and 75 (86%) of these individuals died during the duration of the study. A total of thirteen individuals (14%) were censored. All treatment experienced high mortality rates (90-100%) during the duration of the study (Table 4-1). Development time to different life history stages was also considered and summarized. The length of time for nymphs to reach adulthood did not vary much between treatments (9.6 ± 0.5 days in resistant/seed-treated to 12.4 ± 0.7 days in resistant/untreated). Mean adult longevity differed ranging from 23.0 days (resistant/untreated) to 29.6 days (resistant/seed-treated) (Table 4-1). Sex ratios between treatments ranged from $46.7 \pm 11.4\%$ females (susceptible/seed treated) to $64.3 \pm 11.3\%$ females (susceptible/untreated) (Figure 4-5B).

Discussion

In this study we attempted to determine the effects of both host plant resistance and seed treatment on survival rates and longevity of three different species of soybean aphid natural enemies. Results demonstrated that both a thiamethoxam seed treatment and *Rag1* host plant resistance did not affect the longevity or survival rates for the natural enemies included in this study. The lack of any survival effect from the seed treatment is supported by previous studies where it was demonstrated that thiamethoxam seed treatment typically loses its bioactivity against the soybean aphid between 23-49 days post-planting (McCornack and Ragsdale 2006). The initiation of these survival studies occurred at 58 and 60 days post-planting in 2010 and 2011 respectively. These dates fall outside the expected bioactive period of the neonicotinoid seed treatments. Therefore the lack of negative effects from seed treatment was expected since the bioactivity should have dissipated by the time this study was initiated, although some sublethal effects that might adversely affect longevity of adult natural enemies or impact the developmental time of larvae to adult could have been possible.

These results are contradictory to previous studies that investigated the effects of either seed treatment or host plant resistance on soybean aphid predators. In laboratory and field studies on the effects of neonicotinoid seed treatments, Seagraves and Lundgren (2012) observed decreased abundance of *O. insidiosus* and *N. americoferus* in the field and increased mortality of *O. insidiosus* in the laboratory. This increased mortality during the laboratory studies was not unexpected, however, because unlike our study, their *O. insidiosus* individuals were exposed to young soybean leaves (V2 stage) that still expressed bioactivity against the soybean aphid. Lundgren et al. (2009c) investigated the

effects of direct contact with resistant soybean and concluded that some life history traits of important natural enemies like *H. axyridis* may be affected by resistance traits, but no effects were observed in *O. insidiosus*. While direct feeding of *H. axyridis* on soybean has not been recorded to date, this natural enemy does directly feed on corn. Moser and Obrycki (2009) demonstrated a significant mortality effect of *H. axyridis* larvae feeding directly on neonicotinoid seed-treated corn seedlings, but again, this study was conducted on a much younger plant stage where the neonicotinoid seed treatment was still biologically effective on the pest species.

One limitation of this study was the small sample size for some of the natural enemies. For survival analysis, a larger sample size per treatment of at least 30 is ideal. However, due to the necessity of having same-age individuals available on the start date, providing this number of individuals and maintaining individuals for the duration of the study was not always possible. Therefore, analysis was conducted despite not always reaching the ideal sample size of 30 per treatment, but consideration was made for this limitation by choosing analyses that can accommodate small sample sizes.

The studies were conducted in laboratory utilizing field-collected leaves to assess the effects of field conditions on soybean aphid natural enemies. Preliminary studies were attempted where natural enemies were caged onto field plants, but due to the long duration of the study, high numbers of caged natural enemies either escaped or experienced high mortality rates due to extreme weather (rain, heat, etc.). Therefore, to obtain consistent results, the studies were conducted in laboratory using field-collected soybean leaves to reduce the escape and mortality rate of the natural enemies.

In summary, this study demonstrated no survival effects from either neonicotinoid seed treatment or *Rag1* host plant resistance on soybean aphid natural enemies. Previous studies (including Chapter 3) investigated the field effects of *Rag1* host plant resistance and seed treatment on soybean aphid natural enemy populations, and similar conclusions were made regarding the lack of effect these factors have on the natural enemy community within soybean. Effects on predator density in the field from both of these factors were investigated by Tinsley et al. (2012), and they came to a similar conclusion that throughout the season, natural enemies are relatively unaffected by either management option. Collectively, these results provide further evidence that seed treatment and host plant resistance are compatible with soybean aphid biological control in soybean aphid management. However, this study makes no attempt to elucidate the effectiveness of these management options on the pest species directly. While this study provides evidence of compatibility between seed treatment, host plant resistance, and biological control, efficacy on the soybean aphid must be taken into consideration before utilizing new management options in an IPM program.

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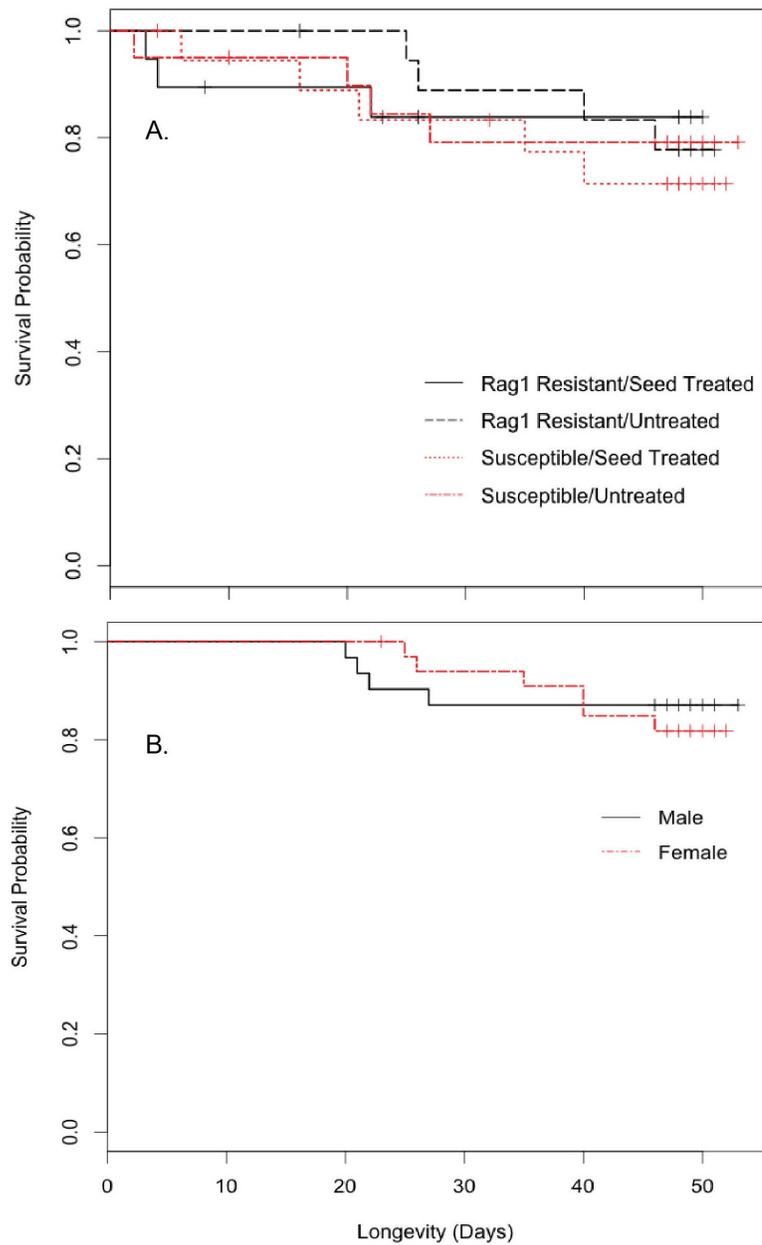


Figure 4-1. Kaplan-Meier survival curves of the aphid predator *Harmonia axyridis* based on seed treatment and *Rag1* host plant resistance treatments (A) and sex across all treatments (B). All individuals were reared under laboratory conditions on field-collected leaves from Rosemount, MN and fed soybean aphid.

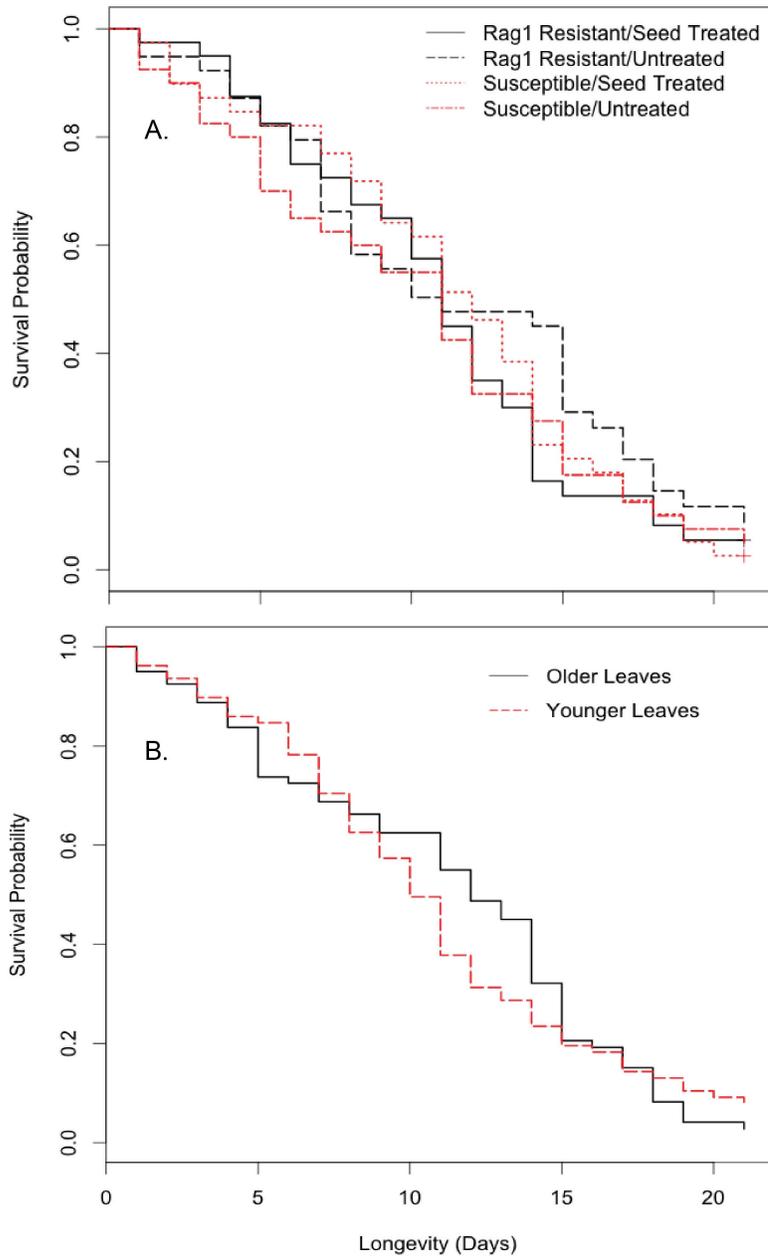


Figure 4-2. Kaplan-Meier survival curves of the aphid predator *Orius insidiosus* based on seed treatment and *Rag1* host plant resistance treatments (A) and leaf age across all treatments (B). All individuals were reared under laboratory conditions on field-collected leaves from Rosemount, MN and fed soybean aphid.

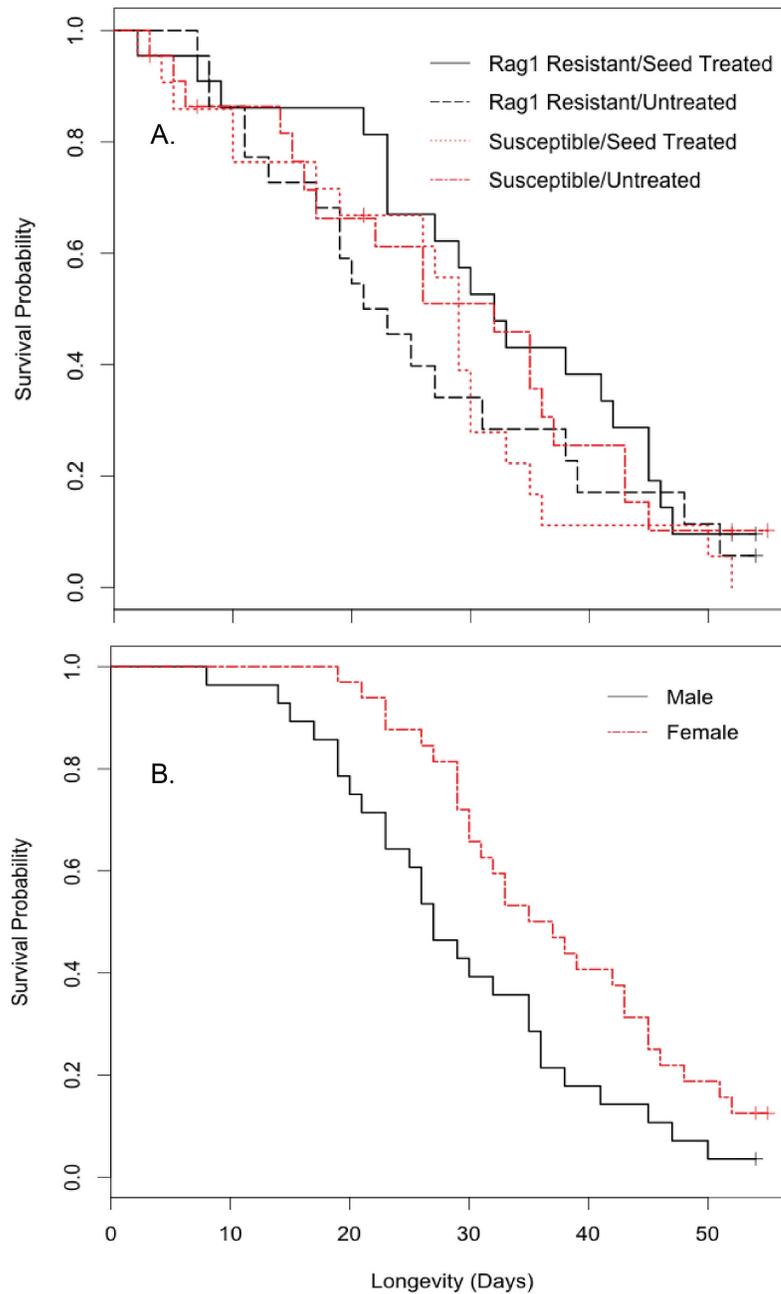


Figure 4-3. Kaplan-Meier survival curves of the aphid predator *Nabis americanoferus* based on seed treatment and *Rag1* host plant resistance treatments (A) and leaf age across all treatments (B). All individuals were reared under laboratory conditions on field-collected leaves from Rosemount, MN and fed soybean aphid.

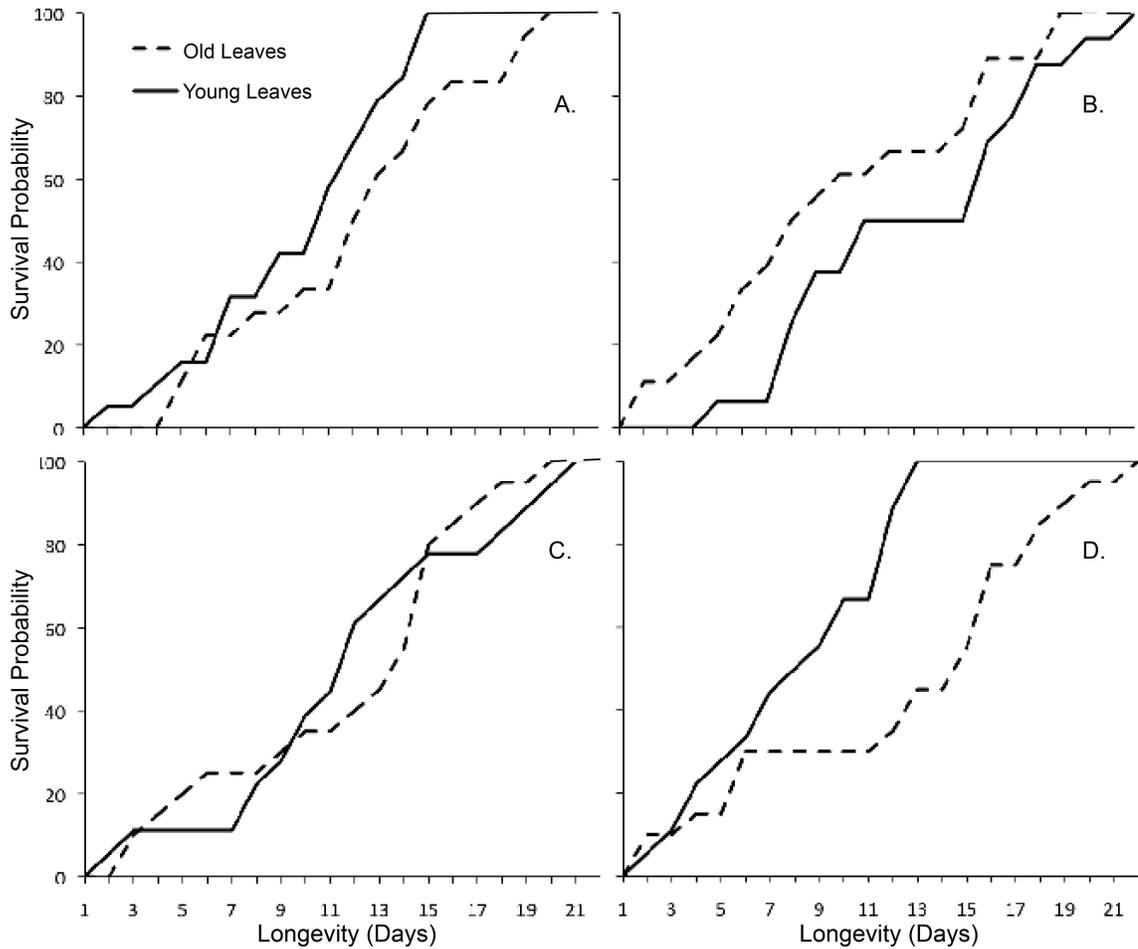


Figure 4-1. Effect of soybean leaf age on adult *Orius insidiosus* reared on leaves from four different treatments combinations of host plant resistance and insecticide seed treatment. Treatments included *Rag1* resistant/seed treated (A), *Rag1* resistant/untreated (B), susceptible/seed treated (C), and susceptible/untreated (D). Leaves were field collected from treatment plots at Rosemount, MN in 2010.

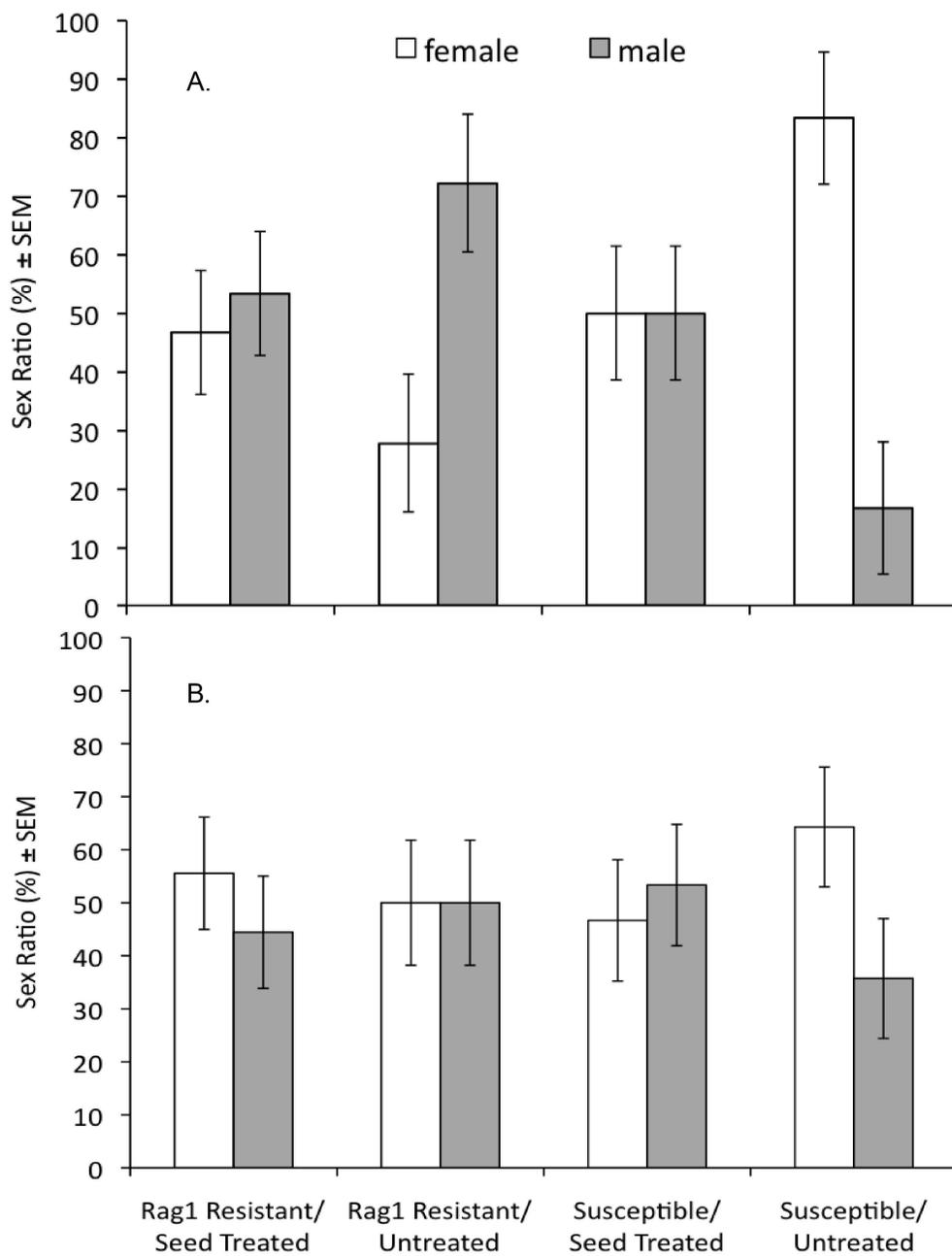


Figure 4-5. Natural enemy sex ratios on the four treatment combinations of *Rag1* host plant resistance and neonicotinoid seed treatment. Sex ratios are for *Harmonia axyridis* (A) and *Nabis americanaferus* (B). Error bars depict the standard error of mean (SEM) for each treatment.

Table 4-1. Average (\pm SEM, *N*) longevity (days) and mortality rates of soybean aphid natural enemies maintained on soybean leaves from four different treatment combinations of host plant resistance and insecticide seed treatment. Overall longevity was calculated using only individuals that died during the study period. Mortality rates represent the percentage of individuals that died during the study period. Experiments were terminated when adults reached 28 days for *Harmonia axyridis*, 21 days for *Orius insidiosus*, and 42 days for *Nabis americanoferus*.

| | Treatment | | | |
|--------------------------------|--|-------------------------------------|------------------------------|---------------------------|
| | <i>Rag1</i> resistant/ Seed treated | <i>Rag1</i> resistant/ Untreated | Susceptible/ Seed treated | Susceptible/ Untreated |
| <i>Harmonia axyridis</i> | | | | |
| Time to Adulthood (days) | 21.00 (0.24, 15) | 21.78 (0.21, 18) | 21.13 (0.38, 16) | 20.61 (0.47, 18) |
| Overall Longevity (days) | 9.67 (6.17, 3) | 34.25 (5.20, 4) | 23.60 (6.22, 5) | 17.75 (5.45, 4) |
| Mortality Rate | 0.24 | 0.26 | 0.29 | 0.21 |
| <i>Orius insidiosus</i> Adults | | | | |
| Time to Adulthood (days) | - | - | - | - |
| Overall Longevity (days) | 10.13 (0.73, 37) | 10.52 (0.96, 34) | 11.00 (0.84, 38) | 9.63 (0.90, 38) |
| Mortality Rate | 0.95 | 0.92 | 0.97 | 0.95 |
| <i>Nabis americanoferus</i> | | | | |
| Time to Adulthood (days) | 9.61 (0.46, 18) | 12.44 (0.74, 16) | 10.31 (0.72, 16) | 11.13 (0.58, 15) |
| Overall Longevity (days) | 29.63 (3.12, 19) | 22.95 (3.03, 19) | 24.89 (3.27, 19) | 25.33 (3.22, 18) |
| Mortality Rate | 0.90 | 0.95 | 1.00 | 0.90 |

Table 4-2. Average (\pm SEM, *N*) longevity (days) of *Orius insidiosus* adults maintained on soybean leaves from four different treatment combinations of host plant resistance and insecticide seed treatment. Overall longevity was calculated using non-censored individuals from the study. *O. insidiosus* adults were reared on lab-raised soybean aphids.

| | Treatment | | | |
|--------------|--|-------------------------------------|------------------------------|---------------------------|
| | <i>Rag1</i> resistant/ Seed treated | <i>Rag1</i> resistant/ Untreated | Susceptible/ Seed treated | Susceptible/ Untreated |
| Old leaves | 11.22 (1.13, 18) | 9.00 (1.33, 18) | 10.95 (1.17, 20) | 11.75 (1.39, 20) |
| Young leaves | 9.11 (0.89, 19) | 5.17 (1.29, 16) | 5.29 (1.25, 18) | 3.68 (0.87, 18) |

**Chapter 5: Assessment of a buckwheat intercrop in organic soybeans for soybean
aphid management**

Introduction

The soybean aphid, *Aphis glycines*, is a significant economic pest of soybean in North America, and since its discovery in the U.S. in 2000, major advances have been made in developing effective management options for this serious pest (Ragsdale et al. 2011). Management options currently available include a number of foliar insecticides, insecticidal seed treatments, and aphid resistant soybean varieties (Hodgson et al. 2012). In addition, significant strides have been made in developing a soybean aphid integrated pest management (IPM) program that utilizes economic thresholds (ET) and economic injury levels (EIL) to determine when to utilize management practices such as foliar insecticides (Ragsdale et al. 2007)

Organic soybean producers cannot avail themselves of all the soybean aphid management options currently available. These soybean producers can only utilize practices approved for use by the National Organic Program (NOP) under the United States Department of Agriculture (USDA), and under these guidelines, insect pest outbreaks should focus first on prevention and mechanical or physical methods of control such as natural enemy refuges and conservation of natural enemies (USDA 2012). Only when these preventative practices fail to control a pest outbreak can curative management options such as organically approved insecticides be utilized for pest management. Many of the effective insecticides frequently used for soybean aphid management in conventional production are not allowed for organic use, and while organically certified insecticides for soybean aphid do exist, their efficacy may be limited (Kraiss and Cullen 2008a, Kraiss and Cullen 2008b) and those that are effective like naturally occurring

pyrethrins may possess a very short residual period. In addition, many of these organically approved pesticides may carry a significant risk of non-target impacts to aphid natural enemies that contribute towards biological control (Bahlai et al. 2010). Overall, few effective options exist in organic soybean production to provide both preventive and curative soybean aphid suppression. The challenges of managing the soybean aphid has made the production of organic soybeans in the United States very difficult. The need for research to develop new management alternatives for insect pests such as soybean aphid in organic systems has become apparent (Zehnder et al. 2007).

Biological control by natural enemies is an important component of a preventative pest control program in organic agricultural systems by providing top-down action of natural enemies on the pest. Insect pest suppression through the conservation of natural enemies has been demonstrated in different cropping systems (Messina and Sorenson 2001, Naranjo et al. 2003, 2004, Obrycki et al. 2009) as well as in soybean for soybean aphid control (Fox et al. 2005, Rutledge and O'Neil 2005, Desneux et al. 2006, Costamagna et al. 2007, Gardiner et al. 2009b, Schmidt et al. 2011). Practicing conservation biological control is therefore particularly important for organic soybean aphid management where few effective therapeutic tactics exist. Soybean aphid natural enemy conservation can be accomplished by increasing the broader landscape diversity (Gardiner et al. 2009b, Brewer and Noma 2010, Schmidt et al. 2011, Woltz et al. 2012), utilizing reduced-risk insecticides (Kraiss and Cullen 2008b, Ohnesorg et al. 2009, Bahlai et al. 2010), and providing alternative crops as insect refugia (Schmidt et al. 2007, Lundgren et al. 2009a, Koch et al. 2012).

Intercropping is the practice of planting multiple crops in an area for a particular purpose such as increasing insect refugia, suppressing weeds, or reducing soil erosion. Intercropping is frequently used in organic farming as a cultural control method for pest management as it can serve as a refuge for natural enemies. Cultural control for insect pest management is defined as the manipulation of the cropping environment in such a way that the environment is made less favorable for development of a particular pest (Pedigo and Rice 2006). Buckwheat, *Fagopyrum esculentum* Moench, is a fast maturing crop that has been used in organic agriculture in numerous ways, including for food production, as a green manure crop, for weed suppression (2010, Creamer and Baldwin 2010), to minimize soil erosion, and as an intercrop as refuge for natural enemies (Platt et al. 1999). Buckwheat thrives in the cooler summer weather of the northern United States although it is a frost-intolerant plant. It may begin flowering in as little time as a week post-planting and has a long flowering period considering it is a rapid-growing crop. If grain harvest is desired, maturity is typically reached in 70-90 days (2010). The plants produce numerous small white flowers whose nectar and pollen make it attractive to a number of insect groups, and buckwheat has been used as a natural enemy attractant in other cropping systems (Platt et al. 1999, Ambrosino et al. 2006). These characteristics of buckwheat make it favorable as an intercrop in organic systems for insect pest management, but the utility of buckwheat is essentially unknown for soybean aphid biological control as it has only been explored in a few other studies known to date (Lee and Heimpel 2008b, Lee and Heimpel 2008a, Woltz et al. 2012).

Integrating control measures such as biological and cultural control may have an additive effect on insect pest suppression. Intercropping with flowering crops such as buckwheat can enhance biological control by attracting natural enemies to a sugary food source and providing additional livable habitat. The purpose of this study was to investigate whether intercropping organic soybeans with flowering buckwheat can be an effective management option for suppressing soybean aphid populations. Our two overall research objectives were to 1) determine if and to what extent the presence of buckwheat can aid in suppression of the soybean aphid and 2) determine if buckwheat can enhance soybean aphid biological control from recruitment of natural enemies to a soybean/buckwheat intercropped system.

Materials and Methods

Field studies

Field plots. Two-year field experiments were conducted in Minnesota during the growing seasons of 2010 and 2011 to assess the impacts of buckwheat on soybean aphid management. In 2010, the study was conducted at two Minnesota locations: the organically certified research area at the University of Minnesota Southwest Research and Outreach Center (SWROC) in Lamberton, MN and in organic farmer fields near Evansville, MN. The study was repeated at Lamberton, MN in 2011. All fields were USDA organically certified and managed under organic management practices.

To assess potential effects of a buckwheat cover crop on soybean aphid and aphid natural enemy populations, soybean and buckwheat were intercropped in replicated plots and fields. At SWROC in Lamberton, 5-foot-wide buckwheat strips were either planted

along the edge of soybean plots in 2010 or within soybean plots in 2011 (Figure 5-1A and B). Based on 2010 results, the location of buckwheat strips was changed in 2011 from border row strips to internal plot strips to better assess the community of soybean aphid natural enemies attracted to buckwheat. Treatments included plots with or without buckwheat. Buckwheat was planted at a rate of 50 lbs seed/acre. Soybean plots at the Lamberton SWROC measured 60-ft x 60-ft with a 15-ft (2010) or 25-ft (2011) fallow area between plots. Plots were replicated four times in a randomized complete block design in both years. In 2011, the Lamberton farmer fields contained a 10-ft-wide hand-planted strip of buckwheat in a soybean field, and aphids and natural enemies were sampled specific distances (0-25 ft, 25-50 ft, 50-75 ft, and 75-100 ft) from the buckwheat plot. At Evansville 25-ft-wide buckwheat strips were planted down the middle of two soybean fields, and a sampling plot was marked off in soybean directly adjacent to the buckwheat strip (Figure 5-1C). Paired fields (with or without a buckwheat strip) were compared, and two pairs of fields were sampled during the growing season. Plots within each field measured 100-ft x 100-ft. To determine whether distance from buckwheat affected aphid and natural enemy abundance, we also sampled specific distances (0-25 ft, 25-50 ft, 50-75 ft, and 75-100 ft) from the buckwheat strip in the Evansville fields. Soybean fields at Evansville were planted between 21 and 23 May 2010 with Pioneer 91M10, an organic soybean seed variety. Lamberton fields and plots were planted 4 June 2010 and 9 June 2011 with Albert Lea organic Viking soybean seed, variety O-2022N. All buckwheat seed used in both years of sampling was obtained from the Albert Lea Seed House (Albert Lea, Minnesota) and were planted at a seeding rate of 130,000

seeds/acre with 30-inch row spacing.

Aphid and natural enemy sampling. Aphid abundance was estimated at each location by conducting destructive whole-plant aphid counts during the growing season. Sampling was conducted bi-weekly at Evansville (2010) and weekly at Lambertson (2010-11). At Evansville, insect sampling was conducted from 22 June 2010 to 18 Aug 2010 on a bi-weekly basis (5 total sampling dates). At Lambertson, insect sampling was conducted from 24 June to 1 Sept in 2010 and 30 June to 8 Sept in 2011 on a weekly basis. The number of plants counted varied in intensity from 20 to 5 depending on the % aphid infestation rate of soybean plants in the treatment plots and was based on the sampling method described by Ragsdale et al. (2007).

Natural enemies were sampled using a combination of sweep net sampling and visual plant inspections. A combination of these sampling methods allowed both stationary and mobile natural enemies to be accounted for in overall natural enemy abundance (Costamagna and Landis 2007, Schmidt et al. 2008). Sweep net sampling consisted of 20 sweeps per plot, and visual inspections were conducted on two 1-meter row sections of soybean per plot whose totals were combined. In 2011, we sampled natural enemies within buckwheat strips to determine the natural enemy community directly associated with buckwheat. Natural enemy eggs observed were counted but were not included in the final analysis of natural enemies.

Yield effects of a buckwheat intercrop were assessed at all sampling locations except the 2011 farmer fields. Plot yield estimates at Evansville were obtained by harvesting two six-foot row lengths per plot. Evansville plots were harvested 8 October

2010. Plot yield estimates at SWROC in Lambertton were obtained by harvesting two yield rows per plot with a plot combine. Lambertton plots were harvested on 15 October 2010 and 15 October 2011.

Data analysis

To assess the effects of buckwheat on soybean aphid and natural enemy populations, a repeated measure analysis of variance (ANOVA) was conducted using the nlme package in R (R 2.14.2 The R Foundation, 2012). Treatments were either the presence or absence of buckwheat strips within a plot (SWROC) or field (Evansville). To assess the effects of increasing distance from buckwheat on soybean aphid and natural enemy populations, a repeated measure ANOVA was conducted to compare insect populations at the four specified distance intervals from the buckwheat. For all analyses, data was $\log(x+1)$ transformed when necessary to meet the normality assumptions of ANOVA. Means were separated using Tukey's HSD test, and a significance level of 0.05 was used for all statistical tests. Interactions between factors were assessed, but only significant interactions are addressed in the results. Yield effects between treatments were assessed with t-tests.

Results

Soybean aphid

Comparisons between buckwheat intercropped plots and soybean-only plots demonstrated no significant differences between aphid densities at any locations sampled in either year (Evansville: $F=0.19$, $df=1,2$, $P=0.70$; Lambertton: $F=2.09$, $df=1,3$, $P=0.24$

(2010), $F=0.39$, $df=1,3$, $P=0.58$ (2011)) (Figures 2A and 3B&C), although trends did show a slight decrease in aphid densities with the presence of buckwheat at two of the location-years (Evansville in 2010 and Lambertton in 2011 (Figures 2A and 3B respectively)). Aphid densities did significantly differ between sampling dates for all location-years (Evansville: $F=185.71$, $df=4,18$, $P<0.001$; Lambertton: $F=220.05$, $df=10,60$ $P<0.001$ (2010), $F=144.17$, $df=9,54$ $P<0.001$ (2011)). Within any given location-year, aphid densities peaked on the same date for both treatments, indicating no lag in aphid colonization or change in aphid population growth due to the presence of buckwheat.

At Evansville, we also investigated whether distance from buckwheat can have an effect on aphid densities. Results showed no consistent trends in aphid density based on distances of 25-ft. to 100-ft. from buckwheat (Figure 5-4A), and statistical analysis confirmed this with no significant differences in soybean aphid density between the distances sampled (up to 100 ft. away from buckwheat) ($F=0.14$, $df=1,2$, $P=0.74$).

Overall in both 2010 and 2011, soybean aphid pressure was relatively low for much of the growing season (Figures 5-2A and 5-3A&B). None of the location-years reached the economic injury level (EIL) of 674 aphids per plant, and the economic threshold (ET) of 250-aphids per plant (Ragsdale et al. 2007) was never surpassed before August. Aphid densities peaked at Evansville on 6 Aug 2010 at 347.7 ± 167.7 and 537.3 ± 156.1 aphids/plant in buckwheat and no buckwheat plots, respectively. At the SWROC in Lambertton, aphid populations peaked on 4 August 2010 at 352.9 ± 115.5 and 386.0 ± 115.4 aphids/plant in the buckwheat and no buckwheat plots, respectively and on 3

August 2011 at 312.8 ± 130.4 aphids/plant and 257.5 ± 92.1 aphids/plant in buckwheat and no buckwheat plots, respectively.

Soybean yields at all location-years demonstrated no significant effects from the presence or absence of buckwheat (Evansville: $t(2)=0.60$, $P=0.61$; Lambertton: $t(6)=0.11$, $P=0.92$ (2010); $t(6)=1.08$, $P=0.32$ (2011)). Yields at Evansville averaged 23.3 ± 3.4 bu/acre in buckwheat plots and 24.9 ± 1.8 bu/acre in control plots. Yields at Lambertton averaged 36.6 ± 4.7 and 35.0 ± 3.7 bu/acre in 2010 and 2011, respectively, in buckwheat plots and 37.3 ± 4.3 and 37.2 ± 2.5 bu/acre in 2010 and 2011, respectively, in control plots.

Natural enemies

To determine if aphid population differences between plots were due to the recruitment of soybean aphid natural enemies, we compared natural enemy abundance between buckwheat and no buckwheat treatments. Abundance was measured as the total number of natural enemies observed per plot from both sampling methods and excluded all egg counts. Results at all location-years demonstrated a lack of significant differences in natural enemy abundance between treatments throughout the season (Evansville: $F=2.16$, $df=1,2$, $P=0.28$; Lambertton: $F=0.013$, $df=1,3$, $P=0.92$, (2010), $F=0.023$, $df=1,3$, $P=0.89$ (2011)) (Figures 5-2B and 5-3C&D). While natural enemy abundance in buckwheat plots at Evansville did demonstrate a slight increasing trend later into the season (ending 6 Aug), it is unclear if this trend continued since we were unable to sample natural enemies on 18 Aug due to poor weather conditions. We also looked at whether distance from buckwheat affected natural enemy abundance at Evansville. When

natural enemy numbers were compared at the different distances, no significant differences were observed ($F=0.31$, $df=3, 11$, $P=0.82$; Figure 5-4B).

In 2011 we compared the natural enemy community directly associated with buckwheat strips to the natural enemies found in soybean. This sampling was done using both sweeps and visual observation of buckwheat and soybean plants. Results showed a difference in the community of soybean aphid natural enemies in buckwheat and soybean (Figure 5-5). Ladybeetles, particularly *H. axyridis*, followed by *O. insidiosus* dominated the natural enemy community in soybean, but in buckwheat ladybeetles were absent and *O. insidiosus* decreased in abundance compared to in soybean. Syrphid flies (family Syrphidae) were the dominant natural enemy in buckwheat, though the density of syrphid flies did not differ between soybean and buckwheat (0.23 ± 0.069 and 0.63 ± 0.17 flies/plot in soybean and buckwheat, respectively).

As a short-season crop, buckwheat flowering may begin three weeks post-planting, and timing of flowering is critical to obtain natural enemy recruitment for enhanced biological control. In this study, buckwheat was first observed flowering 17 June in Evansville and 1 July in Lambertton for 2010. In 2011, buckwheat was planted late at the SWROC on 30 June, and the first flowering was observed mid-July 2011. The timing of buckwheat flowering therefore occurred prior to soybean aphid detection in all plots for 2010 and prior to significant aphid population growth period in 2011.

Discussion

The objectives of this study were to determine if the presence of buckwheat

intercropped with soybean could enhance soybean aphid suppression through increased biological control activity, therefore providing an organic-friendly management option for this soybean pest. We investigated the effects of buckwheat on both soybean aphid and the soybean aphid natural enemy community. Neither soybean aphid populations nor natural enemy populations demonstrated a strong response to the presence of a buckwheat intercrop. While natural enemy populations did respond to fluctuations in aphid populations over the two seasons at both locations (Figures 5-2 to 5-4) and likely played an important role in overall aphid suppression throughout the season, the natural enemies contributing toward biological control did not exhibit a strong response to the presence of buckwheat contrary to what was hypothesized. Since aphid populations did not decrease due to the presence of buckwheat, there was little evidence that natural enemy recruitment occurred due to the presence of buckwheat in this system.

One explanation for the lack of effect from buckwheat on soybean aphid natural enemies may be due to spatial scale. The buckwheat/soybean intercrop used in this study was a small-scale manipulation of the greater landscape. Other studies have shown that the overall landscape composition rather than local habitat manipulation (such as our buckwheat strips) may be more important in affecting natural enemy populations of the soybean aphid (Woltz et al. 2012). This consideration for the broader landscape in developing a pest management program fits well into the holistic management views of organic agriculture overall (Zehnder et al. 2007)

This study demonstrated a diverse community of aphid natural enemies in both buckwheat and soybean (Figure 5-5), but the results also demonstrated a missing guild in

the natural enemy community: parasitoids. While few parasitoids were recovered from the surveys, this natural enemy group has demonstrated a significant effect on soybean aphid control in Asia, where this pest originates (Wu et al. 2004). Currently, a classical biological control program is being attempted for the soybean aphid to introduce parasitoids specific to the soybean aphid to North America (Heimpel et al. 2004, Ragsdale et al. 2011). In addition, the parasitoid *Aphelinus certus* is continually moving westward from Ontario, Canada into the Midwest, and this species has demonstrated the ability to affect soybean aphid populations in Ontario, Canada (Frewin et al. 2010, Heimpel et al. 2010). While natural enemy groups such as coccinellids and syrphid flies have demonstrated an attraction for flowering crops such as buckwheat (Frank and Liburd 2005, Woltz et al. 2012), parasitoids may exhibit the strongest response to the presence of a flowering intercrop. Therefore increasing the parasitoid community in North American soybean could significantly impact biological control of the soybean aphid and further enhance soybean aphid management in organic soybean aphid production.

A significant challenge to this study was the issue of weed control in all plots and fields. Weed suppression in organic agriculture is a major agronomic issue (Wedryk et al. 2012). While the plots used in this study were cultivated for as long as possible, weeds can only be controlled through cultivation when plants are small to reduce damaging the crop itself. Cultivation became impossible once the soybean canopy closed. Not only do weeds compete with the crop for necessary resources, but they can also provide added habitat and floral resources for insects. In this study, the latter was the issue as the weeds

provided numerous floral resources in the soybean plots, potentially compromising the study results by diluting the effect of flowering buckwheat. To compensate for this issue, experimentally removing weeds from the plots could be attempted or alternatively, weed diversity and abundance could be included as additional factors into the study to determine their impacts on aphid and natural enemy populations. This weed issue also addresses the need to approach pest management in organic systems in a very holistic way since one component of the system (weeds) can potentially have both a negative (competition with the crop) and positive impact (recruit natural enemies for biological control) at the same time (Zehnder et al. 2007).

In conclusion, it is unlikely that utilizing relatively small patches of buckwheat in an organic soybean cropping system intercropped with soybean will significantly suppress soybean aphid. While conservation of natural enemies for biological control continues to be an important component of organic soybean aphid IPM, utilizing buckwheat would not be recommended to enhance biological control for organic soybean aphid management.

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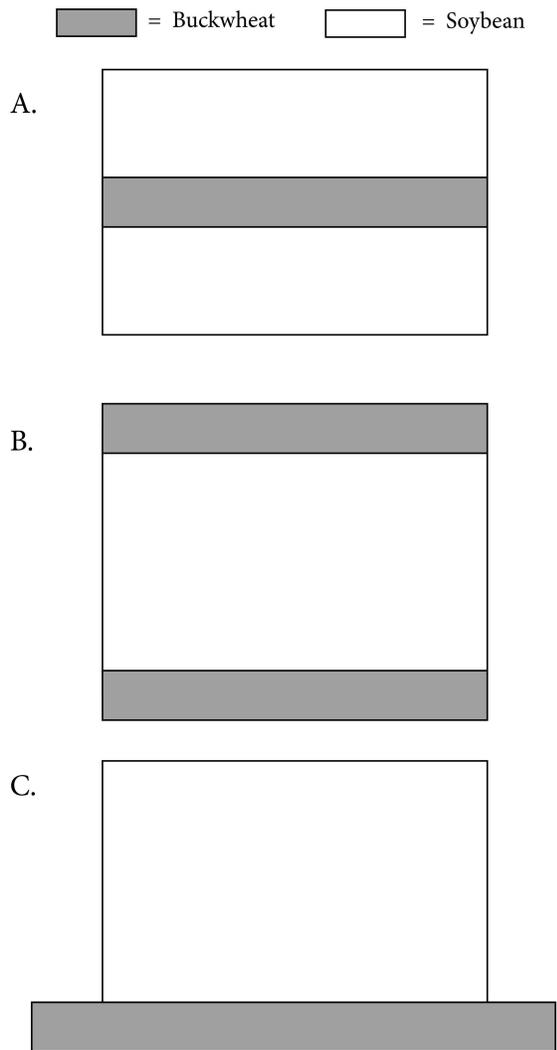


Figure 5-1. Plot design for soybean and buckwheat intercropping within plots at Lamberton, MN SWROC in 2010 (A) and 2011 (B) and Evansville, MN in 2010 (C).

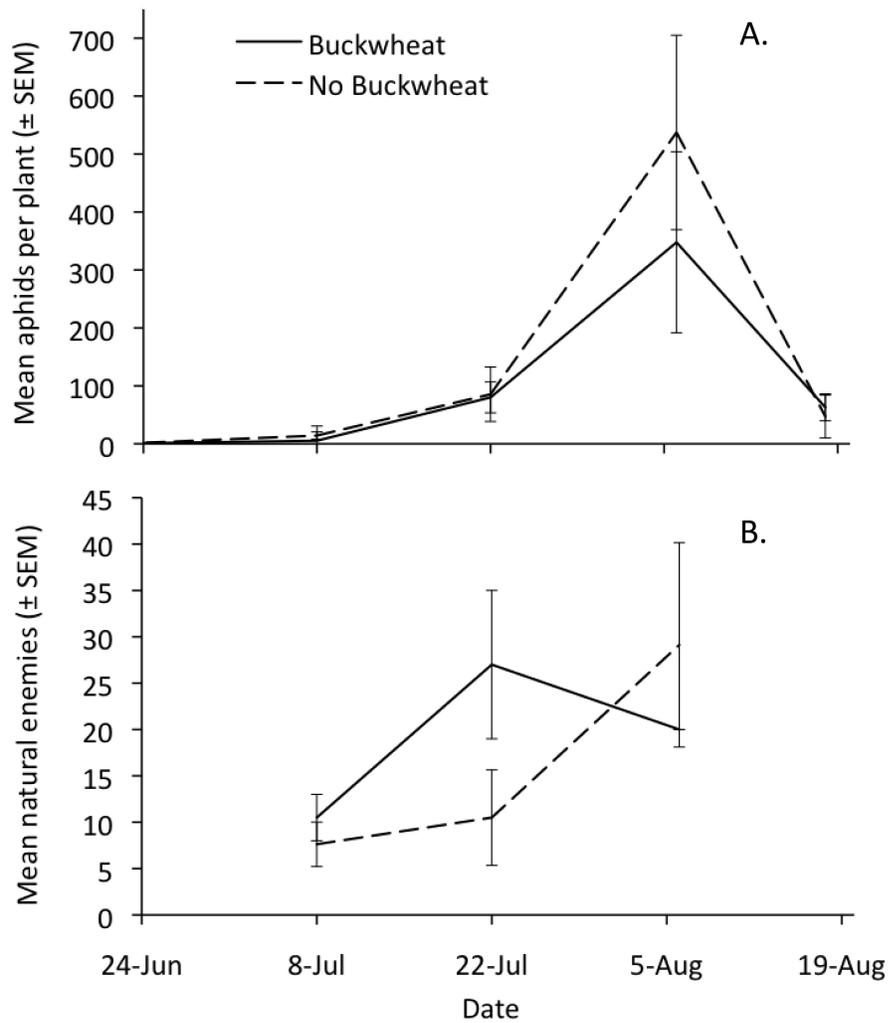


Figure 5-2. Effect of buckwheat on soybean aphid populations (A) and aphid natural enemy populations (B) near Evansville, MN in 2010.

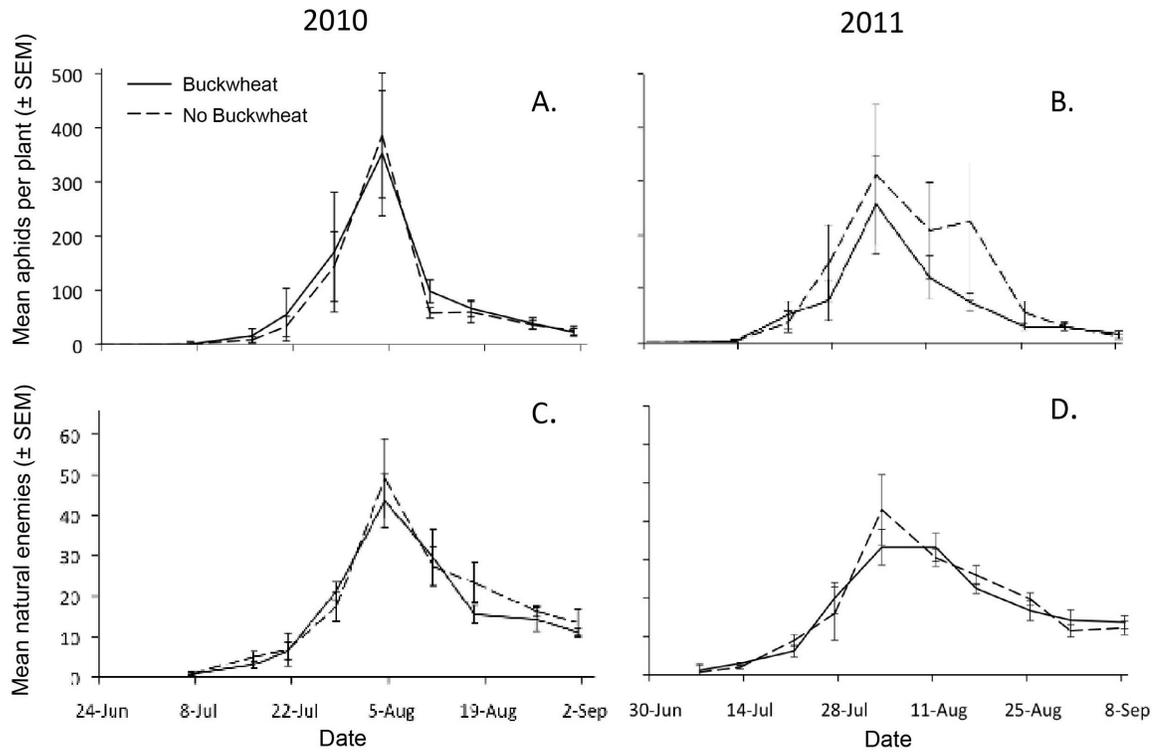


Figure 5-3. Effect of buckwheat on soybean aphid densities (Panels A & B) and aphid natural enemy abundance (Panels C & D) at the SWROC in Lamberton, MN in 2010 and 2011. Error bars indicate the standard error of the means.

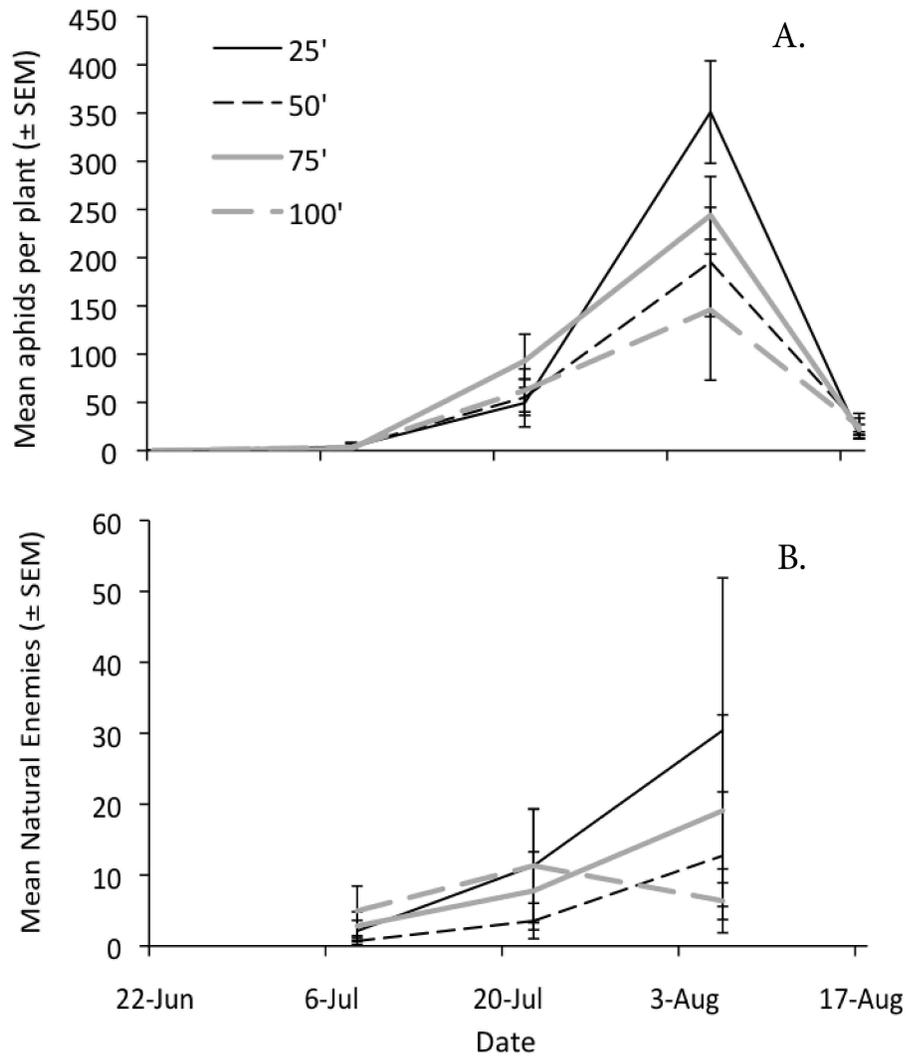


Figure 5-4. Effects of distance (in feet) from buckwheat on soybean aphid (A) and natural enemy population densities (B) at Evansville, MN in 2010.

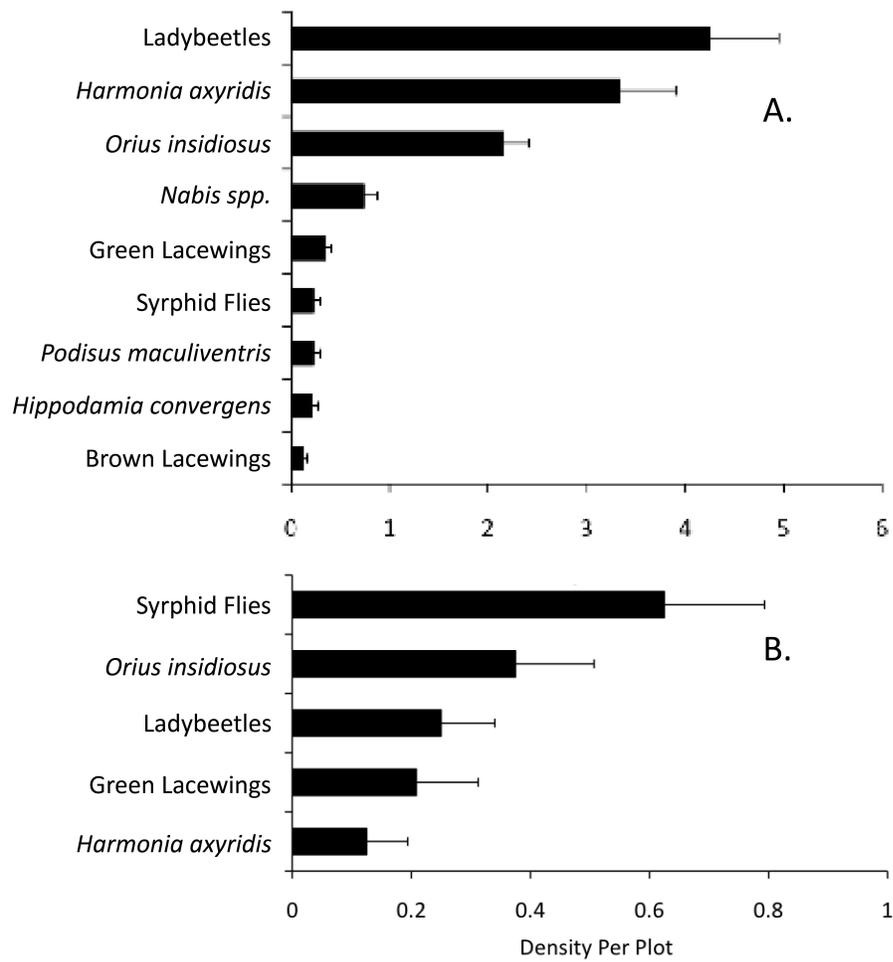


Figure 5-5. Natural enemy densities in soybean adjacent to buckwheat (A) and in buckwheat (B) at SWROC in Lamberton, MN in 2011. Error bars indicate standard error of means.

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