

IMPACT OF *RAG1* APHID RESISTANT SOYBEANS ON *BINODOXYS COMMUNIS*
(GAHAN) (HYMENOPTERA: BRACONIDAE), A PARASITOID OF SOYBEAN
APHID *APHIS GLYCINES* MATSUMURA (HEMIPTERA: APHIDIDAE)

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ABSTRACT

Ghising, Kiran, M.S., Department of Entomology, College of Agriculture, Food Systems, and Natural Resources, North Dakota State University, March 2011. Impact of *Rag1* Aphid Resistant Soybeans on *Binodoxys communis* (Gahan) (Hymenoptera: Braconidae), a Parasitoid of Soybean Aphid *Aphis glycines* Matsumura (Hemiptera: Aphididae). Major Professors: Dr. Janet K. Knodel and Dr. Jason P. Harmon.

After its discovery in North America during the summer of 2000, the soybean aphid, *Aphis glycines* Matsumura (Hemiptera: Aphididae) became a major pest of the soybean, *Glycine max* (L.) Merr., in most soybean growing regions of the United States. The use of insect-resistant plant varieties and natural enemies, important components of Integrated Pest Management (IPM), when applied solely have the potential to be effective measures for controlling soybean aphids. However, resistant host plants may influence natural enemies in beneficial or detrimental ways, thereby altering their effectiveness when the two strategies are combined. Therefore, we investigated how a resistant variety impacts fitness of a biological control agent to understand its compatibility for pest management of the soybean aphid. A near isogenic susceptible soybean variety without the *Rag1* gene and a resistant variety with the *Rag1* gene were used to determine the effect of the *Rag1* on the development and fitness of the soybean aphid parasitoid, *Binodoxys communis* Gahan (Hymenoptera: Braconidae). Before testing for effects of the *Rag1* gene on the parasitoid, we first validated the expression of the *Rag1* gene and confirmed that these plants were resistant to soybean aphids by determining the growth rate of soybean aphids on both resistant and susceptible plants. The soybean aphid population and per capita growth rate were significantly higher when reared on susceptible soybean plants compared to resistant plants. In addition, polymerase chain reaction (PCR) was used to verify the genotypes and the presence of the *Rag1* gene in some of the plants used in the growth rate experiment.

Results of the soybean aphid growth rate experiment combined with the results of the PCR helped to validate the expression of the *Rag1* in the resistant plants used in our experiments. To determine the impact of these resistant plants on parasitoids, the total numbers of mummies (parasitized soybean aphids) produced and adult parasitoid emergence were compared for parasitoids that were given aphid hosts from either susceptible or resistant plants. Parasitoid fitness was measured in terms of parasitoid development time, their body length, and their metatibiae length. We found a higher number of mummies in susceptible soybean plants than in the resistant plants as well as a higher emergence rate of adult parasitoids from the mummies reared on susceptible plants. The development time from mummy to adult parasitoid emergence was only one day longer with aphid hosts from resistant plants compared to susceptible plants. Despite some difference in the size of parasitoids from resistant and susceptible plants, very few parasitoids completed development on resistant plants. In summary, our results indicate poorer establishment and reproductive performance of *B. communis* from soybean aphids on resistant plants compared to soybean aphids on susceptible plants. This suggests that widespread adoption of resistant soybean plants might be detrimental to the overall sustainability of this parasitoid and its ability to help control soybean aphids. We did, however, find that at least some *B. communis* could successfully develop and emerge on soybean aphids from resistant plants, suggesting that there is at least some possibility that the parasitoid could survive and assist in aphid management even if the *Rag1* resistant plants become commonplace. The parasitoid's relative fitness and reproductive output will likely play important roles in ultimately determining the short- and long-term compatibility of utilizing both *B. communis* and resistant soybean plants for soybean aphid control.

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GENERAL INTRODUCTION

The soybean aphid, *Aphis glycines* Matsumura (Hemiptera: Aphididae), is native to Asia and an economically important insect pest of soybeans throughout the soybean growing region of Asia and North America (Rutledge et al. 2004, Wu et al. 2004). Besides causing significant yield loss via direct feeding, soybean aphid is an important vector of several viral diseases (Venette and Ragsdale 2004, Wu et al. 2004, Ragsdale et al. 2007). Despite the costs and potential hazardous effects of insecticides on the environment, chemical control has been the predominant method used for soybean aphid management (Ye et al. 1998, Wu et al. 2004, Hill et al. 2006a). However, viable non-chemical strategies are also desirable in order to create an Integrated Pest Management (IPM) program that exhibits more effective, sustainable, and cost-effective pest control through the use of multiple control tactics. In the case of soybean aphid, potential strategies include the use of insect-resistant plant varieties and biological control agents (Auclair 1989, Harrewijn and Minks 1989, Ragsdale et al. 2004, Hill et al. 2006a).

Various soybean varieties exhibit resistance to various insect pests, including the soybean aphid (Wiseman 1999, Lambert and Tyler 1999, Hill et al. 2004a, b). For example, the variety 'Dowling' showed limited soybean aphid colonization via strong antibiosis (Hill et al. 2004a). Later work further demonstrated resistance in 'Dowling' by showing reduced aphid survival, fecundity, longevity, and development via antibiosis (Li et al. 2004). Hill et al. (2006a, b) identified a single dominant gene known as *Rag1* in 'Dowling' that governs resistance to the soybean aphid. Soybean varieties that express the *Rag1* gene thus have the potential to be resistant to the soybean aphid and help diminish the aphid's negative effects.

The recent introduction of *Binodoxys communis* Gahan (Hymenoptera: Braconidae) is an example of a classical biological control program. *Binodoxys communis* is a parasitoid of soybean aphid in their native range of Eastern Asia. The parasitoid was introduced in the United States in 2007 to help control the soybean aphid (Wyckhuys et al. 2009, Ragsdale et al. 2011). *Binodoxys communis* is capable of high rates of parasitism on the soybean aphid, and it is known to be highly host specific to the soybean aphid (Desneux et al. 2009). These factors make it a good potential candidate to help control soybean aphid population.

While both host plant resistance and biological control have been successful in a wide variety of cropping systems when either is used by itself, limited research has been conducted examining the compatibility of combining both strategies for the soybean aphid management. It is not always possible to predict what will happen when these two strategies are used together because tritrophic interactions between plants, herbivores, and natural enemies may alter natural enemies in positive or negative ways depending on the particular system and its components (Duffey and Bloem 1986, Orr and Boethel 1985). For example, plant characteristics that influence the quality of an insect herbivore may also influence the herbivore's natural enemy because they rely upon that herbivore as a nutritional resource or as a host. For example, an individual resistance gene (A_{10}) present in resistant cultivars of red raspberry conveys resistance via the chemical composition of epicuticular leaf waxes, and is effective against European raspberry aphid, *Amphorophora idaei* Börner (Homoptera: Aphididae) (Keep and Knight 1967, Birch and Jones 1988, Griffiths et al. 2000, Shepherd et al. 1999a, b). The resistant raspberry cultivar negatively influenced the aphid performance in terms of their pre-reproductive period, total reproductive output, life span and the intrinsic rate of increase compared to susceptible

cultivars (Mitchell et al. 2010). In the resistant cultivar, a higher incidence of aphid dropping was observed, and this decreased aphid densities, which negatively affected the attacking behavior of the aphid parasitoid, *Aphidius ervi* Haliday (Hymenoptera: Aphidiinae) (Mitchell et al. 2010).

The primary objective of this study was to examine the effects of the *Rag1* gene of resistant soybean plants on the development and fitness of *B. communis*. The effect of host plant resistance on: 1) the total number of mummies produced, 2) total number of adult parasitoids that emerged, 3) total development time for the adult parasitoid to emerge, 4) adult parasitoid body size, and 5) metatibiae length were measured for both near isogenic soybean lines with the *Rag1* gene (resistant plants) and without the *Rag1* gene (susceptible plants). This study will be an important first step in determining the compatibility of *B. communis* and *Rag1* resistant soybean plants for the management of soybean aphids.

LITERATURE REVIEW

Soybean Production

Soybean, *Glycine max* (L.) Merr., (Rosales: Fabaceae), belongs to the family Fabaceae, and is a legume crop native to Eastern Asia, where it is considered one of the oldest agricultural crops under cultivation (Probst and Judd 1973, Chinese Ministry of Agriculture 2001). Soybeans are used as a major source of oil and protein for human and animal consumption and have several medicinal purposes. Proteins present in the soybean are rich in vitamins and minerals, and contain fibers that help with digestion (Probst and Judd 1973, Wu et al. 2004). Currently, soybean production makes up about 56% of the world oilseed production and ranks as the number one crop in terms of oil production (Soystats 2009).

Soybean was introduced into the United States as early as 1804 for use as hay or silage for feeding hogs and sheep (Probst and Judd 1973). Soybean production was originally limited as a forage crop; however, soybeans are now an important oilseed crop in the United States (Mease 1804, Probst and Judd 1973). In 2009, soybeans were planted on approximately 75 million acres with an annual production of approximately 80 million metric tons that was valued at 27.4 billion USD (Soystats 2009). The United States contributes about 33% of the world's soybean production (Soystats 2009). If different biotic (insects, plant pathogens, nematodes) as well as abiotic (water, drought, temperature) stresses occur during the early reproductive stages of soybean, significant yield loss can occur in soybean (Fehr and Caviness 1977).

Soybean Aphid Origin and Biology

Origin and Invasion in USA

Soybean aphid, *Aphis glycines* Matsumura (Hemiptera: Aphididae), is a small greenish-yellow insects about 1.6mm long with piercing-sucking mouthparts (Wu et al.2004). It is native to Eastern Asia, and has been considered a major insect pest of soybean since early cultivation of soybean plant (Ma 1986, Chinese Ministry of Agriculture 2001, Venette and Ragsdale 2004, Wu et al. 2004). Soybean aphid was first discovered in the United States in Wisconsin in 2000, and can cause yield losses of more than 50% under severe infestations (Ostlie 2001, Venette and Ragsdale 2004). Within three years of the initial discovery, soybean aphid presence was confirmed in 21 states (Alleman et al. 2002, Venette and Ragsdale 2004). Currently, soybean aphids are considered one of the major insect pests of soybean production in the United States (Ragsdale et al. 2011).

Systematics

Soybean aphids belong to the genus *Aphis* within the family Aphididae, subfamily Aphidiinae, and order Hemiptera (Blackman and Eastop 2000). The order Hemiptera has four suborders: the Sternorrhyncha, Auchenorrhyncha, Coleorrhyncha, and the Heteroptera. Aphids are in the suborder Sternorrhyncha, which also contains scale insects, psyllids, and whiteflies (Blackman and Eastop 2000).

Host Range

Soybean aphid is a heteroecious holocyclic species that alternates between two host plant species to complete its life cycle. Several species of buckthorn (*Rhamnus* spp.) serve as a primary overwintering host, whereas soybean is the secondary summer host (Dixon 1998, Ragsdale et al. 2004). Although the life cycle of the soybean aphid is similar in its

native range, in North America several plant species have been reported as primary and secondary host plant species for completing soybean aphid lifecycle (Ragsdale et al. 2004). Dahurian buckthorn, *R. davurica* Pallus, and Japanese buckthorn, *R. japonica* Maxim are the two common species of buckthorn used as its primary hosts in China and Japan, respectively (Takahashi et al. 1993, Hirano et al. 1996). In North America, three species of buckthorn have been identified as a primary hosts, namely common buckthorn, *R. cathartica* L., alderleaf buckthorn, *R. alnifolia* L'Heritier, and lanceleaf buckthorn, *R. lanceolate* Pursh (Voegtlin et al. 2004, Voegtlin et al. 2005, Yoo et al. 2005).

The wild soybean, *Glycine soja* Sieb & Zucc., and cultivated soybean, *G. max* are the two most common secondary hosts of the soybean aphid in China (Wang et al. 1962, Hirano et al. 1996). Even though soybean aphid colonization is not high, several species of *Trifolium* (Fabaceae) can also be used as secondary hosts (Alleman et al. 2002). The chemomechano-receptors present on soybean aphid antennae help identify plant volatile chemicals emitted by soybean and aids migration between buckthorn and soybean (Du et al. 1994, Du et al. 1995).

Life Cycle

After mating in autumn, adult female aphids lay eggs on buckthorn. The preferred oviposition site is between the bud and twig of the plant (Ragsdale et al. 2004). Eggs are very cold hardy, and can survive exposure to temperatures as low as -34°C (Crompton 2007). In the spring, eggs hatch into apterous (wingless) females known as fundatrices that give birth to alate (winged) females via parthenogenesis (Wang et al. 1962, Wang and Ba 1998, Ragsdale et al. 2004, Wu et al. 2004, Voegtlin et al. 2004). In the early summer, these winged females migrate to cultivated soybean fields (Ragsdale et al. 2004). These

females continue producing apterous daughters by parthenogenesis until overcrowding or deterioration of plant quality occurs, after which they begin producing alate female aphids (Wang et al. 1962, Wang and Ba 1998, Ragsdale 2004, Venette and Ragsdale et al. 2004, Wu et al. 2004). These alates disperse and can colonize uninfested soybean fields (Wang et al. 1962, Lu and Chen 1993, Wang and Ba 1998, Ragsdale et al. 2004, Wu et al. 2004). Heavy winds aid the long-range dispersal of alate aphids (Venette and Ragsdale 2004). Li-hua and Rui-lu (1993) examined the role of temperature in the production of alate aphids during late summer with their production increasing at temperatures near 21°C, and declining when temperatures ranged between 27-32°C. As temperature decreases in the fall, apterous females produce a sexual generation that includes both alate males (androparae) and alate females (gynoparae). They leave soybeans and fly to *Rhamnus* spp., where they mate and give birth to apterous females (oviparae) (Wang et al. 1962, Wang and Ba 1998, Glogoza 2004, Ragsdale et al. 2004, Voegtlin et al. 2004, Wu et al. 2004).

Soybean Aphid Damage to Soybean

Soybean aphids can cause significant reductions in soybean yield and seed quality, either directly through plant feeding or indirectly through the transmission of several viral diseases. Soybean aphids feed by inserting piercing-sucking mouthparts into phloem tissue of soybean and imbibe phloem sap (Wang and Ba 1998, Wu et al. 1999). As a result of direct feeding on the phloem sap, soybean aphids cause stunted plant growth, reduced height, reduced pod size, and lower pod quality with fewer seeds per pod (Ragsdale et al. 2007, Rhainds et al. 2007, Beckendorf et al. 2008). When soybean aphid infestations are severe, seed oil content is reduced (Beckendorf et al. 2008). Soybean aphid feeding can reduce root nodule volume of soybean plants by 34%, which ultimately reduces the

abundance of N-fixing bacteria in the nodule and negatively affects the nitrogen fixation rate, thus reducing plant growth (Riedell et al. 2009). Soybean aphids also inject toxic saliva into the plant increasing feeding injury (Miles 1999). Additionally, aphids secrete honeydew, a sugar-rich sticky liquid, that promotes the growth of sooty mold, and inhibits photosynthetic rates in plants (Ostlie 2002, Macedo et al. 2003, Wu et al. 2004).

In part, the extent of the soybean aphid colonization depends upon the growth stage of the plant, with higher aphid populations occurring when plants are infested early in the growing season (Wang et al. 1962, Hirano et al. 1996, Wu et al. 2004). Researchers have found that soybean aphid infestation in the early vegetative growth stages can result in a yield loss of more than 50%. Soybean aphid feeding has typically caused higher yield loss than defoliating insect, such as green cloverworm, *Plathypena scabra* (Fabricius) (Noctuidae: Lepidoptera) (Ostlie 2001, Blackman and Eastop 2002). High soybean aphid densities can also cause significant yield losses when attacks occur during early reproductive stages (Myers et al. 2005b).

Soybean aphid can indirectly damage plants by vectoring viral diseases that can have a significant impact on soybean yield and seed quality (Irwin and Goodman 1981, Zhang and Zhong 1982, Guo and Zhang 1989, Li and Pu 1991, Luo et al. 1991, Quimio and Calilung 1993). In China, soybean mosaic virus (SMV) is widespread and known to spread quickly via aphids in soybean fields (Irwin and Goodman 1981, Quimio and Calilung 1993, Wu et al. 2004). Other viral diseases transmitted by soybean aphids in China include: soybean stunt virus (*Secoviridae: Nepovirus*), soybean dwarf virus (*Luteoviridae: Luteovirus*), abaca mosaic (*Potyviridae: Potyvirus*), beet mosaic (*Potyviridae: Potyvirus*), tobacco vein-banding mosaic virus (*Potyviridae: Potyvirus*),

peanut mottle virus (*Potyviridae: Potyvirus*), peanut stripe potty virus (*Potyviridae: Potyvirus*), and peanut mosaic virus (*Potyviridae: Potyvirus*) (Iwaki 1979). In the United States (U.S.), soybean aphid has been shown to vector soybean mosaic virus and alfalfa mosaic virus in soybean (Hill et al. 2001, Wang and Ghabrial 2002). In addition, soybean aphids vector viruses that threaten productivity of other cropping systems, such as potato virus Y (PVY) in potato, cucumber mosaic virus (CMV) in snap beans, and bean common mosaic virus (BCMV) in dry beans (University of Minnesota 2006, Davis et al. 2005). While searching for preferred hosts, alate aphids with their frequent movement in the field make short shallow probes on non-hosts, an action that maximizes the transmission potential of several viral diseases from diseased plants to healthy non-host plants (Berlandier et al. 1997).

Integrated Pest Management of Soybean Aphid

Integrated pest management (IPM) of the soybean aphid involves the use of multiple control strategies including chemical, cultural, biological control, and host plant resistance (Wang et al. 1962, Chung et al. 1980, Wang and Ba 1998, Li et al. 2000, Sun et al. 2000, Ragsdale et al. 2004, Wu et al. 2004, Fox et al. 2005, Ragsdale et al. 2007). An IPM program is a systematic approach that routinely monitors the pest population densities in the field and encourages the judicious application of insecticides when pest populations reach economically damaging levels, and thus reduces the risks of yield loss and production costs to producers (Fernandez-Cornejo 1998, Olson et al. 2008, Song and Swinton 2009).

Chemical Control

The use of insecticides is the predominant effective strategy for the control of soybean aphids (Wang et al. 1962, Ye et al. 1998, Wang et al. 1998, Li et al. 2000, Sun et al. 2000, Ostlie 2002). Broad spectrum insecticides also can control other soybean insect pests present in soybean fields (Wu et al. 2004). Myers et al. (2005b) reported a 31 % increase in soybean yield when pyrethroids (λ -cyhalothrin) and organophosphates (chlorpyrifos) were applied for soybean aphid control during the reproductive growth stages of the plant.

In Asia, there are numerous insecticides available for soybean aphid control that is usually applied at least four times during a growing season to avoid yield loss (Dai and Fan 1991, Wu et al. 2004). In the United States, the invasion of soybean aphids has led to an increase in the frequency of insecticide applications in soybean (Steffey 2004, DiFonzo 2009, Johnson et al. 2009). For example, in 2000, only 0.1% of soybean acreage in the US was reported to be treated with an insecticide; however, in 2006, insecticide usage increased to 13% due to treating soybean aphid infestations (Ragsdale et al. 2011). The increased use of insecticide has gradually increased costs of soybean production by 16-33 USD per hectare (Ragsdale et al. 2007). In the US, two classes of insecticides, pyrethroids (λ cyhalothrin, esfenvalerate, zeta-cypermethrin, and beta-cyfluthrin) and organophosphates (dimethoate, chlorpyrifos, methyl parathion) are commonly applied as foliar broadcast sprays for soybean aphid control (Ostlie 2002, Glogoza 2004, Eisely and Hammond 2007). In addition, several neonicotinoid insecticides used as seed treatments have systemic activity in the plant, and are effective against piercing and sucking insects such as aphids (Nault et al. 2004, Tomizawa and Casida 2005, O'Neal and Johnson 2010).

Neonicotinoid insecticides registered as soybean seed treatments for control of soybean aphid include thiamethoxam (Cruiser MAXX, Syngenta Crop Protection Inc., Greensboro, NC) and imidacloprid (Gaucho, Bayer Crop Science LP., Research Triangle Park, NC).

Insecticides are applied when aphid populations reach the economic threshold of 250 aphids per plant on 80% of plants, with most frequent application during the late vegetative through the reproductive stages of plant development (Chen and Yu 1988, Ragsdale et al. 2007). Treating soybean at the economic threshold prevents the aphid population from reaching the economic injury level of 675 aphids per plant, which is when significant yield loss and permanent plant injury occurs (Ragsdale et al. 2007).

Due to the high reproductive capacity of soybean aphids when environmental conditions are favorable, field populations can rebound quickly in spite of insecticide application (Myers et al. 2005a). In addition, frequent application of insecticides has accelerated the development of aphid resistance to certain classes of insecticides. For example, China reported soybean aphid resistance to organophosphate insecticides (Huang et al. 1998). Another detriment of insecticide usage is the accumulation of chemical toxins in the environment that may poison humans and wildlife (Ye et al. 1998, Wu et al. 2004). Still another negative impact of extensive broad-spectrum insecticide use is a reduction of the soybean aphid's natural enemies (Gao et al. 1993b, Wang et al. 1993, Qu et al. 1987, Sun et al. 2000). For example, Moser and Obrycki (2009) reported a higher incidence of larval mortality of *Harmonia axyridis* Pallas (Coleoptera: Coccinellidae), a soybean aphid predator, when exposed to seedlings treated with neonicotinoids. Additionally, exposed larvae showed several neurotoxic symptoms such as trembling, paralysis, and loss of coordination (Moser and Obrycki 2009).

Cultural Control

Cultural practices can be an effective alternative pest control method that may reduce the need to apply insecticides (Wu et al. 2004). Some of the examples of cultural practices that help manage the soybean aphid populations are altering planting dates, intercropping, row spacing, trap cropping and multiple cropping systems. These practices may disrupt the availability of the host soybean plants, alter the quality of the plant as a host, or enhance aphid natural enemies (Herzog and Funderburk 1986, Wang and Ba, 1998, Wang et al. 2000). For example, intercropping soybean with maize resulted in a significant increase in soybean yield, as this practice presumably increased the natural enemies population that eventually lowered the soybean aphid densities when compared to sole cropping of soybean (Wang and Ba 1998). A similar study conducted by Schmidt et al. (2007) illustrated that intercropping alfalfa living mulch with soybean increased the natural enemy populations by 45%, which in turn, delayed aphid establishment and helped to maintain aphid populations below economic thresholds. Late-planting soybean facilitates development of the sexual generation and overwintering soybean aphids, whereas early planting may help plants escape early aphid colonization (Ragsdale et al. 2004). Another possible practice is to narrow the spacing between soybean rows, which helps to maintain a closed canopy and conserve natural enemies (Kogan and Turnipseed 1987).

Host Plant Resistance

Genetically-based plant resistance is an important component of IPM programs and may be an effective alternative strategy for pest management of the soybean aphid (Auclair 1989, Harrewijn and Minks 1989). Besides lowering aphid populations directly, using resistant soybean varieties can reduce the dependency on insecticides in soybean

production, thereby conserving natural enemy populations in the field (Ye et al. 1998, French-Constant et al. 2004).

Resistance can be defined as the morphological, chemical or biological qualities possessed by the plant that reduces damage caused by herbivorous insects (Painter 1951). The degree of resistance can be measured by plant qualities that help to prevent colonization, development, and/or subsequent growth of an insect herbivore (Singh and Singh 2005). Painter (1951) categorizes insect resistance into three groups: antibiosis, antixenosis, and tolerance. Antibiosis is the ability of the plant to influence the insect's development, fecundity, growth, survival, or attack frequency, because the herbivore is negatively affected by plant chemicals and morphological characteristics (Painter 1951). Antixenosis is also known as non-preference and is the ability of the plant to avoid colonization or oviposition. This is governed by plant morphological or chemical characteristics, which ultimately affects oviposition and reduces the number of initial colonizers (Painter 1951, Singh and Singh 2005). Tolerance is the ability of the plant to withstand attack and damage without significant economic yield loss (Painter 1951, Kennedy et al. 1987).

Several breeding programs have been initiated in China for the development of soybean varieties that are resistant to insects and diseases (Wu et al. 2004). In China, resistant genes from wild soybean (*G. soja*) have been incorporated into commercial soybean varieties (*G. max*) (Sun et al. 1991). Fan (1988) screened 181 soybean varieties and identified two resistant to soybean aphids. He et al. (1995) observed that resistant plants had lower aphid colonization, and as a result aphids exerted less injury to resistant plants compared to susceptible plants.

In North America, Hill et al. (2004a) discovered varieties with resistance to soybean aphids. Out of the five varieties studied (Palmetto, CNS, PI71506, Dowling, and Jackson), Dowling and Jackson showed strong resistance to soybean aphid colonization in both choice and no-choice tests (Hill et al. 2004a). Hill et al. (2004b) determined Dowling and Jackson operated via antibiosis and had negative effects on the survival and fecundity of soybean aphids. On the other hand, resistance in PI71506 and CNS was attributed to antixenosis, since the variety appeared to repel soybean aphid colonization (Hill et al. 2004a). Li et al. (2004) investigated the possible effects of resistance on fecundity, longevity, maturity, and mortality of soybean aphids reared on susceptible (Pana and Loda) and resistant soybean varieties (Dowling, PI200538, and Jackson). Li et al (2004) discovered that potentially toxic compounds present in resistant soybean varieties altered the feeding behavior of the soybean aphid, which later interfered with aphid metabolism resulting in significantly lower fecundity and longevity, and higher mortality. A single dominant gene, *Rag1*, governs resistance to the soybean aphid in the resistant variety Dowling (Hill et al. 2006a). Resistance to the soybean aphid in Jackson is also due to the presence of a single dominant gene that has the same linkage group as Dowling (M) and may be allelic with *Rag1* (Hill et al. 2006a, b; Hill et al. 2010). Later on, a different gene named *Rag2* was found to be the source of resistance in soybean germplasm, PI243540 (linkage group F) (Mian et al. 2008), whereas the *Rag3* gene was identified in the soybean germplasm accession PI567543C (Zhang et al. 2010). PI567541B and PI567598B showed an antibiosis type of resistance, which was governed by two unidentified recessive genes other than the dominant ones in *Rag1* and *Rag2* (Zhang et al. 2009). In the United States, the release of a soybean aphid resistant variety with the *Rag1* gene has recently been

commercialized and confers antibiotic and antixenosis resistance along with some tolerance (Chiozza et al. 2010).

Morphological or chemical characteristics possessed by resistant soybean cultivars can influence the feeding behavior of soybean aphids (Diaz-Montano et al. 2006). Soybean aphids reared on four resistant soybean varieties (i.e. K1639, Pioneer 951397, Dowling, and Jackson) took longer to reach phloem tissue and spent only about 2-7 minutes ingesting phloem sap in comparison to more than 60 minutes on the susceptible check (KS4202) (Diaz-Montano et al. 2006). Diaz-Montano et al. (2007) postulated that detrimental toxic substances in the phloem sap of aphid resistant soybean plants were responsible for changing the feeding behavior (or stylet penetration) of soybean aphids. Volatile chemicals released from aphid resistant soybean varieties during feeding may affect soybean aphid oviposition behavior, while also assisting in plant defense by attracting predatory insects to feed on aphids (Zhu and Parks 2005). For example, methyl salicylate, a volatile released from soybean plants when attacked by soybean aphids, increased the abundance of several predatory insects, including mites (Acari: Phytoseiidae), minute pirate bugs (Hemiptera: Anthocoridae), big eyed bugs (Hemiptera: Lygaeidae), and lady beetles (Coleoptera: Coccinellidae) (Zhu and Parks 2005).

Soybeans deficient in potassium can have higher soybean aphid populations than normal plants, and reflect the importance of plant nutrition for insect herbivore populations (Myers et al. 2005a, Myers and Gratton 2006, Walter and DiFonzo 2007). Hu et al. (1992) studied the relationship between the nitrogen content in soybean leaves and the occurrence of the soybean aphid. They concluded that the higher nitrogen content of soybean leaves has been correlated with higher soybean aphid populations and thus could potentially

impact resistance. Hu et al. (1993) noticed that lignin levels in the leaves of soybean plants are involved in the chemical defense mechanism of plants against soybean aphids; varieties with high levels of lignin showed greater resistance to soybean aphids.

Biological Control

The successful use of natural enemies for management of soybean aphids in Asia has increased interest in using biological control as part of IPM programs in North America (Rutledge et al. 2004). In the U.S., both generalist and specialist natural enemies are believed to contribute to soybean aphid suppressions (Fox et al. 2004, Liu et al. 2004, Kaiser et al. 2007). In Asia, several beneficial organisms including, pathogens, predators, and parasitoids, are natural enemies of soybean aphids, and many of them are considered to be effective biological control agents (Wu et al. 2004). In this region, several studies have confirmed the importance of predators and parasitoids in helping maintain soybean aphid populations below the economic injury level (Van den Berg et al. 1997, Liu et al. 2004, Wu et al. 2004, Miao et al. 2007). In China and South Korea, soybean aphids are attacked by as many as thirty species of predators, fifteen species of parasitoids, and one pathogen (Wu et al. 2004).

Plant Pathogens that Infect Soybean Aphid. Limited research into fungal pathogens of the soybean aphid has been conducted. In China, *Entomophthora fresenii* (Nowak), a common pathogen, provides effective control of the soybean aphid and is abundant in fields with high humidity and high aphid densities (Chen and Yu 1988).

In North America, only a few species of entomopathogenic fungi have been recorded infecting soybean aphids, and they have had less impact on soybean aphid control than predators (Rutledge et al. 2004). Neilsen and Hajek (2005) identified seven different

pathogenic fungi attacking soybean aphids in New York, with the predominant species being *Pandora neoaphidis* (Remaudie`re & Hennebert) Humber (Entomophthorales: Entomophthoraceae). *Pandora neoaphidis* typically caused the highest infection rate when aphid populations increased early in the season (Nielsen and Hajek 2005). Besides *P. neoaphidis*, other species of fungal pathogens reported include: *Conidiobolus thromboides*, *Entomophthora chromaphidis* (O.F. Burger & Swain), *Pandora* sp., *Zoophthora occidentalis* (Thaxt.) A. Batko, *Neozygites fresenii* (Nowak.) Remaud.& S. Keller), and *Lecanicillium lecanii* (Zimmermann) (Nielsen and Hajek 2005). However, late season infections caused a longer suppression period of soybean aphids because *P. neoaphidis* was able to develop a reservoir of inoculum to infect aphid populations next spring (Nielsen and Hajek 2005). In Minnesota, Koch et al. (2010) reported that three dominant species of pathogenic fungi infected soybean aphids in the field: *P. neoaphidis*, *C. thromboides* and *Zoophthora radicans*.

Predators of Soybean Aphid. Several predatory insects have been reported feeding on the soybean aphid in Asia. In China, common predators include lady beetles, lacewings, and syrphid flies (Gao 1991, Wu et al. 2004). Ma et al. (1986) provided an overview of the soybean aphid predators prevalent in China, which included several coccinellid beetles (seven spotted lady beetle, *Coccinella septempunctata* L.; *Adonia variegata* Goeze; the multicolored Asian lady beetle, *H. axyridis* (Pallas); *Propylaea japonica* (Thunberg); *Scymnus hoffmanni* Weise; and *Coelophore saucia* Mulsant (Dai 1991, Wang et al. 1991, Han 1997). Wang and Ba (1998) determined that *P. japonica* and *H. axyridis* were the most common predatory species in China, and accounted for over 62% and 10% of all predators, respectively. In the field, larvae of *P. quadrfasiatus* attacked and fed upon an average of

53-67 aphids per day, which resulted in an average predation rate of 500-800 aphids during their lifetime (Gao 1991, Gao et al. 1996). Two species of syrphid flies, *Metasyrphus corollae* F. and *Paragus quadrfasiatus* Meigen (Diptera: Syrphidae), were found to be the most common hoverfly species in soybean fields (Gao 1991). Liu et al. (2004) emphasized the importance of parasitoids (*Lysiphlebus* sp.), coccinellids [*P. japonica* and *Scymnus (Neopullus) babai*], and the dipteran predator *Paragus tibialis* (Fallen) for controlling soybean aphid populations. In exclusion experiments using field cage (2 m × 1 m × 1.2 m), Liu et al. (2004) found a 12-fold increase in soybean aphid densities when both parasitoids and predators were excluded (Liu et al. 2004).

In North America, several observations have revealed the importance of the existing predator community for suppressing soybean aphid populations (Fox et al. 2004, Rutledge et al. 2004). Rutledge et al. (2004) identified 43 predatory taxa associated with the soybean aphid during a two-year study conducted in Indiana and Michigan. The dominant predators were the insidious flower bug, *Orius insidiosus* Say (Hemiptera: Anthocoridae) and the Asian ladybeetle (*H. axyridis*), which together comprised over 85% of all predators found. Rutledge et al. (2004) identified *H. axyridis* as a key predator causing more than 85% soybean aphid mortality in no-choice trials. In a field study conducted in Michigan, nine species of predatory flies and six hymenopteran parasitoids were found to complete their life cycle on soybean aphids (Kaiser et al. 2007). The species of predatory flies (Diptera) detected in the field included: *Aphidoletes aphidimyza* Rondani (Cecidomyiidae), *Leucopis glyphinivora* Tanasijtshuk (Chamaemyiidae) and the following hoverflies (Syrphidae) *Allograpta obliqua* Say, *Eupeodes americanus* Wiedemann, , *Sphaerophoria contigua* Macquart, *Eupeodes volucris* Osten Sacken, *Paragus hemorrhous* Meigen, *Toxomerus*

marginatus Say, and *Syrphus rectus* Osten Sacken (Kaiser et al. 2007). Research suggests that predators and pathogens which attack soybean aphids after they migrate to their overwintering host (buckthorn) help minimize aphid populations during the next growing season (Yoo et al. 2005, Nielsen and Hajek 2005).

Parasitoids of Soybean Aphid. Several species of soybean aphid parasitoids (Hymenoptera) are found in China (Wu et al. 2004). In China, Gao (1985) reported that 10-50 % of soybean aphids were parasitized by a common soybean aphid parasitoid, *Lysiphlebia japonica* (Ashmead) (Hymenoptera: Braconidae).

Moreover, *L. japonica* was effective in controlling soybean aphids early in the season with parasitism rates as high as 35%, and this helped decrease aphid populations later in the season (Gao 1994). Liu et al. (2004) reported *Lysiphlebus* as dominant genera of aphid parasitoids that can reduce significant number of aphid density. In Korea, several parasitoids species were found attacking soybean aphids and included *Aphidius absinthii* Marshall (Aphidiidae) and several braconidae: *A. cingulatus* Ruthe, *A. salicis* Haliday, *Ephedrus persicae* Froggatt, *E. plagiator* Nees, *Lipolexis gracilis* Förster, *Lysephedrus validus* Haliday, and *L. japonica* (Ashmead) (Chang et al. 1994). Likewise, Van den Berg et al. (1997) recommended early season conservation of the lady beetle *Harmonia arcuata* (Coleoptera: Coccinellidae) in Indonesia to help minimize the use of insecticides.

The effectiveness of hymenopteran parasitoids for soybean aphid control in Asia illustrates the potential advantages of using parasitoids in North America as classical biological control agents (Heimpel et al. 2004, Liu et al. 2004, Ragsdale et al. 2004, Wu et al. 2004, Wyckhuys et al. 2007b). More than 40 species of parasitoids from China have been identified and brought into quarantine for further study in the United States (Liu et al.

2004, Heimpel et al. 2010, Ragsdale et al. 2011). The majority of parasitoids imported from Asia were *Aphelinus* spp. along with other Braconidae species (Ragsdale et al. 2011).

Hymenopteran parasitoids may be more effective at controlling soybean aphids when used with dipteran predators (Kaiser et al. 2007). Species investigated included several braconidae; *Lysiphlebus testaceipes* Cresson, *Aphidius colemani* Viereck, *Binodoxys kelloggensis* Pike, Starý & Brewer, and *Praon* sp., and aphelinidae; *Aphelinus asychis* Walker, and *Aphelinis albipodus* Hayat & Fatima (Kaiser et al. 2007). However, Nielsen and Hajek (2005) found parasitoids (*Aphidius* sp. and two *Praon* sp.) to be inefficient in causing soybean aphids mortality, because they observed low parasitism rates (7-15%) and high non-emergence rates of adult parasitoids (56%). A similar study conducted by Costamagna et al. (2007) observed the ineffectiveness of the native parasitoid, *L. testaceipes* (Cresson), in controlling soybean aphid population growth compared to generalist predators such as coccinellids.

***Binodoxys communis* as a Parasitoid of Soybean Aphid.** *Binodoxys communis* Gahan (Hymenoptera: Braconidae) is a parasitoid native to China that has recently been introduced for study in North America and is known to be highly host specific, attacking only *Aphis* species (Wyckhuys et al. 2007a, Desneux et al. 2009, Wyckhuys et al. 2009). Desneux et al. (2009) studied the host specificity behavior of *B. communis* on 20 different aphid species from the subfamily Aphidiinae. Of the 20 species, parasitism rates on soybean aphids was more than 50%, while less than 1% parasitism occurred on other aphid species. Later, Wyckhuys et al. (2008a) reported parasitism rates, proportion of successful emergence, days to mummification, and sex ratio of *B. communis* on soybean aphids.

Despite some evidence of the potential effectiveness of *B. communis*, several laboratory studies have discovered ecological factors that may limit its utility as a biological control agent of soybean aphid (Wyckhuys et al. 2007b, 2009). In Minnesota, ants that tended the soybean aphid colonies disrupted *B. communis* access to aphid hosts (Wyckhuys et al. 2007b). Other factors responsible for poor *B. communis* success included: 1) difficulty in identifying overwintering hosts, 2) competition from numerous predators in soybean field, and 3) intraguild predation of parasitized aphids by coccinellids (Chacón et al. 2008, Chacón and Heimpel 2010, Heimpel et al. 2010).

Tritrophic Interactions between Resistant Plants, Herbivores, and Natural Enemies. Using both host plant resistance and biological control simultaneously to suppress insect herbivores does not always result the level of control that one can expect given what each method can perform by itself. One reason is that herbivore-resistant plants may influence the natural enemy, thereby altering natural enemies' ability to control the herbivore. Resistant plants are different than susceptible plants in their morphological, nutritional and/or chemical characteristics (e.g. volatile chemical cues, leaf waxiness, trichome density), and these differences can influence insect herbivore populations by altering herbivore colonization, development time, growth rate, and population density (Ode 2006). In addition, resistant plants can impact individual herbivores by affecting their size as well as nutritional quality, which are later used as hosts as a food source by natural enemies (Bottrell et al. 1998). Since natural enemies depend on insect herbivores as their food source, these different plant characteristics can affect the natural enemies by altering the natural enemies' food (Bergman and Tingey 1979, Price et al. 1980, Hare 1992, Bottrell et al. 1998, Hare 2002). Natural enemies that feed on insect herbivores reared on resistant

plants can experience positive or negative effects on their survival, development, fecundity, and fitness (Farid et al. 1998, Birch et al. 1999, Kalule and Wright 2002, Ode 2006, Cai et al. 2009).

Since parasitoid offspring develop within herbivore hosts, the quality of the host can influence the size, development, and fecundity of parasitoid offspring (van Emden 2002). For example, certain host plants are resistant to the parsnip webworm (*Depressaria pastinacella* (Lepidoptera: Oecophoridae) because those plants contain toxic compounds. These toxic compounds can negatively affect the survivorship of parasitoid wasps (*Copidosoma sosares* (Walker) (Hymenoptera: Encyrtidae) when they attack webworms that have fed on resistant plant varieties (Ode 2004).

Several studies have identified negative effects of resistant plants on biological control agents (Starks et al. 1972, Salto et al. 1983). Examples include a longer developmental time and reduced larval weight of the larval parasitoid (*Microplitis demolitor* Wilkinson) when their host, the soybean looper (*Pseudoplusia includes* Walker), was reared on resistant soybean variety 'PI227687' compared to a susceptible variety (Yanes and Boethel 1983). Similarly, when soybean looper was reared on the resistant soybean variety (PI 227687), parasitoid performance (*Copidosoma truncatellum* Dalman) declined significantly (Orr and Boethel 1985).

Not all effects of resistant plants are negative, however. Kauffman and Flanders (1985) reported increased fecundity of a Mexican bean beetle parasitoid, *Pediobius foveolatus* Crawford, when it fed on the beetle, *Epilachna varivestis* Mulsant, reared on a resistant soybean (Culter 71) compared to susceptible varieties (Bonus, William and Lima Bean 'Henderson Bush').

Identifying effects of resistant plants on natural enemies is only the first step in determining whether the two control strategies will work together better than either one alone. Even if natural enemies don't perform as well on herbivore hosts from resistant plants, they may still be able to help control herbivores to some degree. In the previous example, the combination of host plant resistance and biological control agents were considered compatible and effective despite negative effects on the parasitoid (Yanes and Boethel 1983). Identifying interactions between host plant resistance and biological control agents will, however, help determine potential problems and when it may not be productive to use two control strategies simultaneously.

***BINODOXYS COMMUNIS* (GAHAN) (HYMENOPTERA: BRACONIDAE)
PRODUCTION AND FITNESS ON THE SOYBEAN APHID, *APHIS GLYCINES*
MATSUMURA (HEMIPTERA: APHIDIDAE),
REARED ON SUSCEPTIBLE AND RESISTANT SOYBEANS**

Abstract

A near isogenic susceptible soybean variety without the *Rag1* gene and resistant variety with the *Rag1* gene were evaluated in the laboratory for their effects on the growth rate of soybean aphid, *Aphis glycines* Matsumura, and their impact on fitness of the soybean aphid parasitoid, *Binodoxys communis* (Gahan). The presence or absence of the *Rag1* gene was verified by quantifying soybean aphid growth rates and PCR of leaf tissue using the Satt 435 marker. Parasitoid fitness was measured in terms of total number of mummies produced, parasitoid emergence rate, parasitoid development time, adult body, and hind metatibiae length. Adult parasitoids that attacked soybean aphids reared on susceptible plants produced significantly more mummies. Susceptible plants also produced more adult parasitoids due to more mummies and a higher emergence rate of mummies on susceptible plants compared to resistant plants. Parasitoid development time was one day longer on resistant plants compared to susceptible plants. Although the total number of adult parasitoids that emerged from resistant plants was low, these parasitoids had significantly shorter metatibiae compared to those from susceptible plants. This study reveals that biological control by *B. communis* may be compromised when host plant resistance is also utilized for pest management of soybean aphids.

Introduction

A primary concept underlying integrated pest management (IPM) is the use of multiple pest control strategies (Allen and Rajotte 1990, Kogan 1998). Two common IPM strategies are deploying host plants that are resistant to herbivores and promoting biological control agents that can also help control herbivores (Smith 2005). Combining these two strategies can result in enhanced herbivore suppression compared to using either method alone, although in some cases one of these pest management strategies may negatively influence the other (Beddington et al., 1978, Auclair 1989, Harrewijn and Minks 1989, Bottrell et al. 1998, Dogramaci et al. 2005).

Resistant plants often have chemicals and morphological characteristics that make them less attractive to insect herbivores (antixenosis, non-preference) or negatively influence pest fecundity, survival, or development time (antibiosis) (Painter 1958, Singh and Singh 2005). However, these same attributes can directly or indirectly impact higher trophic levels, often by affecting the quantity and quality of the herbivores that natural enemies rely on to reproduce (Ode 2006). Endoparasitoids that develop within their hosts may be particularly susceptible, as host quality can impact survival of immature parasitoids, development time, and ultimately adult fitness (Barbosa et al. 1982, Duffey and Bloem 1986, van Emden 1995). In addition, parasitoid foraging behavior can be influenced by morphological and chemical attributes of resistant plants, which could ultimately undermine the effectiveness of biological control (Turlings and Benrey 1986, Gould et al. 1991, Hare 1992, Ode 2006).

Since its first detection in North America in 2000, the soybean aphid, *Aphis glycines* Matsumura (Hemiptera: Aphididae), has spread throughout soybean-growing

regions in the north central U.S. and some Canadian provinces, where it is considered one of the most economically important pests of soybean (Alleman et al. 2002, Venette and Ragsdale 2004). Besides causing direct damage to plants, soybean aphids can also vector several viral diseases and excrete honeydew, promoting a fungus known as sooty mold that disrupts photosynthesis (Guo and Zhang 1989, Clark and Perry 2002). Insecticide applications are a common and effective method of soybean aphid control, although drawbacks associated with broad-spectrum pesticide use (e.g. insecticide resistance, non-target effects, environmental contamination) make alternative management strategies desirable (French-Constant et al. 2004, Heimpel et al. 2004, Ragsdale et al. 2007). Classical biological control and host plant are two non-chemical approaches that are currently being explored for soybean aphid management (Heimpel et al. 2004, Hill et al. 2004b, Wyckhuys et al. 2009).

Binodoxys communis (Gahan) (Hymenoptera: Braconidae) is a monophagous aphid parasitoid wasp native to China that has been recently released in the United States on a limited scale for experimental purposes (Wyckhuys et al. 2007b). Due to its high prey specificity, *B. communis* is thought to be more effective than other biocontrol agents at maintaining soybean aphid populations at low levels (Desneux et al. 2009, Wyckhuys et al., 2007b). Plant resistance to the soybean aphid has been advanced with the discovery of cultivars like 'Dowling' and 'Jackson,' which show resistance to the soybean aphid (Hill et al. 2004a). Resistance in the 'Dowling' variety is associated with the *Rag1* gene that limits soybean aphid colonization in no-choice tests and negatively affects the fecundity, survival, longevity, and development of the soybean aphid (Li et al. 2004, Hill et al. 2006a). *Rag1* is currently being bred into commercial soybean lines. Despite the potential associated with

both of these control strategies, there has been little work investigating their effects on each other.

The overall research objective was to compare effects of resistant soybean plants with the *Rag1* gene and susceptible soybean plants without the *Rag1* gene on the development and fitness of the soybean aphid parasitoid, *B. communis*. We first confirmed that our lines differ in their resistance to soybean aphid by measuring the growth rate of aphid populations reared on resistant and susceptible soybean plants and by PCR. We then assessed effects of the *Rag1* gene on the parasitoid by allowing parasitoids to attack and develop on soybean aphids fed on either resistant or susceptible plants. The total number of aphids, mummies, proportion parasitism and successfully emerged adult parasitoids was determined for each plant type. We compared fitness parameters of emerged parasitoids, including: days to adult emergence, adult body length, and hind tibiae length.

Materials and Methods

Experimental Organisms

Soybean aphid and *B. communis* colonies were maintained in the laboratory in the Department of Entomology at North Dakota State University (NDSU) in Fargo, ND. The soybean aphid colony was originally established using aphids collected from a soybean field located at the Prosper Agricultural Experimental Station Farm near Prosper, ND. The *B. communis* colony (Harbin strain) was established with thirty mummies obtained from the laboratory of Dr. George E. Heimpel, University of Minnesota (UMN), St. Paul, MN (Wyckhuys et al. 2007a). Dr. Heimpel's parasitoid colony at UMN was originally established from seven males and thirty-three females of parasitized *A. glycines* (mummies)

that were collected near Harbin and Suihua county of Heilongjiang province, China in late August 2002.

Both insect colonies were maintained and reared on susceptible soybean plants, RG607RR (details below), in a collapsible insect rearing cage (Mega View Science Co. Ltd., Taiwan; Fig. 1), in separate environmental chambers ($25 \pm 2^\circ\text{C}$, 60-80% RH, and L16:D8 photoperiod). To maintain aphid colonies, older soybean plants were replaced with new plants every 10-12 d, while parasitoid colonies were renewed every 12 d with aphid infested susceptible plants from soybean aphid colony.

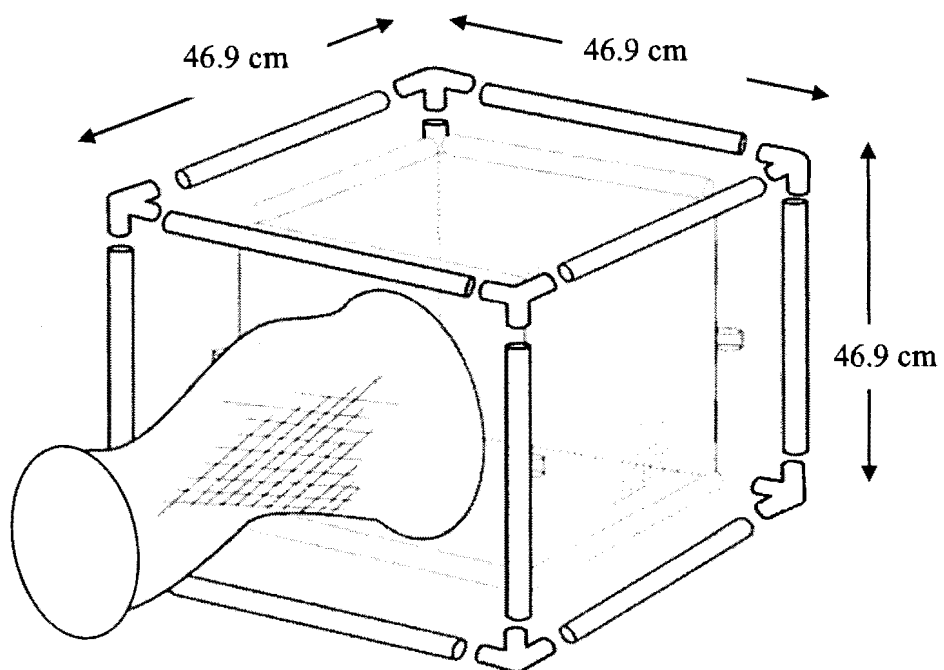


Figure 1. Collapsible insect rearing cage used for maintaining soybean aphid and *B. communis* colonies.

Plant Materials

Two near isogenic lines of soybean were used in experiments: a susceptible plant, RG607RR, and a near isogenic resistant plant with the *Ragl* gene. The source of the *Ragl* gene we used for the experiment was LDXG04018-3 (provided by Brian Diers, University

of Illinois, Urbana-Champaign, IL), which is the result of a cross of Dwight X (Loda X Dowling) (T. Helms, pers. comm.). Later, the cross of RG607RR X LDXG04018-3 was made to form the F₁ generation. The F₁ generation plants were then backcrossed to RG607RR to produce BC₁F₁ (backcrossed F₁ generation) generation.

However, before crossing was made between F₁ plants to RG607RR, the incidence of the *RagI* gene in F₁ plants was confirmed with aphid counts and by molecular markers. Thus, BC₁F₁ plants were backcrossed to RG607RR to form the BC₂F₁ generation. Again, BC₂F₁ plants were screened with aphid and molecular markers. Once again BC₂F₁ plants were selected for crossing to RG607RR to form the BC₃F₁ generation. Later, those BC₃F₁ plants were allowed to self-pollinate to form the BC₃F₂ (backcrossed 3 times to get F₂ generation) generation. After harvesting individual BC₃F₂ plants, the seed of each BC₃F₂ plant was put into a separate envelope to form BC₃F_{2:3} lines, which simply means that the lines are F₃ seed from individual F₂ plants. Then, the seed of each BC₃F₂ plant were planted in a separate row to form F_{2:3} plant-rows. Lines of early maturity were selected and harvested. In 2006-2007, these selected F_{2:3} plant-rows were then screened for the aphid resistance with the help of SSR markers and greenhouse screening using petri dishes on individual leaves with aphids (Hochhalter 2009). In the summer of 2008, replicated yield plots were evaluated in Morris, MN and Prosper, ND where each plot was naturally infested with soybean aphids, and aphid counts and yield were taken (Hochhalter 2009). In 2008-2009, yield and aphid count data were analyzed to confirm that some of the soybean experimental lines showed excellent performance in terms of aphid resistance. Yield of *RagI* near-isogenic lines were comparable to yield of the recurrent parent, RG607RR (T. Helms, unpub. data).

For our experiment, we confirmed the presence of the *Rag1* gene in resistant plants by performing aphid growth rate experiments (See Aphid Growth Section) and by testing individual plants for the allelic state of the *Rag1* using polymerase chain reaction (PCR) (Appendix I). Li et al. (2007) mapped the *Rag1* gene to chromosome 7 (formerly linkage group M) between the markers Satt435 and Satt463. Further research by Kim and Diers (2009) and Kim et al. (2010) confirmed that the *Rag1* is in this interval, and that Satt435 is tightly linked to the *Rag1* locus. PCR was performed using Satt435 primers and the protocol described by Kim and Diers (2009) (For details, see Appendix I). The PCR markers showed that all resistant plants used for our experiment had the dominant *Rag1* allele.

Both the near isogenic soybean lines were grown under greenhouse conditions ($25 \pm 5^\circ\text{C}$, 60-80% RH, and L16:8D photoperiod) in the winter of 2010. Three seeds of each near isogenic soybean lines were planted in 10.2×10.2 cm plastic pots and new plants were planted every week to maintain colonies and perform experiments. The majority of new plants supplied to insect colonies were at the V3 developmental stage (Herman 1988). Pots used for experiments were thinned to one plant per pot.

Aphid Growth

The objective of this experiment was to compare soybean aphid populations grown on resistant and susceptible soybean plants. This study helped validate whether our two near isogenic soybean lines differed in terms of aphid resistance. It also helped us explain differences that we observed in parasitoid performance on susceptible and resistant plants.

Ten plants of each line were used and the plant location was randomized. The experiment was repeated twice for a total sample size of 20 plants per variety. Each

experimental plant was covered with a clear plastic cage (50.8 cm high × 15.3 cm diameter) with a fine nylon mesh at the top of the cage to allow for air flow. Caged plants were kept in trays filled with water to ensure that no aphids were able to enter or exit the bottom of the cage.

Four days after the development of the first set of trifoliolate leaves, aphids were transferred to each experimental plant. Aphids were transferred by first placing several aphid-infested trifoliolate leaves on moistened filter paper in a petri dish under incandescent table lamps. This process encouraged aphids to remove their proboscis from leaves. Walking aphids were transferred to the first trifoliolate leaves of experimental plants using a fine paint brush. We tried to control the aphid density by infesting each experimental plant with a mixture of five nymphs (approximately 3rd instar) and five apterous adults. Aphids were counted on each plant 24 h after initial inoculation and then every other day for 14 d. We ended the experiment after 14 d to coincide with the length of one generation of *B. communis* (Wyckhuys et al. 2008a).

To investigate treatments effects on aphid establishment and reproduction, we calculated the following response variables: total number of aphids 24 h after inoculation, size of the aphid population after 14 d, and per capita growth rate (\log [aphids at day 14/aphids after day 1]). The number of aphids observed after 24 h was comprised of aphids that successfully established on a plant and any nymphs born during the first 24 h. In the first run of the experiment, we did not count small and medium number of aphids separately on each treatment after 24 h of inoculation, but we counted them separately in the second run of the experiment. Potential homogenous growing conditions in the greenhouse were accounted for by placing one resistant plant next to a susceptible plant

within the tray and then each tray tested as a nested blocking variable within the temporal block. Since we found no effect of individual trays we used the temporal block as the only blocking variable. Data were analyzed using two-way analysis of variance (ANOVA) (JMP; SAS Institute Inc. 1989-1999). Soybean aphid densities at the end of the experiment were log-transformed and the total number of aphids after 24 h was square-root transformed for normality and homogeneity of variance to meet assumptions of parametric statistical tests.

Parasitoid Experiment

To better understand how *B. communis* responds to soybean aphids on individual resistant and susceptible plants, we allowed individual parasitoids to attack aphids on one of the two types of soybean (resistant or susceptible).

For rearing adult parasitoids for the experiment, we first took mummies from the parasitoid colony and kept individually in a clear gelatin capsules (Size 0; Rx Co. Ltd, West Fargo, ND, USA, Fig. 2) nested within a 1.5 ml micro centrifuge tube (Brinkmann Instruments Inc., Westbury, NY, USA, Fig. 3). Once parasitoid adults emerged, pair of male and female adults were isolated in glass vials with plaster of Paris (DAP Inc., Baltimore, MD, USA, 21224) at the bottom to maintain moisture, and provided cotton balls (size 5) soaked in a honey solution (4 : 1 water: honey). Female adult parasitoids were distinguished from males via their ovipositor, a tubular structure extending outside the abdomen. Paired adult parasitoids were given the opportunity to mate for 24 h prior to use in the experiment. Female parasitoids used in experiments were naïve (i. e. they never had the opportunity to encounter or attack any hosts until the experiment began).

Aphid density can be important in influencing parasitoid oviposition behavior, parasitism rates and other factors (Wyckhuys et al. 2008), and aphid density is likely to differ between resistant and susceptible plant varieties in the field. We tried to control the aphid density by transferring equal densities of aphids i.e. ten aphid nymphs (approximately 3rd instar) and ten apterous adults to each susceptible and resistant treatment plant using the same techniques as described previously. There were 24 replications per treatment across two temporal blocks. Plants were covered with a clear plastic cage (50.8 cm high × 15.3 cm diameter) with a fine nylon mesh at the top of the cage to allow for air flow (Fig. 4). Cage bottoms were placed in sand-filled aluminum foil plates to ensure that aphids and parasitoids were unable to escape from cages.

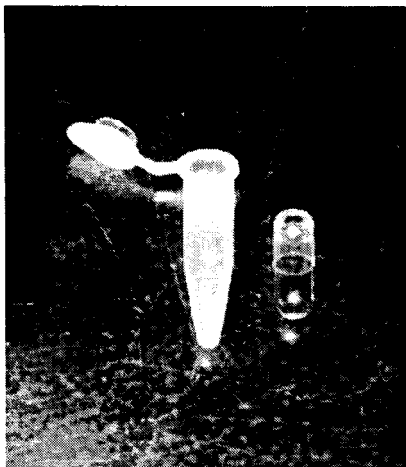


Figure 2. Microcentrifuge and gelatin capsules for keeping individual *B. communis* mummies.

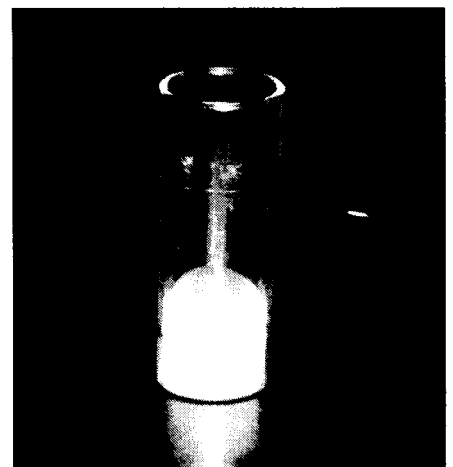


Figure 3. Glass vial with plaster of Paris at the bottom to isolate and mate newly emerged male and female adult parasitoids.

Soybean aphids were given 24 h to establish on either a susceptible or resistant plant after which their densities were assessed. Then, a single mated female parasitoid was

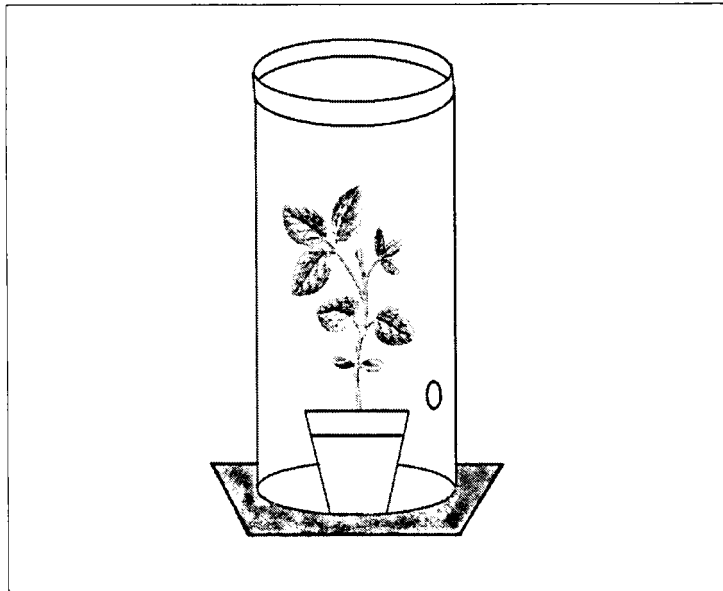


Figure 4. Setup of an experimental plant covered with a clear plastic cage placed on an aluminum foil plate containing sand.

released into each cage and given 24 h to attack aphids. The female parasitoid was removed from the cage after 24 h using an aspirator. When the female parasitoid was removed from the cage, we recorded whether the adult parasitoid was dead, alive, or missing however this information was not informative in explaining any response variables. Parasitized aphids (mummies) were counted every day starting three days after female parasitoids were removed from cages. After mummies were assessed in individual plant, they were counted and removed from the plant using a spatula. The experiment was ended after 15 d to make sure that we did not accidentally count any second-generation mummies that were created with emerged adults that we had missed.

The 15 d estimate came from the fastest development time that we observed any mummy being formed (6 d) and the fastest time we observed an adult emerging from a mummy (9 d). Collected mummies were placed individually into clear gelatin capsules (size 0), and then placed inside 1.5 ml micro-centrifuge tubes. Collected mummies were

maintained at normal room temperature ($25 \pm 5^{\circ}\text{C}$, 60-80% RH, and L16:8D photoperiod) and wasps were allowed to develop until emergence. We checked for adult parasitoids daily, and preserved emerged adults in glass vials with 95% ethanol labeled with treatment, date, time, and parasitoid sex. Development time from attack to mummy formation and from mummy to adult emergence was recorded. We also measured parasitoid body size and the length of their right and left metatibiae using a microscope stage micrometer accurate to one micron. Body size and hind metatibiae are often used for determination of parasitoid fitness, including fecundity and male mating ability (Ode and Strand 1995, Sagarra et al. 2001, Lampert et al. 2010).

To compare the performance of parasitoids on resistant and susceptible soybean plants, we used a number of response variables: (1) total number of mummies produced, (2) proportion of aphids parasitized (number of mummies found on a plant / number of aphids available to parasitize), (3) proportion of adult parasitoid emergence (number of successfully emerged adults from a plant / number of mummies found on that plant), (4) days to mummification, (5) days from mummification to adult emergence, (6) total development time for male and female parasitoids, (7) offspring mean body size (mm), and (8) offspring mean hind metatibiae length (mm). Data were analyzed using two-way ANOVA (JMP; SAS Institute Inc.) with plant identify as the independent variable (susceptible vs. resistant) and a blocking factor used to account for the two different runs of the experiment. All data were checked to meet the assumptions of ANOVA and transformations were made as necessary to data that was not normally distributed. In addition, likelihood ratio tests (adjusted G-tests) were used when comparing count data (number of plants producing mummies vs. not producing mummies in susceptible and

resistant plants; number of adults producing mummies vs. not in susceptible and resistant plants; and number of males vs. females produced across all susceptible and resistant plants). A paired *t*-test was used to compare development time of males and females from the same plant in the susceptible plant treatment, but not for parasitoids from resistant treatment as they were too low in numbers.

Results

Aphid Growth

In the second run of the experiment more aphids successfully established on susceptible plants than on resistant ones (9.9 ± 0.7 vs. 4.4 ± 0.6 ; $t = 5.4$; $df = 1, 18$; $P < 0.0001$), and more newly born aphids were found on susceptible plants than on resistant plants (7.9 ± 1 vs. 4.8 ± 0.7 ; $t = 2.6$; $df = 1, 18$; $P = 0.019$). After 14 d across both blocks, aphid populations were over 30 times greater on susceptible soybean plants than on resistant plant ($F = 139.6$; $df = 1, 37$; $P < 0.0001$; Table 1). Susceptible plants also had a significantly higher number of aphids 24 h after initial inoculation than on resistant plants ($F = 15.5$; $df = 1, 37$; $P = 0.0004$; Table 1).

We accounted for the difference in the number of aphids after 24 h by looking at the growth of aphid populations 24 h after inoculation to the end of the experiment. The susceptible soybean plant had a significantly higher per capita aphid growth rate than on the resistant plant ($F = 102.0$; $df = 1, 37$; $P < 0.0001$; Table 1).

Table 1. Effect of plant resistance on soybean aphid populations.

Treatment	No. of aphids 24 h after initial inoculation (Mean ± SE)	No. of aphids after 14 d (Mean ± SE)	Per capita growth rate ^a (Mean ± SE)
Susceptible	19.5 ± 2***	634.7 ± 68.0***	1.4 ± 0.1***
Resistant	10.7 ± 1.4	19.5 ± 2.8	0.2 ± 0.1

Means within a column are significant when followed by a *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; NS = not significant (ANOVA).

^a Average per capita change in aphid population 24 h after inoculation to the end of the experiment. Data were log-transformed to meet normality assumptions.

Parasitoid Experiment

At the end of the experiment almost 10 times as many mummies were produced on susceptible plants compared to resistant plants ($F = 78.6$; $df = 1, 45$; $P < 0.0001$; Table 2). The difference in mummy production was due to both a difference in the number of plants that successfully produced at least one mummy (23/24 susceptible plants vs. 17/24 resistant plants; $G_{adj} = 5.60$, $df = 1, 46$; $P = 0.018$) and the number of mummies produced on those plants that produced mummies (average \pm S.E.; susceptible plant 22.0 ± 1.5 vs. resistant plant 2.8 ± 1.8 ; $F = 64.99$; $df = 1, 37$; $P < 0.0001$).

This difference in mummy production could have been influenced by the number of aphids available for the parasitoid to attack. Despite starting all plants with the same number of aphids, 24 h after infestation there were 50% more aphids available on susceptible plants than on resistant plants ($F = 34.2$; $df = 1, 45$; $P < 0.0001$; Table 2). However, when we account for these differences in aphids available to parasitize by calculating proportion parasitism (mummies from a plant / aphids available to parasitize), susceptible plants still produced significantly more mummies than resistant plants ($F = 73.6$; $df = 1, 45$; $P < 0.0001$; Table 2).

The total number of adults produced on susceptible plants was almost 20 times greater than the number produced on resistant plants ($F = 34.8$; $df = 1, 45$; $P < 0.0001$; Table 2). Most susceptible plants produced at least one adult parasitoid compared to resistant plants (20/24 susceptible plants vs. 6/24 resistant plants; $G_{adj} = 17.1$; $df = 1, 46$; $P < 0.0001$) and no resistant plants produced more than two adults. Besides susceptible plants having more mummies, the proportion of mummies that developed into adult parasitoids was higher on susceptible plants compared to resistant plants ($F = 5.08$; $df = 1, 37$; $P =$

0.030; Table 2). Controlling for initial differences in aphid density, the number of adult parasitoid produced per aphid was over 10 times greater on susceptible plants compared to resistant ones (0.17 ± 0.03 vs. 0.01 ± 0.005 ; $F = 34.00$; $df = 1, 45$; $P < 0.0001$).

The development time for attacked aphids to become mummies was significantly longer on resistant vs. susceptible plants ($F = 10.7$; $df = 1, 36$; $P = 0.002$; Table 3). Removing those plants that had mummies but no emerged adults, soybean plant treatment did not have any effect on the time it took for adults to develop from mummies ($F = 0.6$; $df = 1, 24$; $P = 0.45$; Table 3); however adults on susceptible plants completed their entire development (attack to adult) about one day faster than those from resistant plants ($F = 13.2$; $df = 1, 24$; $P = 0.001$; Table 3).

Development time of a parasitoid wasps can be influenced by host size, which ultimately manipulates the sex of an individual offspring being produced (Charnov et al. 1981), but in our experiment, male wasps that developed on a susceptible plant developed at the same rate as female wasps on the same plant (males 11.2 ± 0.13 d vs. females 11.0 ± 0.19 d; paired t-test $t = 0.75$; $df = 1, 15$; $P = 0.46$). We could not compare males and females on resistant plants as only one plant produced both males and females, and this plant only produced one adult of each sex. There were more male wasps produced on susceptible plants compared to female wasps (86 vs. 55), but the average proportion of males produced on each plant was not different than 0.5 when looking across susceptible plants (0.59 ± 0.06 ; $t = 1.50$; $df = 1, 8$; $P = 0.15$). There was no difference in the numbers of males and females produced when comparing susceptible and resistant plants (resistant plants: four males and five females; $G_{adj} = 0.89$, $df = 1, 48$; $P = 0.35$).

Table 2. Parasitism rates of adult *Binodoxys communis* on soybean aphids reared from susceptible and resistant soybean plants.

Treatment	No. of mummies (Mean ± SE)	No. of aphids available prior to parasitoid inoculation (Mean ± SE)	Proportion parasitism (Mean ± SE)	No. of adult parasitoid emerged (Mean ± SE)	Proportion of mummies emerged into adult parasitoids (Mean ± SE)
Susceptible	21.2 ± 2.1***	36.2 ± 1.1***	0.6 ± 0.05***	5.8 ± 0.9***	0.3 ± 0.04*
Resistant	2.0 ± 0.5	24.5 ± 1.7	0.1 ± 0.02	0.3 ± 0.1	0.2 ± 0.1

Means within a column are significant when followed by a *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; NS = not significant (ANOVA).

Table 3. Effect of *Rag1* on the development and fitness of the soybean aphid parasitoid, *Binodoxys communis* Gahan (Hymenoptera: Braconidae).

Treatment	Aphids to mummies (days) (Mean ± SE)	Mummies to adult parasitoids (days) (Mean ± SE)	Parasitoid adult development time (days) (Mean ± SE)	Mean body size of adult parasitoids (mm) (Mean ± SE)	Metatibiae length of adult parasitoids (mm) (Mean ± SE)	
					Left	Right
					metatibiae length	metatibiae length
Susceptible	7.5 ± 0.1** (n = 23)	3.6 ± 0.2 ^{NS} (n = 20)	11.1 ± 0.2*** (n = 20)	1.2 ± 0.02 ^{NS} (n = 20)	0.322 ± 0.004* (n = 20)	0.321 ± 0.004** (n = 20)
Resistant	8.7 ± 0.3 (n = 17)	3.5 ± 0.2 (n = 7)	12.4 ± 0.3 (n = 7)	1.2 ± 0.02 (n = 6)	0.297 ± 0.005 (n = 6)	0.282 ± 0.006 (n = 6)

Means within a column are significant when followed by a *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; NS = not significant (ANOVA).

n denotes the sample size of resistant plants used for the calculation.

Body length (mm) and length of the left and right metatibiae (mm) of adult parasitoids were measured as indicators of fitness. The average body size of adult parasitoids was similar for susceptible and resistant plants ($F = 1.5$; $df = 1, 24$; $P = 0.22$; Table 3). In contrast, parasitoids reared from susceptible plants had larger metatibiae compared to parasitoids reared from resistant plants (left hind metatibia: $F = 4.7$; $df = 1, 24$; $P = 0.04$, and right metatibia: $F = 11.6$; $df = 1, 24$; $P = 0.002$; Table 3).

Discussion

Soybean aphid resistant plants with the *Rag1* gene were shown to negatively affect the performance and fitness of the parasitoid, *B. communis*, compared to near isogenic susceptible plants. Fewer mummies and a lower emergence rate of adult parasitoids were observed on resistant soybean plants compared to susceptible soybeans. The few adult parasitoids that did emerge from resistant plants took longer to develop and had shorter metatibiae compared to adults from susceptible soybean plants. Our results are similar to several previous studies, which found that adult parasitoids that emerged from an herbivore on resistant plants had reduced fitness in terms of longer development and smaller size (Bottrell et al. 1998, Ode 2006).

Speculating about the mechanism(s) producing this negative effect of resistant plants on parasitoids is potentially useful for hypothesizing when this type of effect may occur and how it could ultimately affect soybean aphid control. In general, resistant host plants can affect parasitoids through a variety of indirect and direct mechanisms. Host plant resistance can indirectly influence a natural enemy by altering the herbivore's population size, its growth (or death) rate, and the quality of an individual herbivore as a host or prey for the natural enemy that depends on them (Bottrell et al. 1998).

One of the largest negative effects resistant plants are likely to have on parasitoids in the field is a substantial reduction in the number of potential hosts available. We tried to control for this variable by starting all plants with the same number of aphids. Leaving the aphids to establish, however, resulted in a 50% difference in the number of aphids available on plants of each treatment. We chose to maintain this difference rather than manipulating the situation in a way that may have further confounded the treatments. Thus, it is possible that the lower density of aphids on resistant plants resulted in the large observed difference in parasitoid performance. However, it is unlikely that density itself was entirely responsible for our results since most experimental studies at this spatial and temporal scale find that proportion parasitism decreases when more aphids are available (Walde and Murdoch 1988), yet we found a drastic increase in the proportion parasitism on susceptible plants.

A more likely explanation for the large difference in mummy and parasitoid production is related to the mortality rate of soybean aphids on resistant plants. Since we were primarily interested in the net change in aphid populations, we cannot disentangle whether higher aphid populations on susceptible plants were because of aphids living longer or higher reproductive rates. However, we do know that establishment rates of aphids were much higher on susceptible plants (almost 100 percent establishment) than on resistant plants (under 50 percent establishment), which is consistent with higher mortality on resistant plants. Further evidence is provided by Li et al. (2004) who found that soybean aphid longevity decreased on resistant plants with the *Rag1* gene. We speculate that aphids parasitized by *B. communis* were more likely to die before the parasitoid completed development when the aphid was on a resistant plant compared to a susceptible plant. This

mechanism would go a long way in explaining our biggest effect, lower proportion parasitism on resistant plants.

A third indirect mechanism is that soybean aphids are a poorer host for parasitoids when they feed on resistant plants. Feeding on resistant plants may make aphids smaller, less nutritious, or even potentially toxic (Li et al. 2004, Diaz-Montano et al. 2007). All of these factors could result in lower fitness for the parasitoid developing in that aphid and potentially help make it more likely that a parasitized aphid will perish before the parasitoid completes its development (Kaufman & Flanders 1985, Werren et al. 1992, Ode 2006).

All of the mechanisms mentioned thus far work indirectly against the parasitoid by altering the aphid, yet it is also possible that resistant plants have a direct negative effect on parasitoids as well. Lundgren et al. (2009) discovered a direct effect of reduced adult longevity for a key predator, *Harmonia axyridis* (Palas), when it was exposed to resistant soybean varieties containing the *Rag1* gene. More generally, some resistant plant characteristics directly influence natural enemy host searching behavior, host accessibility and aphid dropping/falling behavior where the aphid falls from the plant (Grevstad and Klepetka 1992, Hare 1992, Bottrell et al. 1998). The mechanisms that govern such effects may be due to morphological features, such as glandular trichomes, or plant chemistry (Kauffman and Kennedy 1989, Van Lanteren and de Ponti 1991). Another explanation is that sensory cues required to locate its host are modified on resistant plants, thereby altering the parasitoid's effectiveness (Wäckers and Lewis 1994).

In summary, *B. communis* was able to successfully reproduce and survive on soybean aphids on resistant soybeans; however, parasitoid production and fitness were

negatively affected by the *Rag1* aphid resistant plants. Establishment and success of *B. communis* as an effective biological control agent of soybean aphid may be more problematic if resistant plants become widely grown in the field. These negative effects may be even stronger if we account for an overall lower population of soybean aphids caused by resistant plants. This means that in all likelihood, the combination of resistant plants and biological control by *B. communis* will be less effective than one would expect given their individual performance. However, our results alone cannot fully evaluate whether greater soybean aphid control will be achieved by using both strategies as opposed to just using one. The parasitoid's relative fitness and reproductive output as well as the availability of suitable hosts from refuges or resistant biotypes could all play important roles in ultimately determining the compatibility and utility of employing both *B. communis* and resistant soybean plants for soybean aphid control.

GENERAL SUMMARY AND CONCLUSIONS

The soybean aphid, *Aphis glycines* Matsumura, is a major invasive pest of soybean that is native to Eastern Asia. Host plant resistance and biological control are both potential components of a sustainable integrated pest management approach for control of soybean aphid. More detailed studies are needed on tritrophic interactions and the potential compatibility between host plant resistance and biological control in soybean production systems. We examined the effect of host plant resistance using near isogenic soybean lines with the *Rag1* gene on a parasitoid, *Binodoxys communis* Gahan, as the biological control agent of soybean aphid. Our study revealed that *B. communis* can successfully develop on soybean aphids reared on the resistant soybean plant. However, parasitism was much lower when aphids were reared on resistant plants compared to when aphids were reared on susceptible soybean plants. Several negative effects of resistant soybeans were also observed on parasitoid fitness. In summary, resistant plants with the *Rag1* gene interfered with parasitoid performance in terms of mummy production, parasitoid adult emergence rate and development time, and next-generation adult metatibiae length. Therefore, combining *B. communis* and aphid resistant plants containing the *Rag1* gene will likely provide less control of soybean aphids than one would expect given their individual effects on soybean aphids.

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APPENDIX I. POLYMERASE CHAIN REACTION (PCR) SHOWING THE EXPERIMENTAL PLANT GENOTYPES

The purpose of this study was to verify the genotypes, and the presence of the *Ragl* gene of the individual plants used in the parasitoid experiment. The susceptible plant used was RG607RR, and the source of the resistant *Ragl* gene was LDXG04018-3 (from Brian Diers, Univ. IL), a result of a cross of Dwight X (Loda X Dowling) (For details, see Plant Materials section in Materials and Methods). In 2008, the near-isogenic resistant breeder's line was screened in the field for aphid resistance, yield, and other agronomic traits. This line showed excellent aphid resistance and was comparable in yield to the recurrent parent (T. Helms, unpub. data).

Materials and Methods

Twelve aphid susceptible plants (RG607RR) and 12 aphid resistant plants were tested. Prior to aphid infestation, a 1 cm² section of a fully expanded leaf was clipped from each plant with a small dissecting scissors and placed in individually labeled 2 ml centrifuge tubes. Scissors were cleaned with a 10% bleach solution after each sample was taken. Samples were immediately placed in a -85° C freezer prior to DNA extraction. DNA was isolated from each sample using the Power Plant™ DNA isolation kit (Catalog # 13200 - 50, Mo Bio, Carlsbad, CA). Polymerase chain reactions (PCR) were performed using Satt435 primers (M. Funada, pers. comm.) using the cycle profile detailed in Kim and Diers (2009). PCR products for each sample were stored in a -20°C freezer for one week prior to conducting gel electrophoresis, which was conducted using a 3% SFR (Super Fine Resolution)-agarose gel at 190v with a 2 h running time (K. Kim, pers. comm.). DNA from susceptible and resistant plants was alternated across the gel for genotype

comparisons. The completed gel was viewed in a UV-light box and photographed using a digital camera.

Results

The PCR product showed one bright upper band for homozygous dominant genotypes, one bright lower band for homozygous recessive genotypes for susceptible plants, and faint upper and lower bands for heterozygous genotypes. In Fig. 5, the genotype of each plant used in one of the experiments is shown. The first well of the gel was left blank. Even-numbered lanes are susceptible plants and odd-numbered lanes are resistant plants. The PCR tube for lane 4 (from a susceptible plant) popped open in the thermal cycler and the contents evaporated. Lane 7 shows faint upper and lower bands (barely visible in print), indicating that this was a heterozygous plant. Based on aphid counts during the course of the fitness trait experiment, this



Figure 5. Photographic image of SFR (Super Fine Resolution)-agarose gel following electrophoresis of soybean marker Satt435 produced from the *Rag1* aphid resistant soybean line (odd numbered lanes), and the susceptible soybean line (even numbers).

plant exhibited aphid resistance. The band in lane 24 is very faint and is not visible in print, possibly because of human error when extracting the DNA or preparing the PCR product.

Conclusions

Samples taken from plants from the susceptible seed source all had susceptible genotypes, while those taken from plants from the resistant seed source all had the resistant genotypes. The presence of a single heterozygous plant among the 12 plants selected from the resistant seed source indicates that the recessive allele is present within this line, but at a low frequency.

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