THE INDIRECT AND DIRECT EFFECTS OF TEMPERATURE AND HOST PLANT RESISTANCE ON POPULATION GROWTH OF SOYBEAN APHID (*APHIS GLYCINES*) BIOTYPE 1

by

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Abstract

Temperature has an important indirect impact on pest populations. Direct effects occur, but also may result from temperature-induced changes in plant quality, including the expression of host plant resistance traits. Therefore, I examined both indirect and direct effects of temperature on biotype 1 soybean aphids (SBA), *Aphis glycines*, on a *Rag1*-resistant soybean variety and compared the effects with a susceptible variety to gain a better understanding of how temperature impacts SBA. Four aphid responses were evaluated: preimaginal development, survival to adulthood, number of progeny produced, and adult longevity.

In the first experiment, I grew soybean seedlings to the V-0 stage at 25°C and then conditioned them for 0, 3 or 5 days at 20° or 30°C before infesting with a single first instar SBA at each of the two experimental temperatures. Based on previous literature for SBA, I hypothesized that conditioning plants at the lower temperature would cause resistance to break down and that longer exposure would exacerbate the effect. Results showed that conditioning soybeans to 20°C significantly reduced SBA survival, and the effect on survival increased with longer conditioning. Conditioning plants to 30°C had no significant effect on SBA survival. However, estimated population growth decreased as conditioning time increased at 30°C and this effect was also observed at 20°C. Thus, plant resistance may have increased at both temperatures.

The second experiment compared SBA responses, including population growth, at four temperatures (15, 20, 25, and 30°C) on a *Rag1*-resistant and susceptible soybean variety. I predicted that SBA fitness would be lower at all temperatures on resistant soybeans, but the magnitude of differences between cultivars would not be uniform across temperatures. Results

indicated that both temperature (highest and lowest) and plant resistance detrimentally affected SBA fitness. There was also a significant interaction between the two variables with respect to SBA survival. Survival was lower and development rates were slower on the resistant cultivar. SBA required more degree-days to develop on resistant soybeans compared to the susceptible cultivar.

This information will aid soybean producers in implementing a cost-efficient IPM strategy involving *Rag1* resistant soybeans to combat SBA under a range of temperatures.

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Dedication

I dedicate my work to my niece, Chloe Hough, to whom I have tried to be a role model by showing her that hard work, kindness, creativity, and integrity are the keys to success. I would also like to dedicate this to my Uncle Bud who passed away during my Master's program. At my graduation party for my B.S. Biology degree, he told me, "You are officially the smartest person in the family now."

Chapter 1 - Introduction

Soybean (*Glycine max* (L.) Merr.) plays an important part in our everyday life, from food to biofuel to the environmentally friendly soy candles filling homes with pleasant scents. In 2012, 76.1 million acres of soybean were planted in the United States (USDA, 2013a), 4 million of which were planted in Kansas with a state yield of over 83 million bushels (Kansas Department of Agriculture, 2012). During the past sixteen years, a new invasive pest species -- the soybean aphid (SBA), *Aphis glycines* Matsumura -- has become an adversary for soybean farmers. It made its first appearance in North America in 2000, arriving either from China or Japan on an airline passenger or in horticultural cargo (Anonymous, 2000; Obermeyer et al., 2000; Vennette and Ragsdale, 2004). SBA has a wide indigenous range in eastern Asia which includes China, Indonesia, Japan, Korea, Malaysia, the Philippines, Taiwan, and Thailand (Hodgson and Heidel-Baker, 2013; Tilmon et al., 2013). Currently, SBA resides in the Midwest and most of the Northeastern and Southern United States, but has the potential to expand its geographic range (Pioneer Hi-Bred, 2011).

SBA is capable of causing yield losses of more than 50% in soybean (McCornack et al., 2004). The types of damage this pest can cause include stunting, leaf distortion, and reduced pod set, which results when the aphids insert their piercing-sucking mouthparts into the phloem. Feeding also decreases chlorophyll content (Li et al., 2004; Diaz-Montano et al., 2007). In addition to direct feeding injury, SBA promotes the growth of sooty mold when it excretes honeydew onto the plant tissue (Li et al., 2004). Finally, this pest can also serve as a vector of diseases such as alfalfa mosaic virus, bean yellow mosaic virus, tobacco ringspot virus, cucumber mosaic virus, potato virus Y, and soybean mosaic virus in both Asia and North America (Wu et al., 2004; Diaz-Montano et al., 2006).

To effectively manage SBA, its biology must be understood. For example, SBA has several characteristics that contribute to rapid population growth, including the ability to develop from first instar to adult (through four nymphal stages) in 5 to 7 days, and a fecundity that ranges from 20 to 75 progeny. Thus, SBA populations can double in less than two days under ideal conditions (McCornack et al., 2004). SBA can reproduce parthenogenetically (asexually) and even have telescopic generations where the progeny are ready to bear their own young before they are born. However, its holocyclic life cycle includes both a sexual and asexual reproductive period. SBA is heteroecious, which means it can develop on alternate and unrelated host plants (Hill et al., 2012). As late summer or fall approaches, SBA flies to the winter host, common buckthorn (*Rhamnus cathartica* L.) located in shelterbelts or woody areas, where they mate and lay eggs, which overwinter (Iowa State University, 2007). In the spring, seasonal environmental cues such as increasing day length and temperature prompt alate development and movement of mature aphids from their overwintering or primary host (common buckthorn) to the summer or secondary host (soybean), which occurs after 2 to 3 generations. Soybean is also the secondary host for SBA in Asia, but they colonize on Japanese buckthorn (*Rhamnus japonica* Maxim) and Dahurian buckthorn (*Rhamnus davurica* Pallus) for primary hosts in their native region (Takahashi et al., 1993; Hodgson and Heidel-Baker, 2013). In late summer, biotic and abiotic factors, such as plant quality and overcrowding, promote alate development and movement from soybean to common buckthorn, which occurs after about 15 generations on the summer host (Hill et al., 2012). The range of SBA may continue to expand, depending on changes in host plant distribution, possibly resulting from climate change. For example, common buckthorn grows in the Northern, Midwestern, and Western parts of the United States, but has recently spread into the South (USDA, 2015). Soybeans are grown throughout the Eastern United States,

but concentrated mostly in the Midwest (USDA, 2013b). SBA are unlikely to move into the Southwest because it does not do well in arid climates (Rice, 2006).

The usual way to control SBA is with an insecticide; the use of these products has increased 130-fold since SBA made their first appearance in the U.S. (Hodgson and Heidel-Baker, 2013). Timing of insecticide applications is crucial since SBA has multiple hosts (Hesler and Dashiell, 2007). According to the Kansas State University soybean management guide, there are currently seventeen different insecticide options for SBA (Whitworth et al., 2013). Organophosphates and pyrethroids are effective against SBA, as are neonicotinoids; but use of the latter class as both a seed and foliar treatment increases the risk of insecticide resistance developing due to it being a persistant, systemic insecticide (Chandrasena et al., 2011). Seed treatments alone are not effective with large SBA populations during the soybean reproductive stage and they only result in a minor increase with small SBA populations, so they are considered to be an "insurance policy' (Hodgson and Heidel-Baker, 2013). Therefore, farmers are encouraged to use 'best management' practices and spray when SBA reaches an economic threshold of 250 aphids per plant, which is usually at the early reproductive stage of the soybean (R1-R3) (Kaiser et al., 2007). It is not recommended to spray insecticides if SBA are found later in the season because the soybean plant is becoming a less suitable resource since SBA populations will be declining. It is also not recommended to spray pesticides more than necessary, nor use them as a sole control method due to both economic and environmental costs. For example, pesticides have the potential to harm non-target species, run off into streams, seep into the ground water, and allow biomagnification to take place. Even though it is advised to spray when SBA have reached their economic threshold during the earlier stages of soybean

growth, this poses a threat to pollinators, such as honey bees, during the flowering stage (Ohio State University, 2013).

There are other options for controlling SBA such as biological control, which involves natural enemies as pest-suppressive agents (Heimpel et al., 2004; Desneux et al., 2006; Ragsdale et al., 2011; Hodgson and Heidel-Baker, 2013). However, it is uneconomical to periodically release predators and parasitoids, and naturally-occurring biological control is usually integrated with chemical control due to the inconsistency of natural enemies to reduce and maintain SBA below the economic threshold (McCornack and Ragsdale, 2006). Although the effects of biological control can be unpredictable, there are numerous predators that feed upon soybean aphids. Predominant predators include multicolored Asian lady beetle (*Harmonia axyridis* Pallas), minute pirate bug (*Orius insidiosus* Say), predatory flies (*Aphidoletes aphidmyza* Rondani and *Allograpta obliqua* Say), and carabid beetles (*Elaphropus anceps* Le Conte, *Clavina impressefrons* Le Conte, *Bembedion quadrimaculatum* Say) (Hill et al., 2012). Although SBA resistance in soybean can have an impact on adult longevity in lady beetles, they are considered to be the most important biological control agent for SBA since the winged adult males and females travel from soybean to buckthorn, both of which are host plants of SBA (Hodgson and Heidel-Baker, 2013). According to a study by Desneux et al. (2006), without predators, soybean aphid populations increased 7.7 fold and only 2.9 fold when predators were present, which indicates that biological control does play an important role in managing SBA. Predators are more effective when soybean aphids are in a clumped distribution with small populations, but the distribution is random when soybean aphids first arrive in soybean fields (Desneux et al., 2006). Six hymenopteran parasitoids and nine dipteran parasitoids have been found to attack soybean aphids (Kaiser et al., 2007). Complementing the resident parasitoids,

two species of parasitoids have been imported from Asia to enhance biological control of *A. glycines*: *Aphelinus albipodus* Hayat and Fatima (Hymenoptera: Aphelinidae) and *Lipolexis gracilis* Forster (Hymenoptera: Branconidae) (Heimpel et al., 2004).

Because resistance to insecticides will occur over time, and biological control has variable efficacy, another alternative control method for SBA is host plant resistance. The three mechanisms of host plant resistance are antibiosis, antixenosis, and tolerance (Smith, 2006; J. C. Reese, Personal Communication). Antibiosis is an antagonistic relationship between two organisms where the metabolic substances of one harms the other. Antibiosis negatively impacts pest biology, causing a reduction in mortality, fecundity and/or longevity which decreases reproductive fitness. Antixenosis (also known as non-preference) functions by altering the behavior of an organism by another organism or its metabolic substance (UN Food and Agriculture Organization, 2015). Antixenosis affects the behavior of the pest, specifically its preference for plants; the comparison is usually made between a susceptible and a resistant plant (J. C. Reese, Personal Communication). Antixenosis may also be characterized as a plant possessing unattractive stimuli (e.g., color, odor, physical features) or lacking the attractive stimuli the pest seeks (Groves, 2015). Tolerance is defined as plants being able to withstand and recover from insect damage equal to that of a susceptible plant; tolerant plants do not negatively impact pest or beneficial arthropods.

Soybean lines have been bred to be more resistant to SBA with the use of *Rag* genes, exclusive to the SBA (Hill et al., 2006; Bansal et al., 2013), which is both constitutive and induced (Chiozza et al., 2010). Resistance and susceptibility form a continuum, so one source of resistant soybean germplasm may be more suitable in suppressing SBA than another. Depending on the mechanism(s) and level (strength) of resistance, these plant defenses do not necessarily

kill SBA in the sense that the insects die immediately after feeding upon a resistant soybean plant; but they may make it more difficult for SBA to survive due to antibiotic and/or antixenotic effects. Depending on the genotype of a given soybean line, one or both effects can occur. Tolerance is also a type of resistance, except it is defined as plants being able to withstand and recover from insect damage equal to that of a susceptible plant, but it is not the result of a resistance gene.-Antibiosis can cause the mortality rate to increase, longevity to decrease, and a reduced number of progeny, which is the case for *rag1c* (line PI 567541B) and *rag4* (PI 567541B) (Hill et al., 2012). However, reduced numbers of SBA nymphs could also indicate that the insects did not die, but dispersed to another plant; this could be an indication of antixenosis since the behavior of SBA was affected. Because both antixenosis and antibiosis negatively impact pest fitness, it may be difficult to distinguish which mechanisms are driving the SBA resistance. For example, in a recent study Hesler and Dashiell (2007) noted that the lack of SBA nymphs could have been due to an antibiotic chemical, inability for nymphs to settle (antixenosis), or both. In the soybean line PI 567543C, which contains the *Rag3* resistance gene, antixenosis is the only mechanism of resistance expressed (Hill et al., 2012). Some soybean lines exhibit both types of resistance where the plants are bred to contain more than one SBA resistance gene; this is referred to as "stacking" the genes. (Li et al., 2004). For example, both *Rag1* and *Rag2* genes both primarily confer antibiosis, but *Rag2* resistance is also expressed as antixenosis. And in at least one popular soybean line with *Rag1* resistance, 'Dowling', a popular soybean line containing *Rag1*, both types of resistance occur (Hill et al., 2012).

Currently, there are six known SBA resistance genes: *Rag1*, *rag1c*, *Rag2*, *Rag3*, *rag4*, and *Rag5* (Hill, et al. 2012). Depending on the soybean aphid biotype, the dominant gene may or may not be effective against it. For example, *Rag1* is successful in reducing the growth and

development of biotype 1, but biotype 2 can overcome its antibiotic effects. *Rag1* is effective against biotype 1 and 3. *Rag2*, *rag1c*, *Rag3*, and *rag4* work against biotypes 1 and 2. Biotype 3 can overcome both genes combined (Wiarda et al., 2012). The genetic mapping of *rag1c* is in the same region as *Rag1* and *rag4* is in the same region as *Rag2* (Zhang et al., 2009; Hill et al., 2012). *Rag5*, the most recently discovered *Rag* gene, is effective against biotypes 1 and 2, but not biotype 3 (Bansal et al., 2013); it was found near *Rag2*, but exhibited antixenosis, while *Rag2* involves antibiosis (Jun et al., 2012). However, SBA resistant soybeans may interfere with biological control. SBA resistance was shown to reduce adult longevity in the lady beetle *Harmonia axyridis*, whereas longevity of the minute pirate bug *Orius insidiosus* increased (Lundgren et al., 2009). In cases of antibiotic resistance that reduces SBA survival, parasitoids may not survive because they are unable to fully develop before host SBA die (Ballman et al., 2012). However, soybeans with the *Rag1* gene itself do not appear to have a direct adverse effect on natural enemies (Li et al., 2008) and choosing a variety that controls SBA, but does not harm natural enemies, is possible (Bottrell and Barbosa, 1998).

Temperature has a profound effect on all life forms (Precht et al., 1973; Logan et al., 1976). It is especially important in poikilotherms, including insects and plants, whose body temperatures depend on ambient conditions and respond directly to the amount of heat energy available (Precht et al., 1973; Sharpe and Demichele, 1977). Temperature also helps to determine the seasonal timing of pests, and how quickly and to what extent populations develop. Thus, understanding temperature-development relationships is important for making predictions about pest populations. Typically insect growth and development in herbivores is directly temperature-dependent, but only within a favorable range, which varies depending on the species and geographic origin (Bernays, 1991).

With respect to the SBA, there have been a few studies focused on the effect of temperature on its biology. McCornack et al. (2004) studied temperature effects on biotype 1 SBA on V-0 stage susceptible soybean seedlings to determine the optimal temperature for growth and reproduction of this pest. Prior to that study, Hirano et al. (1996) had also investigated the effect of different temperatures on SBA on susceptible soybeans. Responses measured by Hirano et al. included: development, survival rate, reproduction, intrinsic rate of increase, and longevity. Tests were done both in exclusion cages and open field plots. Richardson et al. (2011) found that reproductive traits in SBA were 'plastic' under a range of temperatures in that they changed over time with successive generations. In contrast, adaptation did not occur with respect to development or longevity. Because SBA are known to have originated in a temperate climate, they have been shown to respond more favorably under moderately low temperatures. That is, even though soybean aphids exhibit faster development and a shorter generation time at higher temperatures, they do not live as long, have as long of a reproductive life, or as high fecundity (Hirano et al., 1996; McCornack et al., 2004).

Plants produce chemicals for both structural and physiological functions. With respect to physiology, some phytochemicals including proteins, carbohydrates, and lipids have a direct role in the production of tissues that allow plants to grow and reproduce (Schoonhoven et al., 2005). Others serve a defense function to help plants protect themselves against insect herbivores and pathogens. While temperature is a key factor in the growth of living organisms, including plants (Precht et al., 1973), it also controls which chemicals are produced and at what level or magnitude (Went, 1953). Plant chemistry also varies with plant phenology which is directly correlated with temperature-dependent growth and development. Thus, different plant growth stages will have different chemical compositions, and plants at different stages of development

can react differently to temperature (Went, 1953). Temperatures experienced at different times of the season or geographically can also cause variation in the quality or quantity of phytochemical production (Maxwell and Jennings, 1980). Temperature extremes can cause stress that results in a depression in plant growth as chemicals used for primary production are reallocated to synthesize defense chemicals. In this way, temperature-driven variation in nutrients (e.g., sugars, amino acids, proteins) and defense chemicals that are both qualitative (toxins) and quantitative (digestibility-reducing) plant substances will have a large impact on herbivores (Denno and McClure, 1983). Temperature-induced changes may even alternate between day and night in ways that alter insect feeding times (Schoonhoven et al., 2005). While temperature has been shown to modify the expression of plant defense, including traits associated with host plant resistance, it is important to keep in mind that indirect effects of temperature on insect fitness that are mediated by changes in plants may be the net result of combined changes in plant defense chemicals, nutrients, and even plant structure which can facilitate or impede feeding, especially for insects with chewing mouthparts (Denno and McClure, 1983; Chiozza et al., 2010).

How temperature interacts specifically with host plant resistance is a question of practical importance for pest managers and producers. A review of the literature indicates that temperature influences resistance to both insects (DeBarro, 1992; Warren and Anderson, 2013) and plant pathogens (Hobbs et al., 2012). For SBA, only two previous studies (Richardson, 2011; Chirumamilla et al., 2014) examined the influence of temperature on soybean resistance to SBA (see below). In plant-insect systems, resistance may break down or become enhanced at low or high temperatures (e.g., Jackai and Inang, 1992; Thindwa and Teetes, 1994; Harvey et al. 1994). For example, Chen et al. (2014) found that exposing wheat cultivars to low temperatures

increased the level of plant resistance to Hessian flies. In contrast, both Richardson (2011) and Chirumamilla et al. (2014) provided evidence that *Rag1* resistance to biotype 1 SBA weakens at lower temperatures based on a comparison of differences between resistant and susceptible plants over a range of temperatures. Sometimes resistance does not change over a fairly broad range of temperatures. For example, Chirumamilla et al. (2014) found that *Rag1* resistance to biotype 1 SBA remained the same at 21 and 28˚C. However, Richardson et al. (2011), who worked with SBA biotypes 1 and 3 showed that one of the resistant soybean lines tested became more susceptible at 28°C. Therefore, additional studies are needed to determine if and how plant resistance to SBA (and other pests generally) is modified by temperature, and how these responses relate to the genetic source of resistance.

The question of whether plant resistance influences temperature-dependent growth and development is not well-known in insects, including the SBA, but a couple of studies have measured degree-days for SBA on susceptible soybeans. McCornack et al. (2004) computed the number of accumulated degrees-days required for development and reproduction of SBA under several temperatures on a susceptible cultivar. Desneux et al. (2006) compared accumulated weekly degree-days relative to population growth of SBA in clumped vs. random distributions on susceptible soybeans and found no differences. Even though these studies provide useful information, it is important to understand the relationship between temperature and SBA development on both resistant vs. susceptible soybeans because pest managers use thermal threshold values and degree-day constants derived from these relationships to predict the seasonal timing and population growth of pests. Therefore, if plant resistance delays development, then the number of degree-days required for insects to develop will not be the same as the number of degree-days on susceptible plants. Thus, predictions may be inaccurate.

The research reported here focused on examining interactions between host plant resistance and temperature involving a soybean line containing the *Rag1* gene and Biotype 1 SBA. My specific objectives were to 1) test if conditioning resistant plants to a lower and higher temperature (20 and 30˚C, respectively) before infesting them with SBA influenced the expression of resistance; 2) determine if temperature modified levels of resistance by comparing individual SBA responses, as well as demographic (life table) parameters, on resistant and susceptible soybeans over a range of temperatures between 15 and 30°C, including temperatures known to induce stress to SBA; and 3) evaluate if temperature-development relationships and thermal constants of the SBA were the same or differed when reared on resistant versus susceptible plants.

Chapter 2 - Temperature conditioning of soybean seedlings influences expression of resistance to soybean aphid (*Aphis glycines*) Biotype 1

Abstract

The use of soybean varieties that confer resistance to the soybean aphid (SBA), *Aphis glycines* Matsumura, represents a potentially important part of the IPM strategy for this invasive pest. However, little is known about whether plant exposure to temperature alters the expression of resistance. To test for indirect, plant-mediated temperature effects on SBA, individual soybean seedlings containing the *Rag1* gene were exposed to either low (20°C) or high (30°C) temperature for different durations (0, 3 or 5 days) at 25˚C prior to infestation with a single first instar biotype 1 SBA. I hypothesized that conditioning plants to lower temperatures would cause resistance to break down, and that the effect would be enhanced by longer exposure to higher temperatures. Four aphid responses were evaluated: pre-adult development time, survival to adulthood, lifetime progeny produced per female, and adult longevity. When plants were conditioned at 20˚C for periods up to five days, there were no statistically-significant effects on SBA development or adult longevity. However, percent survival and numbers of progeny decreased significantly as plant conditioning time increased, suggesting that the expression of *Rag1* resistance was enhanced by longer plant exposure under low temperature. In contrast, conditioning plants at 30°C had no significant effect on any of the individual SBA responses. However, the finite rate of population increase at 30˚C became lower as the duration of plant conditioning time increased. This same trend was observed at 20˚C. The effect was most significant for the low temperature treatment and the longest conditioning time. Survival was significantly lower at 30°C compared to 20˚C, indicating that the higher temperature had a direct

adverse effect on SBA. These results suggest that SBA respond directly to abiotic stress, but that host plant resistance may be enhanced at lower, and possibly higher, ambient temperatures.

Introduction

The soybean aphid (hereafter SBA), *Aphis glycines* Matsumura, first appeared in North America in 2000, and most likely originated from either China or Japan (Hill et al., 2001). It has now spread throughout much of the Midwest, but is not well established in the eastern U.S. (Ragsdale et al., 2011). The establishment of SBA in the United States is possible due to the presence of its primary host, the common buckthorn *Rhamnus catharica* L., which is also an invasive species hailing from eastern Asia (Takahashi et al., 1993; Hodgson and Heidel-Baker, 2013). SBA is capable of causing yield losses of more than 50% in soybean (*Glycines max* (L.) Merr.) (Ragsdale et al., 2007). The types of damage associated with SBA feeding include stunting, leaf distortion, and reduced pod set. The piercing-sucking style of feeding also decreases the chlorophyll content of soybean plants (Li et al., 2004; Diaz-Montano et al., 2007) and contributes to sooty mold growth through the production of honeydew (Li et al., 2008). In addition, SBA is a vector of plant disease, including alfalfa mosaic virus, bean yellow mosaic virus, tobacco ringspot virus, and soybean mosaic virus in both Asia and the United States (Wu et al., 2004; Diaz-Montano et al., 2006).

Several control tactics are used by producers to help maintain at acceptably low population levels. The most common method is to apply insecticides (McCornack and Ragsdale, 2006). However, the majority of insecticides, including the pyrethroid, lambda-cyhalothrin, and the organophosphate, chlorpyrifos, have negative repercussions for the environment as well as for non-target species, including natural enemies and pollinators (DiFonzo, 2013). In addition, overuse of pesticides increases the risk and incidence of pests developing resistance. For

example, neonicotinoid pesticides are used both as a seed and foliar treatment, thus doubling the exposure to SBA (Chandrasena et al., 2012). Biological control by resident and imported natural enemies (Heimpel et al., 2004) can help in the natural suppression of SBA populations (Ragsdale et al., 2011); but it cannot be relied upon as the sole control tactic (Desneux et al., 2006).

Another non-insecticidal approach for controlling SBA is host plant resistance. Employing resistant soybeans to combat SBA has several advantages, including ease of use and general effectiveness. However, depending on the resistance mechanisms involved, resistant plants may or may not be compatible with natural biological control. For example, SBA resistance was shown to reduce adult longevity in the lady beetle *Harmonia axyridis*, whereas longevity of the minute pirate bug *Orius insidiosus* increased while feeding on SBA on resistant leaves (Lundgren et al., 2009). Currently, six SBA-resistant genes have been identified: *Rag1, rag1c, Rag2, Rag3, rag4, and Rag5* (Hill, et al. 2012). *Rag1* is effective against biotypes 1 and 3. *Rag2*, rag1c, Rag3, and rag4 work against biotypes 1 and 2 (LaBarge, 2011). The genetic mapping of rag1c is in the same region as *Rag1*, and *rag4* is in the same region as *Rag2* (Zhang et al., 2009; Hill et al., 2012). *Rag5* (a newly-proposed gene) is effective against biotypes 1 and 2, but not biotype 3 (Bansal et al., 2013). This new gene was found near *Rag2*, but exhibited antixenosis; whereas *Rag2* exhibits antibiosis (Jun et al., 2012). *Rag1* is the most commonly used resistant gene in varieties sold in Iowa (McCarville et al., 2012), and a study performed at Iowa State University reported far fewer SBA and higher yields on *Rag1*-resistant lines than on susceptible lines (McCarville et al., 2012). Studies by Diaz-Montano et al. (2006, 2007) showed that soybeans containing the *Rag1* gene are associated with both antibiosis and antixenosis in that SBA take twice as long to reach the phloem to feed and plants do not experience chlorophyll loss, and progeny production is reduced.

Temperature has multiple direct effects on insect life histories, including alterations of growth and development, survival, reproduction, and sometimes dormancy (Logan et al., 1976; Bauerfeind and Fischer, 2013). In herbivorous insects, temperature can indirectly affect insect population growth and fitness by causing changes in host plant quality (Went, 1953; Denno and McClure, 1983) or the amount of resource available (Precht et al., 1973). Separating direct from indirect temperature effects can be difficult, especially in hemipterans, which must remain in contact with plants to feed. Thus, very few prior studies have definitively isolated indirect plant-mediated effects of temperature (Gijzen et al., 1996; Chen et al., 2014).

The expression of plant resistance to insects can vary with environmental conditions, including temperature. Extreme temperatures can alter the effect that plants have on insect pests by either diminishing (Wood and Starks, 1972; Salim and Saxena, 1991; Harvey et al., 1994; Richardson, 2011; Chirumamilla et al., 2014; Chen et al., 2014) or strengthening (Thindwa and Teetes, 1994) resistance. Sometimes, temperature does not have an apparent effect on resistance (Jackai and Inang, 1992). Despite the existing literature, little is known about how environmental temperature affects SBA-plant interactions and only two studies have investigated the effect of temperature on the expression of *Rag1* resistance to the soybean aphid (Richardson, 2011; Chirumamilla et al., 2014). Richardson (2011) and Chirumamilla et al. (2014) found that plant resistance breaks down at a lower temperature, but Chirumamilla et al. (2014) reported no change in the expression of resistance within a range of moderate to higher temperatures. Neither study addressed the possibility that exposing soybeans to conditioning temperatures prior to infestation might alter the level of resistance to SBA. Previously, Sosa (1979) and Chen et al. (2014) had shown that the length of plant exposure to low temperatures could reduce resistance to the Hessian fly, *Mayetiola destructor*. Therefore, the primary goal of my study was to

determine whether the length of time soybean seedlings were exposed to moderately low and high temperatures modified the level of *Rag1* resistance by observing SBA responses (development, survival, progeny production, adult longevity). My experimental approach was able to independently evaluate indirect effects of temperature on plant resistance from direct effects on SBA fitness. Based on previous studies (Richardson. 2011; Chirumamilla et al., 2014), I predicted that *Rag1* resistance would decrease as soybean seedlings were conditioned for longer times at the lower temperature, but that conditioning would have no effect on resistance at the highest temperature.

Materials and Methods

Plant and Insect Cultures

Biotype 1 soybean aphids were obtained from a colony maintained in the Department of Entomology at Kansas State University. The SBA used in the experiment were kept on seedlings of a susceptible soybean line (SD01-76R). These plants were grown in conetainers inside a cage constructed of plexiglass, mesh, and wood (55.9 x 35.6 x 88.9 cm [L x W x H]). Prior to initiating experiments, soybeans from a line containing the *Rag1* gene (LD(05)-16060), to which biotype 1 SBA are resistant, were grown from seed for 7 to 9 days in pots (7.6 x 10.2 cm [H x D]) in an environmental growth chamber with a photoperiod of 16:8 (light:dark) h, light intensity of 273.1 ± 33.0 W/m^2, and a relative humidity of 75.6 ± 1.6%. The temperature was set to 25°C (actual recorded temperature: 25.6 ± 0.06°C [mean ± SE]). This temperature is close to the range of temperatures (19-22°C) that soybeans normally experience at this stage of development in Kansas. The media used to propagate the resistant soybean seedlings was Metro-Mix 360 (Sun Gro Horticulture, Bellevue, WA) potting soil and four seeds were planted in each pot. After germination, all but one same size soybean seedling was removed from each pot. During

propagation and throughout the experiment, seedlings were watered daily or when the soil at the pot surface was completely dry. Caution was taken not to overwater the seedlings to prevent fungus from growing on the soil.

Experimental Procedures

The experiment was repeated three times between 31 July 31 and 22 October 2013 using biotype 1 SBA and a resistant soybean line (LD(05)-16060). The experiment was run in four environmental growth chambers; two Percival Model I36VLC8 (Percival Scientific, Inc., Perry, IA) and two Conviron Model A1000 (Conviron, Inc., Winnipeg, Manitoba CA). One of each type of growth chamber was set to 20˚C, and the other two were set to 30˚C. These two temperatures were chosen to include both a lower and higher temperature that were equidistant from the soybean rearing temperature. In addition, 30˚C had previously been shown to cause stress in SBA (McCornack et al., 2004). The actual temperatures measured in the growth chambers were 20.7 ± 0.05˚C and 30.0 ± 0.03˚C. The light intensity was 83.3 ± 9.9W/m^2 for the Percival chambers and 341.0 ± 17.1W/m^2 for the Conviron chambers. Despite the differences in light intensity, statistical analysis revealed no significant effect due to growth chamber type and room location (see Materials and Methods). A photoperiod of 16:8 (light:dark) was used for both temperatures throughout the experiment.

Before placing SBA on the resistant plants, potted seedlings were transferred to growth chambers under each temperature for 0, 3 or 5 days; the 0 day treatment served as a control (no temperature conditioning of the plants before inoculation with SBA). Seedlings used in tests were initially in the V-0 stage, which occurs 7 to 9 days after seeds are sown, and when plants are about two inches tall and have cotyledons that are cupped around the unifoliate leaves (McCornack et al., 2004).

Inoculation consisted of placing two adult SBA on each resistant soybean seedling for 24 hours, either at 20 or 30˚C under a 16:8 photoperiod, after which the adults and all but one nymph were removed using a fine (#000) camel hair paintbrush. Thereafter, all experimental seedlings were inspected daily to determine if nymphs were alive and changes in life stage were recorded. It is difficult to distinguish between individual SBA life stages. Therefore, I divided soybean aphid life stages into three categories: 1^{st}-2^{nd}, 3^{rd}-4^{th} and adult. To maintain a consistent plant height, the soybean seedlings for each temperature and conditioning treatment were replaced every three days with new V-0 stage seedlings by transferring each SBA from the older seedling to the newer seedling. Replacement seedlings were pre-conditioned to the same temperature and duration as previous seedlings in the same treatment. When SBA began to produce progeny, the number of offspring per reproductive adult was recorded and nymphs were removed daily; I continued this procedure until all adult SBA adults died.

Experimental Design and Statistical Analyses

The experiment was arranged in a randomized complete block design (RCBD) with a split-plot and replication on the split-plot. Temperature was the whole-plot treatment factor (two levels: 20 and 30°C) and room (two rooms, each containing two growth chambers of different make and model) was the blocking factor. Temperatures were randomized to growth chambers within each room. Conditioning time (three levels: 0, 3, and 5 days) was the split-plot treatment factor with 12 pots per conditioning time, thus giving a total of 36 pots per growth chamber and a total of 144 pots for the entire experiment. This experiment was repeated three times (range of dates noted above). Temperature and conditioning treatment were considered fixed effects, and time repeat (experiment) and room were considered random effects. Location within growth chamber (shelf) was randomized to contain plants of all three conditioning treatments and was

evaluated in the initial analysis because more than one brand of growth chamber was used and their shelf sizes differed. Initial analyses showed that shelf was never significant ($P > 0.05$), either as a main effect or in interactions, for any response, and was therefore excluded from the final analysis presented here. In addition, the room variance component was consistently 0, indicating that room variability was very much smaller than time variability and was therefore also excluded from the final analysis.

The individual numerical responses (development time [1^{st} instar nymph to adult], total number of progeny per adult [lifetime progeny], and adult longevity) were analyzed using the SAS (V 9.4) MIXED procedure with the Restricted Maximum Likelihood (REML) method of model fitting because the residuals passed tests for normality. Proportions of SBA surviving to adult emergence were analyzed using the GLIMMIX procedure with the default pseudo-likelihood method, using the binomial distribution and logit function. (Note that GLIMMIX's default pseudo-likelihood method of model fitting gives the same results as the MIXED REML method when the normal distribution is being used.) Results from the GLIMMIX analyses included: REML estimates and approximate Wald test statistics for the random effect variance components; type 3 F-tests to test fixed effects (i.e., the temperature and conditioning main effects and their interaction); lsmeans and standard errors for all fixed effects; and pairwise comparisons for the conditioning treatment at the $P \leq 0.05$ level. The variance component for time repeat was never significant, indicating that variation in aphid responses did not change due to the experiment being run three times over the course of several months.

Four SBA population responses were computed from the observed data: preimaginal development, survival (newly deposited nymph to adult), adult longevity, and fecundity. The individual response data were used to compute three life table statistics: finite rate of population

increase (λ), net reproductive rate (R_o), and mean generation time (T), which is quantified as the time from the 1st instar to when 50% of progeny are produced. The life table statistics were analyzed using Program R and graphed using SigmaPlot (Systat Software, Inc., San Jose CA). The input data for Program R (R i386 3.1.2) consisted of the age, px (age-specific survival rate from age x to age x+1), mx (number of female offspring per female of age x), and the standard error for both values. The output consisted of the mean, standard deviation, and confidence intervals for each set of data to be tested. The confidence intervals were created using the bootstrap method, a test that involves random sampling with replacement, at 1,000 iterations.

Results

A table summarizing the statistical values for the main effects and interaction for each SBA response is show in Table 2.1.

Preimaginal Development Time

Both temperature and plant conditioning time had a significant effect on SBA development ($P < 0.002$ and 0.035, respectively). However, there was no significant ($P < 0.921$) temperature by conditioning time interaction, suggesting that the relative differences in development among conditioning times were similar for both temperatures. At both temperatures, development time became progressively shorter as the length of plant conditioning increased (Figure 2.1). SBA development was significantly shorter when plants were conditioned for 5 days compared to no conditioning ($P \leq 0.011$), but differences in development were not significant ($P \leq 0.299$) between 0 and 3 days, and marginally non-significant ($P \leq 0.077$) between 3 and 5 days (LS Means Test). On average, SBA took about 2 days longer to develop at 20˚C than at 30˚C (Figure 2.2). The average number of days for SBA to develop from 1st instar nymph to adult ranged from 5.71 to 7.54 days at 20˚C, and from 3.78 to 5.22 days at 30˚C.

Survival

Temperature had a non-significant ($P < 0.094$) effect on SBA survival (data pooled over all plant conditioning times), whereas conditioning time had a highly significant ($P < 0.0001$) effect. There was also a significant temperature by conditioning time interaction ($P < 0.020$) indicating that the effect of plant conditioning time on survival differed between the two temperatures. This difference was confirmed when conditioning times were analyzed at each temperature using a simple main effects procedure (slice test); results showed a highly significant effect of conditioning time on survival at 20˚C ($P < 0.0003$), but not at 30˚C ($P < 0.976$). The percentage survival was consistently low at 30˚C among all conditioning treatments and showed little variability between treatments. At 20˚C, SBA survival was greatest in the treatment with no conditioning and declined markedly as conditioning time increased (Figure 2.2).

Progeny Production

Temperature had a highly significant effect on the number of SBA progeny produced ($P < 0.001$). The mean number of progeny produced at 20˚C (10.6) was three times higher than at 30˚C (3.15) (Figure 2.3). Conditioning time had no effect ($P < 0.467$), and there was also no significant interaction between temperature and conditioning time ($P < 0.571$). Despite the lack of significance, there was an apparent trend for fewer progeny to be produced as conditioning time increased at 20˚C (Figure 2.3).

Adult Longevity

Temperature had a significant effect on adult longevity of SBA ($P < 0.004$), but conditioning time did not ($P < 0.797$). There was also no significant interaction between temperature and conditioning time ($P < 0.948$). There was a trend for a shorter lifespans at 20˚C

as conditioning time increased (Figure 2.4). However, this trend may be coincidental. Averaged over all conditioning times, adult lifespans were markedly shorter for SBA at 30˚C (2.87 days) than those at 20˚C (5.46 days).

Demographic Responses

At 20˚C**,** SBA experienced positive population growth under the 0- and 3-day conditioning treatments as evidenced by λ values greater than 1.00. However, under the 5-day conditioning treatment population growth was either stable or decreased slightly (Figure 2.5). The highest population growth rates at 20˚C occurred on plants that received no conditioning or 3 days of conditioning, and the differences between the two treatments were not significant ($P > 0.05$). On the other hand, there was a highly significant ($P < 0.001$) decrease in population growth in the 5-day treatment compared to the 3-day treatment at 20˚C (Figure 2.5). At 30˚C, SBA population growth rates were lower, for each conditioning time, than those observed at 20˚C (Figure 2.5). Population growth at 30˚C decreased in a linear pattern as days of plant conditioning increased. In the no-conditioning treatment (0-day), population growth was slightly positive, but it was negative at 3 and 5 days of conditioning (Figure 2.5). There was no significant difference in λ values between the 0- and 3-day treatments, whereas significant differences occurred between the 5-day treatment and the other conditioning treatments.

At 20˚C, the net reproductive rates (R_o) for SBA were positive under the 0- and 3-day conditioning treatments, and stationary for the 5-day conditioning treatment. Although there was a progressive decrease in R_o as the length of conditioning time increased, and the net reproductive rate for the 0-day conditioning treatment was almost twice as high (~ 8 nymphs) as the 3-day treatment (~ 4 nymphs). Differences in R_o were only significant between 3 and 5 days under 20˚C, which had a replacement rate of only 1 nymph/female, and the shorter conditioning

treatments (Figure. 2.5). At 30˚C the net reproductive rate at 0 days of conditioning was close to 0, and they were slightly negative at 3 and 5 days. However, the difference in R_o values was not significant between 0 and 3 days, but both of these treatments had significantly higher net reproductive rates than the 5-day conditioning treatment (Figure 2.5).

On average, mean generation times were about 2 days longer at 20˚ than at 30˚C (Figure 2.5). At 20˚C the mean generation times were similar for the 0- and 5-day conditioning treatments (about 10 days); but both had significantly longer generation times compared to the 3-day conditioning treatment (Figure 2.5). At 30˚C mean generation times ranged from 7 to 8 days but there were no significant differences among the three conditioning treatments (Figure 2.5).

Survivorship and Fecundity Schedules

There were large differences in SBA survivorship among plant conditioning treatments at 20˚C. Survivorship decreased the most sharply (i.e., in the shortest time) when resistant seedlings were conditioned for 5-days; it was intermediate in the 3-day treatment, and the decline in survivorship was the most gradual (i.e., slowest decrease over time) in the 0-day treatment (no temperature conditioning) (Figure 2.6). In contrast, when seedlings were conditioning for 0, 3, or 5 days at 30˚C, survivorship decreased quickly in all treatments (Figure 2.6). However, survivorship appeared to decrease slightly faster in the 5-day conditioning treatment compared to the other treatments (Figure 2.6).

With respect to the schedule of fecundity, there were obvious difference between temperatures and among conditioning treatments. At 30˚C, the majority of progeny were produced within two weeks, whereas at 20˚C progeny were distributed more evenly over more than three weeks (Figure 2.6). At 20˚C, the fewest progeny were produced in the 5-day

conditioning treatment; furthermore, no progeny were recorded on about half of the days. In contrast, the highest mean fecundity was observed in the 0-day treatment with peak numbers of progeny produced in the third week of reproduction (Figure 2.6). At 30˚C, the distribution of progeny over time was more similar among plant conditioning treatments. However, between days 5 and 11 mean fecundity was higher in the 0-day treatment than in the 3- or 5-day conditioning treatments (Figure 2.6).

Discussion

Temperature had a predictable direct effect on all four SBA life history traits measured in this study. When SBA were exposed to high temperature (30˚C), they developed faster, but had lower survival, compared to aphids reared at 20˚C. At the higher temperature, there were also fewer progeny produced and longevity was less than at the lower temperature. Previous work by McCornack et al. (2004) showed that optimal temperature for SBA is 27˚C, and that net fecundity, life expectancy, and overall population growth decreased below and above 25˚ and 30˚C, respectively. These findings are consistent with the lower SBA survival rates and lower population growth parameters I observed in all conditioning treatments at 30˚C relative to 20˚C.

Temperature also affected SBA indirectly via plant-mediated direct effects on resistant plants. When soybean seedlings containing the *Rag1* gene were propagated at 25˚C and then held at either 20 or 30˚C for different durations prior to infestation with SBA, preimaginal development became shorter at both temperatures as conditioning time lengthened. In contrast, survival to adulthood decreased as conditioning time increased. However, the effect of plant conditioning on survival was documented only at 20˚C. Exposing soybean seedlings to 30˚C prior to infestation may have influenced plant quality, thus indirectly impacting SBA responses. This is possible because soybeans have been shown to experience stress at temperatures at or

above 30˚C (McCornack et al., 2004), and other researchers have documented either increased resistance (Thindwa and Teetes, 1994) or a breakdown in resistance (Sosa and Foster, 1976; Tyler and Hatchett, 1983) under higher temperatures. However, because SBA survival was very low at 30˚C in all conditioning treatments--likely due to direct thermal stress-indirect effects on SBA responses related to changes in levels of plant resistance traits may have been obscured. Conditioning time had no statistically significant effect on either progeny production or longevity at either temperature. However, at 20˚C there was a trend for both traits to decrease as conditioning time increased.

A review of the literature indicates that the expression of plant resistance can be altered at high and low temperatures, and that resistance may either strengthen or weaken at fixed temperatures at both ends of the spectrum. In the greenbug *Schizaphis graminum*, resistance breaks down at lower temperatures (Schweissing and Wilde, 1979; Harvey et al., 1994). In contrast, Chen et al. (2014) found that resistance in the Hessian fly *Mayetiola destructor* was strongest under lower temperatures below (20-22˚C) based on larval survival. The same general result was observed for Hessian flies by Sosa and Foster (1976) and Tyler and Hatchett (1983), although the range of temperatures and wheat cultivars tested differed slightly. In a separate experiment I ran to compare the effects of different temperatures on SBA on resistant and susceptible soybeans, I was not able to show an increase in plant resistance at lower fixed temperatures (15 or 20˚C) (see Chapter 3). However, in the present experiment when plants were conditioned at 20˚C at the V-0 stage after growing them from seed to that stage at 25˚C, SBA survival decreased and continued to do so up to 5 days of plant conditioning. These results suggest that temperature-conditioned soybean seedlings had an elevated level of plant resistance. My results are similar to earlier reports by Sosa (1979) and Chen et al. (2014) that decreasing

temperature enhances the expression of resistance to the Hessian fly in wheat. Also consistent with my study, Sosa (1979) showed that the level of resistance was related to the amount of time plants were exposed to lower temperature.

An interesting aspect of how temperature influences plants is that the effect on resistance traits can be reversible. For example, Sosa (1979) was able to restore resistance to Hessian flies in wheat plants by giving them a short exposure to low temperature (18˚C) after rearing them at high temperature (27˚C). Reciprocally, short exposure to the higher temperature reverted plants to susceptibility after rearing at low temperature. As in my study, the strength of resistance or susceptibility was proportional to the length of time plants experienced the switch in temperature. Similarly, Harvey et al. (1994) noted that sorghum resistance to greenbugs could be restored by subjecting them to a 20/28˚C thermoperiod after a constant 20˚C. A general conclusion is that plant responses to temperature can be highly plastic with respect to the expression of plant resistance traits. Moreover, if one considers the multiple direct effects that temperature has on insects, it is clear that pest population growth is the end result of a complex, dynamic, set of interactions between temperature, plants, and plant pests.

The decreases in SBA survival as plant conditioning time lengthens at 20˚C suggests that antibiotic resistance was strengthened. However, other SBA life history traits did not respond to plant conditioning in a predictable manner. As plant conditioning time at 20˚C lengthened, SBA development became shorter and there were non-significant trends for decreased progeny production and adult longevity. In comparison, at 30˚C survival was uniformly low for all conditioning times (likely due to direct temperature effects on SBA), but consistent with the 20˚ results SBA development became progressively faster as the length of plant conditioning increased. These offsetting responses indicate that indirect plant-mediated effects of temperature

on insect resistance need to be evaluated for multiple life history traits including survival, and that the net effect of temperature-induced changes in plant resistance should be viewed with respect to overall population growth. With respect to the SBA, when individual demographic responses were integrated into life tables, the results showed a decrease in both the net reproductive rate and finite rate of population increase as conditioning time lengthened. For example, at 20˚C, SBA population growth was positive with no conditioning, and it did decrease significantly at 3 days of conditioning. However, when plants were conditioned at this lower temperature for 5 days, no population growth was observed (Figure 2.5). At 30˚, population growth was slightly positive with no conditioning, but became increasingly negative the longer plants were conditioned at the higher temperature. There was a significant effect of plant conditioning at 5 days on population growth despite the fact that none of the conditioning treatments significantly affected any of the individual SBA responses at 30˚C. This contradiction may be due to a cumulative effect of small reductions in selected traits – survival and progeny, for example – which impacted estimated population growth rates. If so, it is reasonable to assume that soybean resistance may have increased at both higher and lower temperature. The mean generation time was predictably longer at 20˚C than 30˚C, but did not appear to be influenced by the duration that seedlings were exposed to each temperature prior to infestation with SBA.

Because field temperatures fluctuate greatly, both diurnally and over longer periods, it remains to be determined whether temperature conditioning of plants prior to natural infestation would affect host plant resistance enough to impact SBA populations. Soybean aphids reproduce quickly and their doubling time can be around 1.5-1.9 days on susceptible plants in 20-30˚C, with their optimal temperature for development at 27.8˚C (McCornack, et al., 2004). Therefore,

ephemeral effects may be insignificant, especially in the southern part of the Great Plains where spring temperatures may fluctuate considerably. However, in more northern areas, persistent cool spring weather could have a complementary effect on SBA suppression if host plant resistance were elevated at the same time populations were developing slowly due to lower temperatures. Further investigation of direct and indirect effects of temperature on SBA may lead to better predictions that could assist soybean producers.

Figures and Tables

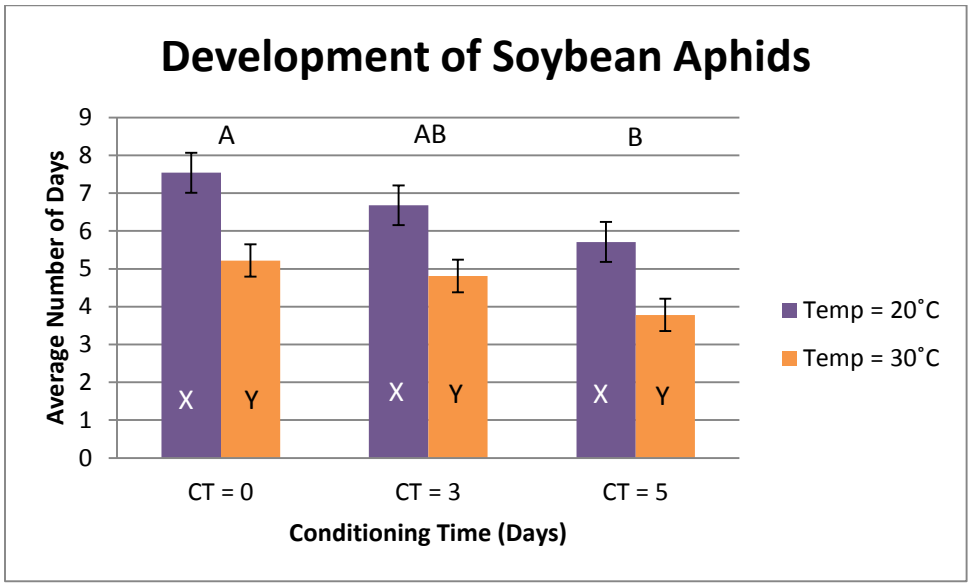

Figure 2.1 Mean ± SE number of days for biotype 1 soybean aphid nymphs to reach adulthood when *Rag1*-resistant soybean seedlings were pre-conditioned for 0, 3 or 5 days at 20 or 30˚C. Range of observations (low-high) for the three trials: 0-day treatment = 11-22, 3-day treatment = 5-11, 5-day treatment = 3-10. Treatments that do not share a common letter are significantly different ($P < 0.05$). X-Y denotes differences in development between temperatures for each conditioning treatment. A-B denotes statistical differences among conditioning treatments (pooled for both temperatures because there was no significant temperature by conditioning treatment interaction).

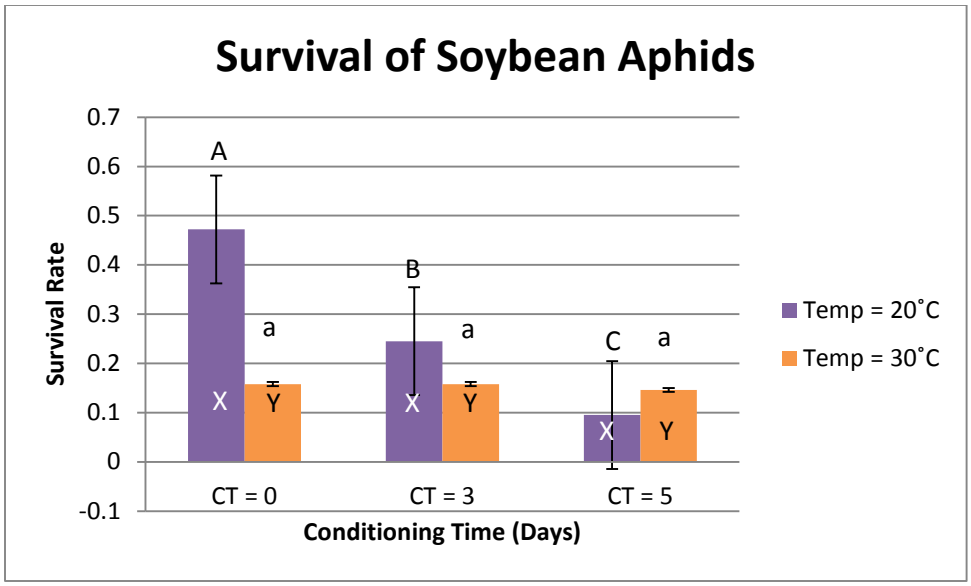

Figure 2.2 Mean ± SE number of biotype 1 soybean aphid nymphs surviving to adulthood when *Rag1*-resistant soybean seedlings were pre-conditioned for 0, 3 or 5 days at 20 or 30˚C. Range of observations (low-high) for the three trials for each treatment combination: 20˚, 0 days = 7-18, 3 days = 5-7, 5 days = 2-3; 30˚, 0 days = 4-4, 3 days = 3-5, 5 days = 1-7. Treatments that do not share a common letter are significantly different ($P < 0.05$). Capital letters (A-C) denote statistical differences among conditioning times at 20˚C; lower case letters (a) indicate there were no significant differences among conditioning times at 30˚C.

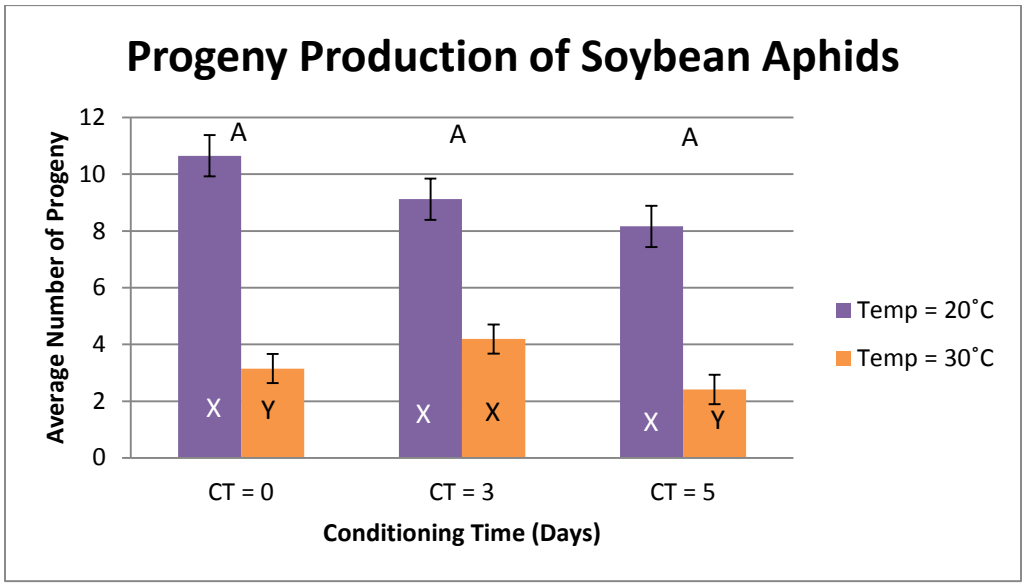

Figure 2.3 Mean ± SE number of progeny produced by biotype 1 soybean aphid adults when *Rag1*-resistant soybean seedlings were pre-conditioned for 0, 3 or 5 days at 20 or 30˚C. Range of observations (low-high) for the three trials: 0-day treatment = 11-22, 3-day treatment = 5-11, 5-day treatment = 3-10. Treatments that do not share a common letter are significantly different ($P \leq 0.05$). X-Y denotes differences in progeny production between temperatures for each conditioning treatment. There were no significant differences in progeny production among conditioning times (denoted by 'A').

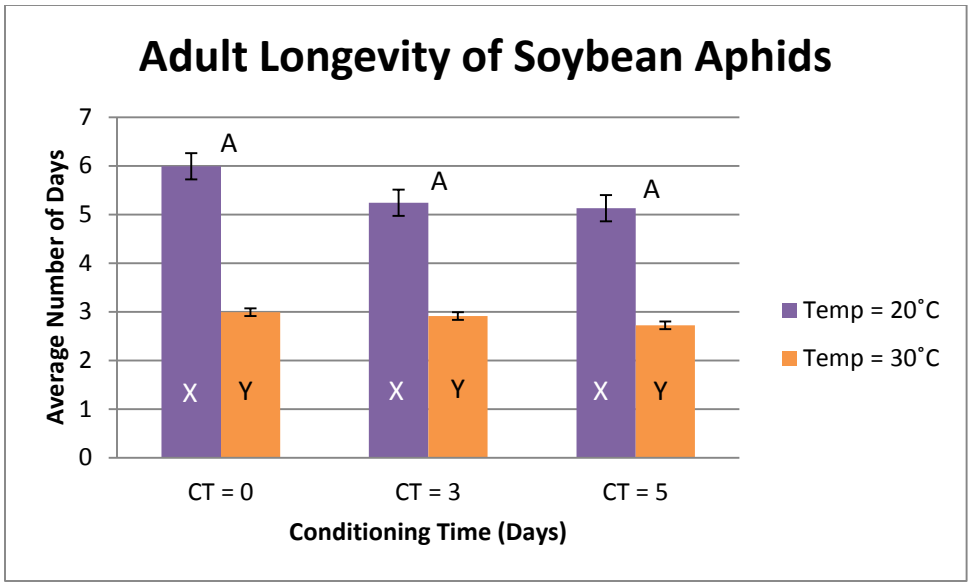

Figure 2.4 Mean ± SE number of days biotype 1 soybean aphids lived after reaching adulthood when *Rag1*-resistant soybean seedlings were pre-conditioned for 0, 3 or 5 days at 20 or 30˚C. Range of observations (low-high) for the three trials: 0-day treatment = 11-22, 3-day treatment = 5-11, 5-day treatment = 3-10.Treatments that do not share a common letter are significantly different ($P \leq 0.05$). X-Y denotes differences in adult longevity between temperatures for each conditioning time. There were no significant differences in adult longevity among conditioning times (letter symbol 'A' represents both temperatures because there was no significant temperature by conditioning time interaction).

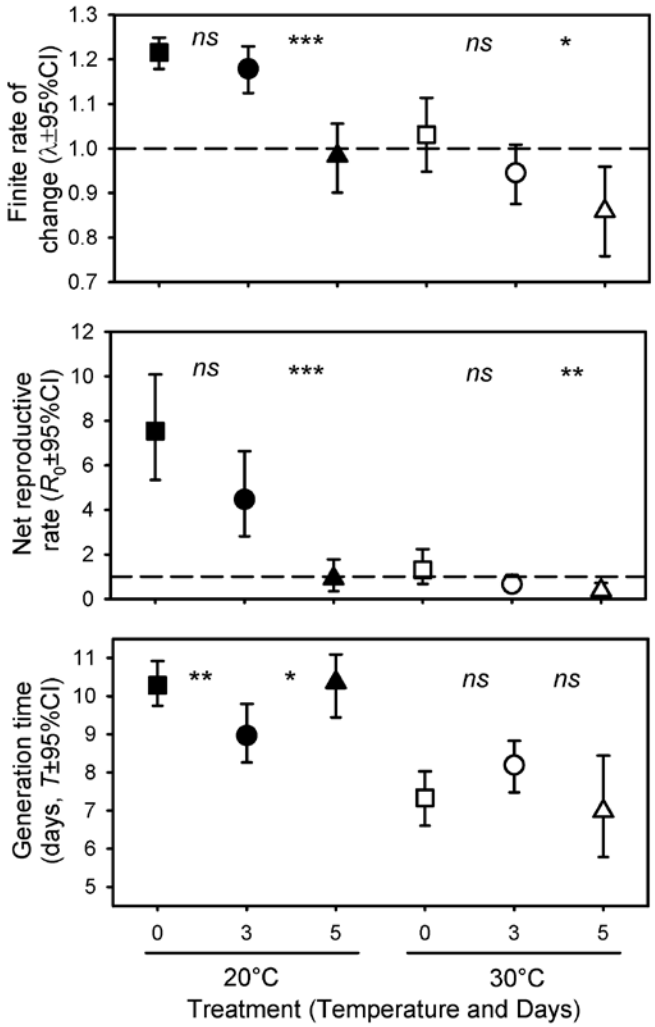

Figure 2.5 Demographic statistics for biotype 1 soybean aphids when soybean seedlings with *rag1* resistance were pre-conditioned for 0, 3 or 5 days at 20 or 30˚C. For finite rates of increase and net reproductive rates, means above dashed line indicate an increase; below the line represents a decrease. Mean generation times are in days. Number of observations (N): finite rate of increase for both temperatures and all conditioning treatments = 72; net reproductive rate: 20˚C, 0-days = 34, 3-days = 18, 5-days = 7; 30˚C, 0-days = 12, 3-days = 12, 5-days = 11; mean generation time: 20˚C, 0-days = 34, 3-days = 18, 5-days = 7; 30˚C, 0-days = 12, 3-days = 12, 5-days = 11. Within temperatures, conditioning times separated by *, **, or *** are significant at the P <0.05, 0.01 and 0.001 level, respectively.

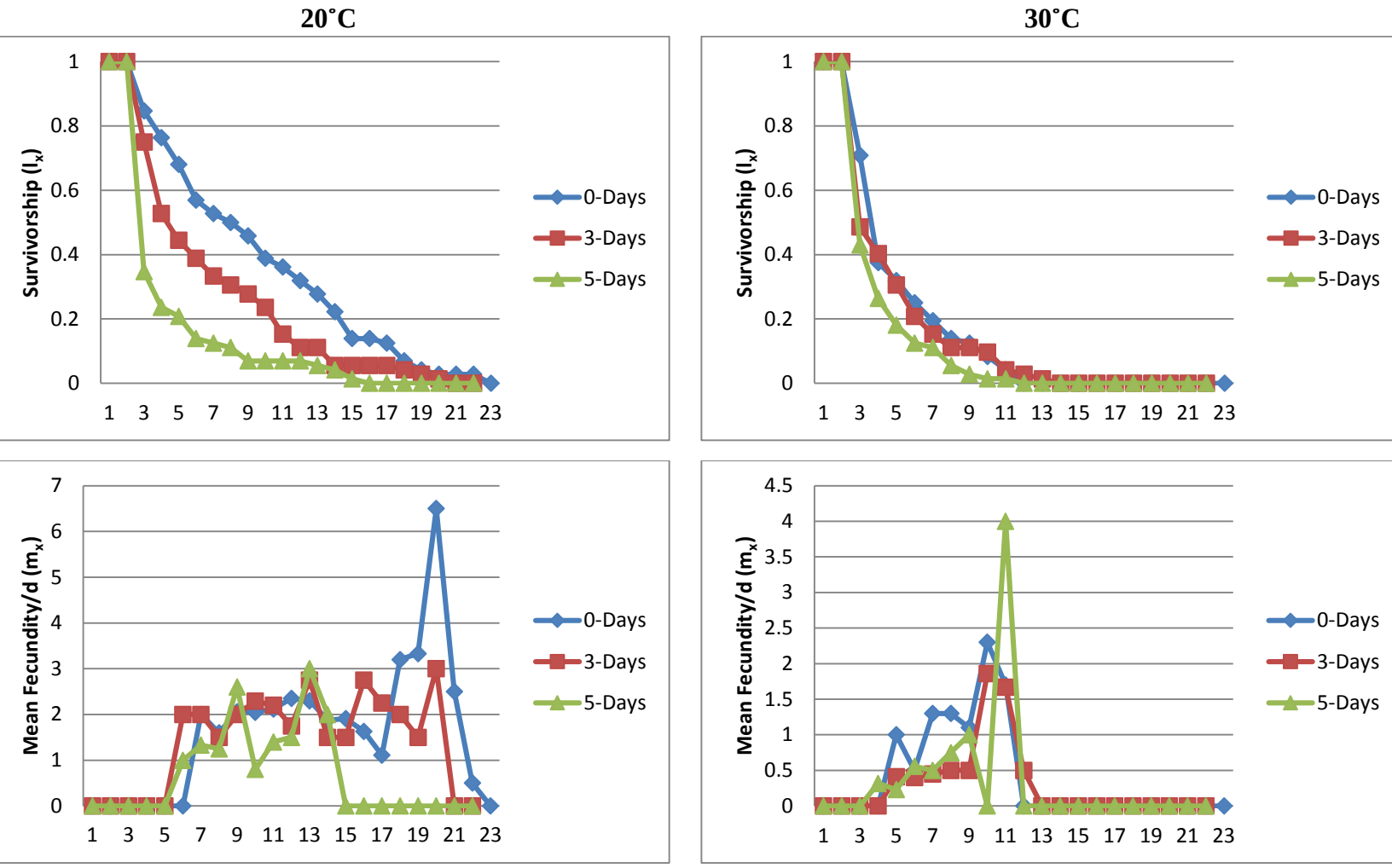

Figure 2.6 Survivorship and mean fecundity as a function of calendar age for soybean aphids on plants conditioned for 0, 3, or 5 days at either 20˚C or 30˚C.

Development				
	P-Value	**F Statistic**	**df_N**	**df_D**
Temperature	0.0021	19.8	1	8
Conditioning Time	0.0353	4.15	2	16
Temp X Conditioning	0.921	0.08	2	16
Survival				
	P-Value	**F Statistic**	**df_N**	**df_D**
Temperature	0.0944	3.74	1	7
Conditioning Time	0.0111	5.05	2	40
Temp X Conditioning	0.0205	4.29	2	40
Progeny				
	P-Value	**F Statistic**	**df_N**	**df_D**
Temperature	0.0008	27.51	1	8
Conditioning Time	0.4667	0.8	2	16
Temp X Conditioning	0.5706	0.58	2	16
Longevity				
	P-Value	**F Statistic**	**df_N**	**df_D**
Temperature	0.0036	16.5	1	8
Conditioning Time	0.7974	0.23	2	16
Temp X Conditioning	0.9484	0.05	2	16

Table 2.1 Main effects, interactions, and statistical output for soybean aphids on susceptible soybean seedlings that were pre-conditioned for 0, 3 or 5 days at 20 or 30˚C.

Survival				
Temperature	**P-Value**	**F Statistic**	**df_N**	**df_D**
20˚C	0.0003	9.94	2	40
30˚C	0.9763	0.02	2	40

Table 2.2 Significance of the effect of conditioning time on survival at either 20˚C or 30˚C.

Chapter 3 - The effect of temperature and host plant resistance on population growth of soybean aphid (*Aphis glycines*) biotype 1

Abstract

Soybeans expressing the *Rag1* gene for resistance have been shown to be effective against biotype 1 of the soybean aphid (SBA), *Aphis glycines*. However, few studies have investigated the interaction between temperature and host plant resistance on SBA populations (Richardson, 2011; Chirumamilla et al. 2014). In addition to direct effects of temperature on SBA population growth, I predicted that the expression of plant resistance would break down at lower temperatures, based on the literature and my previous experiment (Chapter 2). I also predicted that resistant plants would not only affect temperature-dependent development in SBA differently from susceptible plants, but that the effects would be asymmetrical across a range of experimental temperatures. To test these predictions, SBA were reared in growth chambers on seedlings of a susceptible and resistant soybean line under four temperatures: 15, 20, 25, and 30°C. Results showed that both temperature and plant resistance affected SBA fitness and there appeared to be an interaction of the two factors for some SBA responses (e.g., survival and development rate). In addition, there was evidence that the level of plant resistance increased at higher but not lower temperature. These findings will be useful for making predictions of SBA populations on resistant plants under different seasonal temperatures.

Introduction

In 2000, the soybean aphid *Aphis glycines* Matsumura, made its first appearance in North America and Canada, and originated from eastern Asia (Hill et al., 2001; Tilmon et al., 2013). SBA currently resides in the Midwestern region of the United States. However, it is present

sporadically in parts of the Eastern and Southern U.S. and its distribution continues to grow (Pioneer, 2015). The SBA has a heteroecious life cycle. Its primary (summer) host is soybean (*Glycines max* (L.) Merr.). However, it uses buckthorns as a secondary host during its overwintering period. In North America, SBA colonizes common buckthorn, *Rhamnus carthartica* L., whereas in its native Asian range, it uses the Japanese buckthorn *Rhamnus japonica* Maxim and Dahurian buckthorn *Rhamnus davurica* Pallus for overwintering (Takahashi et al., 1993; Hodgson and Heidel-Baker, 2013). SBA have help moving from their primary to secondary host when storms occur and strong winds transport them to soybean fields.

SBA is capable of causing yield losses of more than 50% in soybean (McCornack et al., 2004). Feeding causes stunting, leaf distortion, and reduced pod set. The piercing-sucking style of feeding also decreases the chlorophyll content of soybean plants (Li et al., 2004; Diaz-Montano et al., 2007) and contributes to sooty mold growth through the production of honeydew (Li et al., 2004). In addition, SBA is a vector of disease, such as alfalfa mosaic virus, bean yellow mosaic virus, tobacco ringspot virus, cucumber mosaic virus, potato virus Y, and soybean mosaic virus (Diaz-Montano et al., 2006; Tilmon et al., 2013).

Several control tactics are used by producers to help maintain SBA at acceptably low population levels. Producers tend to apply insecticides as their method of controlling SBA (McCornack and Ragsdale, 2006), which increased 130-fold since the arrival of SBA in the U.S. (Hodgson and Heidel-Baker, 2013). However, a majority of pesticides have negative repercussions for the environment as well as for non-target species, including natural enemies and pollinators. In addition, overuse of pesticides increases the risk and incidence of pests developing resistance. For example, neonicotinoid pesticides are used both as a seed and foliar treatment, thus doubling the exposure to SBA (Chandrasena et al., 2012). Seed treatments alone

are not effective with large SBA populations during the soybean reproductive stage and they do not make much of a difference in terms of yield with small SBA populations, so they are considered more of an “insurance policy’ (Hodgson and Heidel-Baker, 2013). Biological control by resident and imported (Heimpel et al., 2004) natural enemies can help in the natural suppression of SBA populations (Ragsdale et al., 2011); but it cannot be relied upon as the sole control tactic (Desneux et al., 2006).

Another non-pesticidal approach for controlling SBA is host plant resistance. Currently six SBA-resistant genes have been identified: *Rag1, rag1c, Rag2, Rag3, rag4, and Rag5* (Hill, et al. 2012). *Rag1* is effective against biotypes 1 and 3, while *Rag2*, *rag1c*, *Rag3*, and *rag4* work against biotypes 1 and 2 (LaBarge, 2011). The genetic mapping of *rag1c* is in the same region as *Rag1*, and *rag4* is in the same region as *Rag2* (Zhang et al., 2009; Hill et al., 2012). *Rag5* (proposed) is effective against biotypes 1 and 2, but not biotype 3 (Bansal et al., 2013); it was found near *Rag2*, but exhibited antixenosis, while *Rag2* involves antibiosis (Jun et al., 2012). *Rag1* is the most commonly used resistant gene in varieties sold in Iowa (McCarville et al., 2012) and the most widely tested SBA resistant gene in the literature. It has also been widely used in the North Central U.S. states since it began to be sold commercially for Roundup Ready soybean in 2010 (Hodgson and Heidel-Baker, 2013). A study performed at Northwest Research farm at Iowa State University resulting far less SBA on *Rag1* resistant lines than susceptible and a higher yield from *Rag1*-resistant lines (McCarville et al., 2012). Researchers at Kansas State University have shown that soybeans containing the *Rag1* gene are associated with both antibiosis and antixenosis in that SBA take twice as long to reach the phloem to feed and plants do not experience chlorophyll loss, and progeny production is reduced (Diaz-Montano et al., 2006, 2007).

Employing resistant soybeans to combat SBA has several advantages, including ease of use and general effectiveness. However, depending on the resistance mechanisms involved, resistant plants may or may not be compatible with natural biological control. For example, SBA resistance was shown to reduce adult longevity in the lady beetle *Harmonia axyridis*, whereas longevity of the minute pirate bug *Orius insidiosus* increased (Lundgren et al., 2009). Although SBA resistance can have an impact on adult longevity in lady beetles, they are still the most important biological control agent for SBA since the larvae move from soybean to buckthorn, both host plants of the SBA (Hodgson and Heidel-Baker, 2013). In cases of antibiotic resistance that reduces SBA survival, parasitoids may not survive because they are unable to fully develop before host SBA die (Ballman et al., 2012). However, soybeans with the *Rag1* gene itself do not appear to have a direct adverse effect on natural enemies (Li et al., 2008) and choosing a variety that controls SBA, but does not harm natural enemies, is possible (Bottrell and Barbosa, 1998).

Temperature has multiple direct effects on insect life histories, including growth and development, survival, reproduction, and sometimes dormancy (Logan et al., 1976; Bauerfeind and Fischer, 2013). In herbivorous insects, it is common for temperature to affect insect population growth and fitness by causing changes in host plant quality (Schalk et al., 1969; Tang et al., 1999) or the amount of resource available (Precht et al., 1973). Temperature can also alter resistance within a plant, which in turn, can determine the host plant quality.

The expression of plant resistance to insects can vary with environmental conditions, including temperature. It can cause pest resistance to become enhanced (Thindwa and Teetes, 1994), break down (Wood and Starks, 1972; Salim and Saxena, 1991; Harvey et al., 1994; Richardson, 2011; Chirumamilla et al., 2014; Chen et al., 2014), and sometimes a change will

not occur (Jackai and Inang, 1992), but all the types of results still create a better understanding of what may happen and when during a growing season in terms of a producer making pest management decisions. How host plant resistance influences temperature-dependent growth and development has not been measured for SBA on resistant soybean varieties in terms of degree days, and there are few studies on how temperature alters the expression of *Rag1* resistance for the soybean aphid (Richardson, 2011; Chirumamilla et al., 2014). Richardson (2011) recorded *Rag1* resistance breaking down at a lower temperature, whereas, Chirumamilla et al. (2014) reported no change in resistance between a moderate and a higher temperature. Despite using the same set of experimental temperatures, both studies yielded different results. In general, little is known about how the environmental temperature affects SBA-plant interactions. The overall goal of my study was to determine whether ambient temperature modified the level of *Rag1* resistance in soybean seedlings. Based on previously published data for SBA (Richardson, 2011; Chirumamilla et al., 2014), one would predict that lower temperatures should cause *Rag1* resistance to break down. I also predicted that development on the resistant soybean line would be slower than on the susceptible line, and that differences in development would not be the same at all temperatures, resulting in different thermal constants for both the lower thermal threshold (t) and the degree-day constant (K).

Materials and Methods

Plant and Insect Cultures

Biotype 1 soybean aphids were obtained from a colony maintained in the Department of Entomology at Kansas State University. The SBA were reared on seedlings of a susceptible soybean line (SD01-76R) in an environmental growth chamber 25.6 ± 0.06°C, and a photoperiod of 16:8 (light:dark) h with light intensity of 273.1 ± 33.0 W/m^2 , and a relative humidity of 75.6

± 1.6%. Seedlings of the soybean line (LD(05)-16060), which contains the *Rag1* gene for SBA resistance, were also grown in the same growth chamber. Plants from both the susceptible and resistant lines were grown from seeds for 7 to 9 days in pots (7.6 x 10.2 cm [H x D]) containing Metro-Mix 360 (Sun Gro Horticulture, Bellevue, WA). Four seeds were planted in each pot. After germination, thinning was done so that each pot contained only one healthy seedling. The 7- to 9-day growth period was sufficient for seedlings to reach the V-0 stage, at which plants are about five centimeters tall and have cotyledons that are cupped around the unifoliate leaves. During propagation and throughout the experiment, seedlings were watered daily or when the soil on the surface was completely dry. Caution was taken not to overwater the seedlings in order to prevent fungus from growing on the soil.

Experimental Procedures

The experiment was repeated four times between 14 March 2014 and 6 November 2014. In each repetition of the experiment, potted seedlings from both the resistant and susceptible (control) soybean lines were inoculated by transferring two adult SBA from the colony to each seedling and leaving them for 24 hours after which the adults and all but one nymph were removed. Transfers were done using a fine (#000) camel hair paintbrush. Equal numbers of each type of seedling (resistant and susceptible) were then placed in each of four environmental growth chambers (Percival Scientific, Inc., Model I36VLC8, Perry, IA or Conviron, Model A1000, Winnipeg, Manitoba CA) assigned to either 15, 20, 25, or 30°C. The actual temperatures measured in the growth chambers were 15.3 ± 0.02, 20.7 ± 0.05, 25.3 ± 0.02, and 30.0°C ± 0.03 and average light intensity was 83.3 ± 9.9W/m^2 for the Percival chambers and 341.0 ± 17.1 W/m^2 for the Conviron chambers. Thereafter seedlings were inspected daily to determine if nymphs were alive and, if so, in what life stage. Because it was difficult to distinguish between

individual SBA life stages, three age classes were used: 1st-2nd, 3rd-4th and adult. To maintain consistently high plant quality, every three days the soybean seedlings in each temperature treatment were replaced with new V-0 stage seedlings by transferring individual SBA from the old to new seedlings. When SBA began to produce progeny, the number of offspring per reproductive was recorded and removed from the seedling daily. This procedure continued until the adult SBA died.

Experimental Design and Statistical Analyses

The design of this experiment was a Latin Square Design (LSD) with a split-plot and replication on the split-plot. For the whole plot, temperature was the whole-plot treatment factor (four levels: 15, 20, 25 and 30°C) and the LSD row and column factors were growth chamber and time repeat, each with 4 levels. For the split-plot, plant type (two levels: resistant and susceptible) was the split-plot treatment factor with 20 pots per plant type, thus giving a total of 40 pots per growth chamber for a total of 160 pots. In trial 1, contamination by lacewing larvae eliminated SBA from several susceptible plants early in the experiment. These were replaced with an excess of three plants to ensure that the minimum number was maintained, which resulted in 23 (vs. 20) replications for a total of 163 pots for the entire experiment. Temperature and plant type were considered fixed effects, and chamber and time repeat were considered random effects. Based on results from Experiment 1, location within growth chamber (shelf) was ignored in the analysis.

The numerical responses (preimaginal development time [1st instar nymph to adult emergence], total number of progeny per adult, and adult longevity) were analyzed using the MIXED procedure in SAS (V9.4) with the Restricted Maximum Likelihood (REML) method of model fitting. Residuals were evaluated and found to be somewhat non-normal but symmetric.

Therefore, given the large sample size, these results based on the normal distribution are considered valid. Proportions of SBA surviving to adult emergence were analyzed using the GLIMMIX procedure with the default pseudo-likelihood method, using the binomial distribution and the logit function. (Note that GLIMMIX's default pseudo-likelihood method of model fitting gives the same results as the MIXED REML method when the normal distribution is being used.) Results from MIXED and GLIMMIX analyses included: REML estimates and approximate Wald test statistics for the random effects' variance components; type 3 F-tests to test fixed effects, i.e., the temperature and conditioning main effects and their interaction; lsmeans and standard errors for these fixed effects; pairwise comparisons for a significant temperature effect, using unadjusted (LSD) p-values at $P \leq 0.05$ level; and simple effects (using the "slice" option) to test the difference between the two plant types at each temperature when the temperature by plant type interaction was significant. The variance components for both growth chambers and time repeat were never significant, indicating that variation in aphid responses did not change due to differences in growth chambers or the experiment being run four times over the course of several months.

Data from the four individual SBA responses were used to compute three life table statistics: finite rate of population increase (λ), net reproductive rate (R_o), and mean generation time (T). In addition, developmental times at each temperature were converted to developmental rates (1/days) and thermal constants were derived for each plant type (resistant and susceptible) using the standard degree day formula:

$$K = d(T-t)$$

where K is the degree-day constant, d is the average number of days for preimaginal development, T is the growth chamber temperature, and t is the lower developmental threshold. Means and standard errors were generated using the K values at each of the four temperatures as estimates. The days for SBA development were averaged over the four time trials for each plant type and converted to rates by taking the reciprocals (1/days). To compute the lower thermal thresholds, development rates were plotted against temperature for each trial, and then lines were fit by eye through the plotted points. The point at which each line intersected with the X (temperature) axis was considered to be the estimated lower thermal threshold (t). This lower thermal threshold is defined as the temperature at which all development, including biochemical processes, cease due to insufficient heat energy. Means and standard errors for t were computed for both plant types (resistant and susceptible) using the four trial estimates. To determine whether the degree-day constants (K and t) differed significantly when SBA were reared on resistant and susceptible plants, the individual values obtained for each temperature and trial, respectively, were used as a source of variation and a two-tailed t-test was run on the means and variances using the GraphPad Software and the QuickCalcs automatic t-test (http://www.graphpad.com/quickcalcs/ttest1.cfm).

The life table statistics were analyzed using Program R (R i386 3.1.2) and graphed using SigmaPlot. The input data for Program R consisted of the age, px (age-specific survival rate from age x to age x+1), mx (number of female offspring per female of age x), and the standard error for both values. The output consisted of the mean, standard deviation, and confidence intervals for each set of data to be tested.

Results

A table summarizing the statistical values for the main effects and interaction for each SBA response is show in Table 3.2.

Developmental Rate

Both temperature and plant type had a highly significant effect on SBA preimaginal development ($P < 0.001$ and 0.0005, respectively), but there was no significant interaction ($P < 0.1524$) suggesting that the relative difference in developmental rate across temperatures was the same for both plant types. However, when the data were subjected to the test of effect slices procedure, there were significant differences in development rate between susceptible and resistant soybeans at 25 and 30˚C ($P < 0.0003$ and 0.0313, respectively), but not at the two lower temperatures (Figure 3.1). For both susceptible and resistant soybeans, there was a direct relationship between temperature and the rate of SBA development.

Preimaginal Development Time

Both temperature and plant type had a significant effect on SBA preimaginal development ($P < 0.0017$ and 0.0123, respectively), but there was no significant interaction ($P < 0.7520$), indicating that the relative effect of temperature on development was the same for both plant types. Development time becoming progressively shorter as temperature increased, but the differences were only significant between 15 and 25˚C. (Figure 3.2). Development of SBA was significantly longer on resistant soybeans than on susceptible plants at all temperatures. The difference between the average development time of soybean aphids on susceptible and resistant plants among the variety of temperatures was 0.85 days. The average number of days for SBA to

develop from 1st instar nymph to adult ranged from 5.17 to 12.3 days on resistant plants, and from 4.66 to 11.6 days on susceptible plants.

Survival

Both temperature ($P < 0.0054$) and plant type ($P < 0.0001$) had a significant effect on SBA survival. There was also a significant temperature by plant type interaction ($P < 0.0151$). At all temperatures, percentage survival was significantly higher on susceptible plants (Figure 3.3). On average, SBA survival was 44 % higher on susceptible plants than on resistant plants. For both plant types, SBA survival increased between 15 and 25˚C. However, at 30˚C survival decreased significantly on resistant soybeans, whereas on susceptible soybeans survival remained high (Figure 3.3). A comparison of SBA survival on susceptible and *Rag1*-resistant plants at various temperatures showed a larger decrease in survival on resistant plants compared to susceptible plants 30˚C than at 25˚C, suggesting that resistance may be enhanced at the higher temperature (Table 3.1).

Progeny Production

Both plant type and temperature had significant main effects on the number of SBA progeny produced ($P < 0.0001$ and 0.0299, respectively), but there was no significant interaction between the two factors ($P < 0.0957$). The number of progeny produced on resistant soybean plants was consistently much lower than on susceptible plants. The only significant difference within temperatures was between the lowest temperature (15˚C) and the two highest temperatures (25 and 30˚C) (Figure 3.4). Although there was no significant difference between the lower temperatures and higher temperatures, there appears to be a biological difference. The middle temperatures appear to be most optimal for SBA progeny production as opposed to either of the extremes on susceptible plants. For resistant plants, the amount of progeny produced was

fairly consistent for all temperatures except for 30˚C where there only an average of 2.14 progeny.

Adult Longevity

Temperature had a significant effect on longevity of SBA development ($P < 0.0127$), as did plant type ($P < 0.0001$). However, the interaction between temperature and plant type was not significant ($P < 0.4155$). There was a trend for longevity to become shorter as temperature increased. However, the only significant difference was between 15 and 30˚C (Figure 3.5). Adult lifespans were markedly shorter at all temperatures on resistant soybean seedlings. The difference in longevity of SBA on susceptible and resistant plants (averaged over all temperatures) was 7.5 days. The average longevity of SBA adults ranged from 2.5 to 13.6 days on resistant plants, and from 7.9 to 20.7 days on susceptible plants.

Thermal Constants

There was a significant difference in degree-days between SBA on susceptible and resistant plants ($P < 0.0115$). On average, development took 20 degree days longer for SBA on resistant (118.3 ± 4.8 DD) than susceptible (96.6 ± 3.6 DD) plants in this study. The lower thermal threshold was 7.03 ± 0.97˚C for SBA on susceptible plants and 5.8 ± 1.53˚C on resistant plants.

Demographic Responses

SBA population growth was positive on both resistant and susceptible soybeans at all four temperatures as evidenced by λ values greater than 1.00 (Figure 3.6). However, on resistant soybeans rates of growth were consistently low, with slight positive increases observed between 15 and 25˚C followed by a decrease to nearly zero growth at 30˚C (Figure 3.6). On susceptible

plants, changes in population growth rate were larger than on resistant plants, but the pattern was similar with increases between 15 and 25˚C and a decrease between 25 and 30˚C (Figure 3.6). When SBA population growth was compared between resistant and susceptible plants, differences were highly significant ($P < 0.001$) at all temperatures except for 15˚C (Figure 3.6). The optimal temperature for SBA population growth was 25˚C with the highest lambda values noted at that temperature for both plant types.

On susceptible plants, the net reproductive rates (R_o) were all positive, ranging from ~20 to ~60 females per female; they were highest at 20 and 25˚C (Figure 3.6). In contrast, on resistant plants mean R_o values were consistently low (< 10 females per female) and, at 30˚C the net reproductive rate was essentially zero. R_o values were significantly ($P < 0.001$) higher on susceptible soybeans compared to resistant soybeans at all temperatures (Figure 3.6).

There was a steady decrease in mean generation time (T) for SBA on both susceptible and resistant plant types with increasing temperature. Mean generation times ranged from 7 to 14 days. As temperature increased, the decline in mean generation time was slower on susceptible plants than on resistant plants. Differences in T between resistant and susceptible soybeans were significant, but only at 20 and 25˚C (Figure 3.6).

Survivorship and Fecundity Schedules

As predicted, SBA survivorship was much lower at all temperatures on resistant soybeans than on the susceptible line (Figure 3.7). Two other patterns emerged in comparing survivorship curves among temperatures and between plant types. First, survivorship was lowest on both types at 30˚C, but lowest on the resistant line. Second, there were larger differences (a wider spread) in survivorship among temperatures on the susceptible line compared to the resistant line

(Figure 3.7). When survivorship was plotted as a function of degree-days (Figure 3.8), differences among temperatures were more apparent for both plant types. Specifically, on a degree-day basis, survivorship appeared to be inversely related to temperature, with the lowest survivorship on both plant types occurring at 30˚C (Figure 3.8).

When the reproductive schedules were compared, there was no consistent pattern for mean fecundity on susceptible soybeans during the early period; but more progeny were produced at 20˚C during later adult life than any other temperature (Figure 3.7). In contrast, the reproductive schedules on the resistant soybean line were not distinctly different among temperatures (Figure 3.7). Between plant types, most of the reproduction on the resistant cultivar was concentrated in a shorter period of time (ending around day 31) compared to the susceptible line where progeny continued to be produced until day 46 (Figure 3.7). When daily mean fecundity was divided by degree-days and plotted on a degree-day scale (Figure 3.8), the reproductive schedules between plant types and among temperatures within plant types was similar to when the data were plotted as a function of calendar days (cf. Figures 3.7 and 3.8).

Discussion

SBA responded to temperature and resistant soybean plants as expected. On the resistant soybean line, the rate of population increase was lower at all temperatures than on susceptible soybeans. Reduced population growth rates on resistant plants appeared to be most strongly linked with lower survival of immatures as well as reduced progeny and adult longevity. In addition, development to adulthood was consistently longer on resistant plants than on susceptible plants. Although differences in development were not statistically significant, they likely contributed to differences in rates of population increase. Soybean lines with *Rag1* resistance are considered to be antibiotic (Diaz-Montano et al., 2006, 2007). However,

antixenosis also may be involved. For example, it takes longer for SBA to reach the sieve element, if they reach it at all, while feeding on resistant varieties (Diaz-Montano et al., 2007). When the SBA are able to feed, they have the ability to alter the amino acid composition of the soybean plant in their favor (Chiozza et al., 2010). If the SBA were not able to feed properly at the higher temperatures and consumed less, then this could have contributed to their reduced developmental rate and survival.

On both plant types, SBA exhibited temperature-dependent responses for all traits except progeny production, which peaked at 20 and 25˚C and was lower below and above those temperatures. However, a linear relationship in responses was observed only between 15 and 25˚C. As temperature increased within this range, SBA developed faster and survived better; but adult lifespans were shorter. Both the finite rates of increase and net reproductive rates increased with increasing temperature, while mean generation times decreased. On susceptible soybeans, large differences in demographic traits were observed among temperatures, which contrasted with SBA-resistant plants where finite rates of increase and net reproductive rates were consistently low at all temperatures, exhibiting only slight increases between 15 and 25˚C. Although generation times became shorter with increasing temperature on both susceptible and resistant soybeans, the effect of plant resistance on SBA resulted in a slow rate of decline.

SBA performance varied considerably between plant types and among life history traits between 25 and 30˚C, indicating a complex interaction between temperature, plants, and insects. Longevity and progeny production decreased from 25 to 30˚C on resistant and susceptible plants, suggesting that both adult traits responded directly to rising temperature. However, pre-adult survival was affected both by temperature and the type of plant on which SBA was reared. On resistant soybeans, there was a large drop in SBA survival between 25 and 30˚C. The generally

poor performance of SBA on both resistant and susceptible plants at 30˚C indicates that SBA are not well-adapted to this higher temperature. This conclusion is supported by the findings of others (Hirano et al., 1996; McCornack et al., 2004). However, SBA survival on susceptible soybeans was equally high at 25 and 30˚C. The fact that survival decreased significantly at 30˚C, but only on resistant soybeans, suggests that antibiotic resistance may be elevated at this higher temperature. In the greenbug *Schizaphis graminum*, Thindwa and Teetes (1994) also found an increase in host plant resistance under higher temperatures. In contrast, studies with the Hessian fly have reported a breakdown in plant resistance at higher temperatures (Sosa and Foster, 1976; Tyler and Hatchett, 1983; Chen et al., 2014). With respect to SBA, Richardson (2011) and Chirumamilla et al. (2014) compared differences in population growth of biotype 1 on *Rag1*-resistant and susceptible soybeans between a moderate (21˚C) and high (28˚C) temperature and determined there was no change in the level of resistance. However, the highest temperature tested in those studies was lower than my highest temperature (30˚C). It is possible that these resistant soybean lines only respond after temperatures have reached a critical upper threshold.

Low or decreasing temperatures can also have variable effects on plant resistance, in some cases strengthening it (Chen et al., 2014; Chapter 2 this thesis), in others causing resistance to break down (Wood and Starks, 1972; Schweissing and Wilde, 1979; Harvey et al., 1994). In SBA, Richardson (2011) and Chirumamilla et al. (2014) observed a smaller difference in population numbers between resistant and susceptible soybeans at 14˚C compared to 21˚C and concluded that *Rag1*-resistance was reduced or lost at lower temperature. My study did not evaluate changes in SBA population numbers. But there was a four-fold reduction in the difference between finite rates of population increase between resistant and susceptible soybeans at 15˚C compared to 20˚C, which is consistent with the other studies and might be considered

indirect evidence for a breakdown in plant resistance. However, a comparison of life history traits did not reveal conclusive evidence that resistance was weaker at the lowest temperature. Specifically, although the difference in the number of progeny produced between plant types was smaller at 15˚C than at 20 or 25˚C, there was no change in relative rates of survival between resistant and susceptible plants among temperatures. In fact, Richardson and Chirumamilla et al.'s claim for reduced resistance at low temperature contrasts with my conditioning experiment, which showed that SBA resistance increased when plants experienced a decrease in temperature between 25 and 20˚C (see Chapter 2). It is unclear why the results of these studies differed given the similar range of temperatures. One possibility is that different soybean lines respond differently to temperature. Previous studies used the *Rag1* line LD05-16611, whereas my experiments were done with LD05-16060. Alternatively, even though the SBA biotype was the same in all studies, there may have been genetic differences in the populations that affected how the SBA responded to soybean plants. A more likely explanation is that because previous studies relied on general differences in population numbers rather than specific demographic responses (as my study did), the conclusion by Richardson (2011) and Chirumamilla et al. (2014) that resistant plants became more susceptible at low temperature may not be true. That is, limited heat energy available for SBA development and reproduction at low temperature could have produced a similar pattern of population growth on resistant and susceptible plants, thus overriding effects due to host plant resistance.

Resistant soybeans had a substantial effect on the demographic performance of SBA. When SBA were reared on susceptible soybeans, finite rates of increase and net reproductive rates were positive and increased across most of the temperature range. In contrast, population growth on resistant soybeans was barely positive, and adults were able to replace themselves by

only a slim margin. At the highest temperature, 30˚C, there was a net negative effect on demographic performance compared to the middle temperature range for both plant types. But whereas the finite rates of increase and net reproductive rates at 30˚C were positive on susceptible soybeans, they exhibited no growth on resistant plants. The optimal temperature for SBA population growth on both plant types was 25˚C, which is the same temperature that McCornack et al. (2004) reported as optimal on a susceptible soybean line.

SBA developed more slowly, produced fewer progeny, had a lower survival rate to adulthood, and a shorter adult longevity at all temperatures on resistant soybeans, indicating that plant resistance had multiple and significant depressive effects on SBA populations. In addition, when SBA developmental rates were plotted against temperature, the resulting slope for the resistant line was smaller than for the susceptible line. Using the inverse relationship between slope and the degree-day constant (Logan et al., 1976), biotype 1 SBA required ~25% more degree-days to complete development on resistant plants than on susceptible plants. The relationship between temperature and development was not symmetrical across temperatures between resistant and susceptible plants, suggesting that differences in population growth rate on resistant and susceptible plants would not be uniform over the range of ecological temperatures that SBA experience in the field.

In my experiment, SBA development on the susceptible soybean line was directly related to temperature, with significantly shorter development times observed at 30˚C compared to 20˚C. I also found that SBA required fewer degree-days to complete development on the susceptible soybean (96.6 ± 3.6 DD) compared to the resistant soybean (118.3 ± 4.8 DD). The larger degree-day requirement on resistant plants is expected since resistant soybeans appeared to delay SBA development at most temperatures.

A comparison of the thermal constants I found for SBA on susceptible soybeans with those derived from data published by McCornack et al. (2004) for another susceptible soybean line revealed some differences. McCornack et al. (2004) had combined SBA development rates at temperatures they studied with those published by Hirano et al. (1996) and then plotted all values to produce a linear curve. I calculated the slope and then estimate the thermal constant (K) by taking the reciprocal of the slope. This resulted in 76 accumulated degree-days required for SBA to develop to adults in the combined McCornack-Hirano studies. This value is less than what I calculated (96.6 ± 3.6 DD). The linear regression and fit for the merged McCornack-Hirano data had a good fit, so it is possible that the SBA population in my study has a different temperature-development relationship from those in previous studies. This would not be unexpected given differences in geographic location (McCornack et al., Minnesota; Hirano et al., Morioka, Japan; Hough, Kansas) and the fact that my study was done 10-15 years after the previous research.

The lower thermal threshold I derived for SBA on susceptible plants (5.80˚C) was ~1˚ higher than on the resistant line (7.03˚C); but the difference is likely not statistically significant based on an overlap in standard errors. Regardless, any difference in threshold temperatures on resistant and susceptible plants would have a negligible effect on SBA population development in the field. While differences in threshold temperatures were small between plant types, considerable variation exists among studies for SBA on susceptible soybeans. McCornack et al. (2004) initially predicted a lower thermal threshold of 5.6˚C, which is lower than the one in my study. However, Hirano et al. (1996) gave 9.5˚C as an estimate, and McCornack et al. pooled their temperature data with those of Hirano et al. to generate a threshold temperature of 8.6˚,

which is more than two degrees higher than my estimate, but falls within a 95% confidence interval of both mine and McCornack's lower thresholds.

My findings for the SBA extend our general understanding of how temperature affects host plant resistance because I was able to show not only that resistance appears to increase under higher temperature, but that it may also be enhanced if plants experience a decrease in temperature within only a moderate range of lower temperatures. Salim and Saxena (1991) showed that resistance in rice to the planthopper *Sogatella furcifera*, broke down both at high and low temperatures. However, my study is the first to document enhanced resistance under both high and decreasing temperature.

My research also has practical applications for SBA management. The thermal constants I derived for resistant and susceptible soybeans will allow producers to better predict SBA occurrence and population development using degree-days regardless of whether they are planting a resistant or susceptible variety of soybeans. In addition, although it is difficult to separate the direct and indirect effects of temperature on SBA, it appears that high temperature stress may interact with *Rag1* resistance to detrimentally impact SBA populations. How low temperature might affect the expression of host plant resistance traits in the field is less clear because of the contradictory evidence. Further investigations are needed to compare SBA responses under fixed and decreasing temperatures over different temperature ranges, and using pre-infestation plant conditioning. These experiments should include temperature reversal treatments and a control treatment where the temperature stays constant throughout the plant conditioning and throughout the SBA lifespan. As for plant type, a susceptible variety could be added, as well as, other *Rag* resistant soybean cultivars or SBA biotypes. This approach should determine whether resistance strengthens, weakens, or remains constant under different thermal

regimes. Even so, the fact that plant resistance can be quickly reversed by increasing or decreasing temperature leaves a large unanswered question concerning the net impact of temperature on host plant resistance given the frequent temperature fluctuations common to the Midwest where most U.S. soybeans are grown.

Climate change is forecast to have multiple direct and indirect effects on plants and animals in all ecosystems, including crop pests (Backlund et al., 2008; Walthall et al., 2012). In regards to global agriculture, changes in temperature and rainfall pattern can shift the range of insects and alter their number of generations within a growing season. Their development is dependent on temperature based on their thermal requirements; so my data may contribute to understanding if and whether future ambient temperature changes may impact the expression of plant resistance to SBA in addition to direct temperature effects on SBA population growth.

In summary, my research is a small piece, but it adds crucial information to the overall knowledge base for SBA pest management. Experimentally, I showed that the level of resistance appears to change when the soybeans and SBA are exposed to a variety of ambient temperatures. I also quantified differences in degree-days required for SBA development on resistant and susceptible soybeans. Together, these results should lead to better predictive models for soybean producers about the impact of host plant resistance and temperature on SBA populations, thus giving growers a better idea of when to scout and whether pest control action is needed. With more refined information, producers can save time and money while maintaining high yields.

Figures and Tables

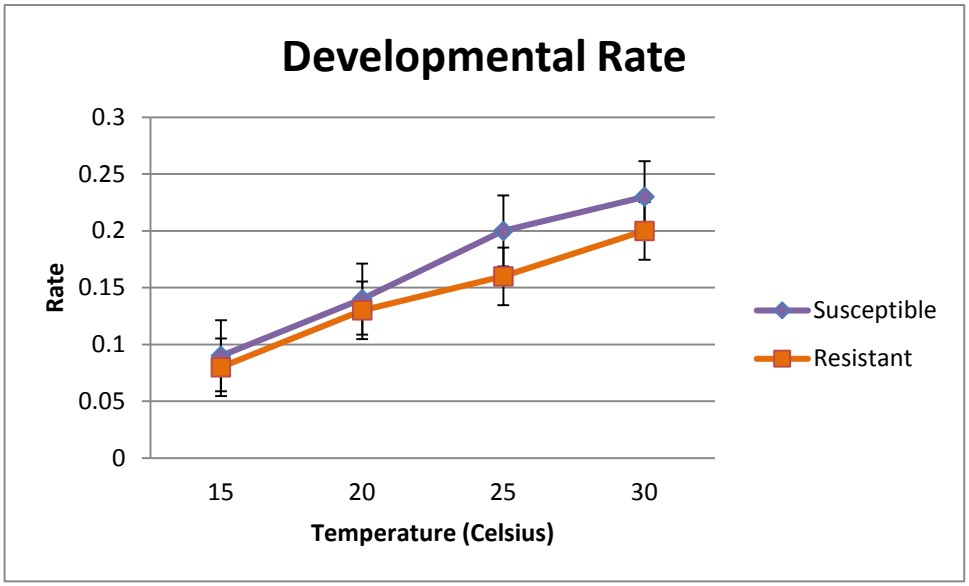

Figure 3.1 Mean ± SE rate of development for biotype 1 soybean aphid nymphs to reach adulthood on susceptible or *Rag1*-resistant soybean seedlings under 15, 20, 25, or 30˚C. Range of observations (low-high) for the four trials for each treatment combination: 15˚C: susceptible: 7-13, resistant: 1-6, 20˚C: susceptible: 14-15, resistant: 6-9, 25˚C: susceptible: 15-19, resistant: 8-10, and 30˚C: susceptible: 16-19, resistant: 2-4.

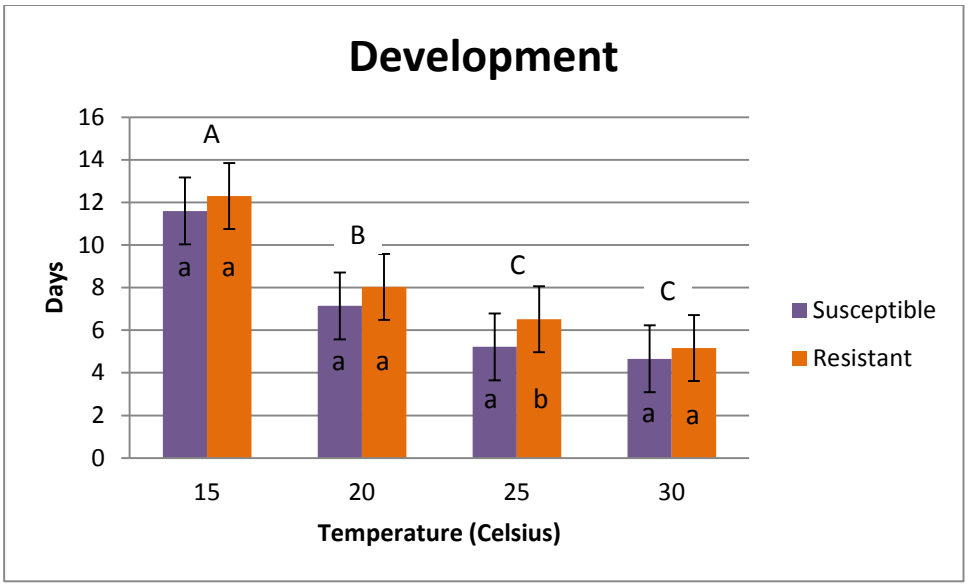

Figure 3.2 Mean ± SE number of days required for biotype 1 soybean aphid nymphs to reach adulthood on susceptible or *Rag1*-resistant soybean seedlings under 15, 20, 25, or 30˚C. Range of observations (low-high) for the four trials for each treatment combination: 15˚C: susceptible: 7-13, resistant: 1-6, 20˚C: susceptible: 14-15, resistant: 6-9, 25˚C: susceptible: 15-19, resistant: 8-10, and 30˚C: susceptible: 16-19, resistant: 2-4. Capital letters denote statistical difference among temperatures with development times pooled for the two plant types. Lower case letters denote statistical difference between plant types within each temperature.

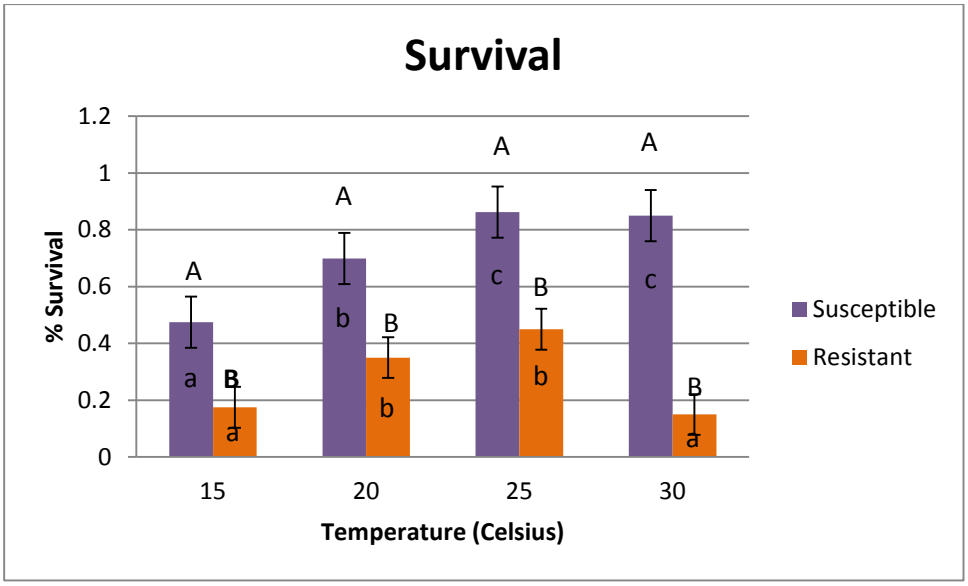

Figure 3.3 Mean ± SE number of biotype 1 soybean aphid nymphs surviving to adulthood on susceptible or *Rag-1*resistant soybean seedlings under 15, 20, 25, or 30˚C. Range of observations (low-high) for the four trials for each treatment combination: 15˚C: susceptible: 7-13, resistant: 1-6, 20˚C: susceptible: 14-15, resistant: 6-9, 25˚C: susceptible: 15-19, resistant: 8-10, and 30˚C: susceptible: 16-19, resistant: 2-4. Lower case letters inside bars denote statistical differences in survival among temperatures for each plant type. Capital letters on top of bars denote statistical differences between plant types at each temperature.

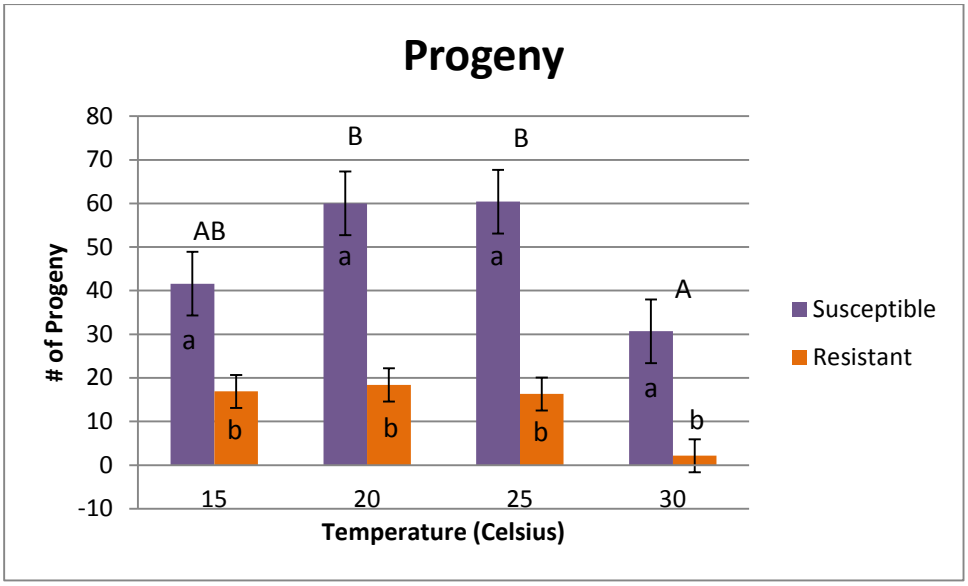

Figure 3.4 Mean ± SE number of progeny produced by biotype 1 soybean aphid adults on susceptible or *Rag1*-resistant soybean seedlings under 15, 20, 25, or 30˚C. Range of observations (low-high) for the four trials for each treatment combination: 15˚C: susceptible: 7-13, resistant: 1-6, 20˚C: susceptible: 14-15, resistant: 6-9, 25˚C: susceptible: 15-19, resistant: 8-10, and 30˚C: susceptible: 16-19, resistant: 2-4. Capital letters denote statistical difference in progeny produced among temperatures with data pooled for the two plant types. Lower case letters denote statistical difference between plant types within each temperature.

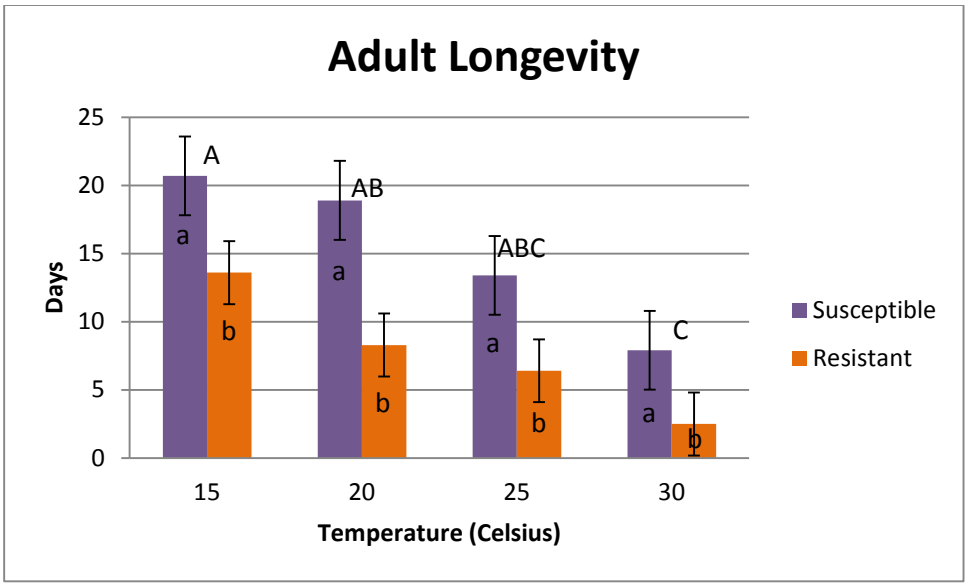

Figure 3.5 Mean ± SE number of days biotype 1 soybean aphids lived after reaching adulthood on susceptible or *Rag1*-resistant soybean seedlings under 15, 20, 25, or 30˚C. Range of observations (low-high) for the four trials for each treatment combination: 15˚C: susceptible: 7-13, resistant: 1-6, 20˚C: susceptible: 14-15, resistant: 6-9, 25˚C: susceptible: 15-19, resistant: 8-10, and 30˚C: susceptible: 16-19, resistant: 2-4. Capital letters denote statistical difference in adult longevity among temperatures with data pooled for the two plant types. Lower case letters denote statistical difference between plant types within each temperature.

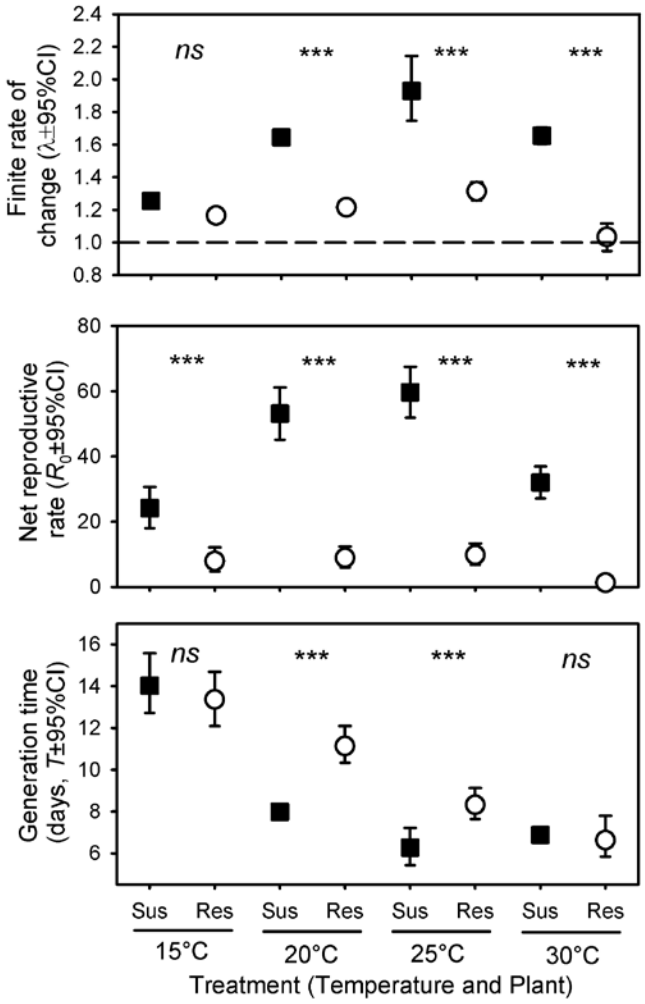

Figure 3.6 Demographic statistics for biotype 1 soybean aphids on susceptible or *Rag1* resistant soybean seedlings under 15, 20, 25, or 30˚C. For finite rates of increase and net reproductive rates, means above dashed line indicate an increase; below the line represents a decrease. Mean generation times are in days. Number of observations (N): finite rate of increase for both plant types and all temperatures 80-83; net reproductive rate: susceptible, 15˚C = 38, 20˚C = 58, 25˚C = 69, 30˚C = 68; resistant, 15˚C = 14, 20˚C = 28, 25˚C = 36, 30˚C = 12; mean generation time: susceptible, 15˚C = 38, 20˚C = 58, 25˚C = 69, 30˚C = 68; resistant, 15˚C = 14, 20˚C = 28, 25˚C = 36, 30˚C = 12. Within temperatures, conditioning times separated by *, **, or *** are significant at the P <0.05, 0.01 and 0.001 level, respectively.

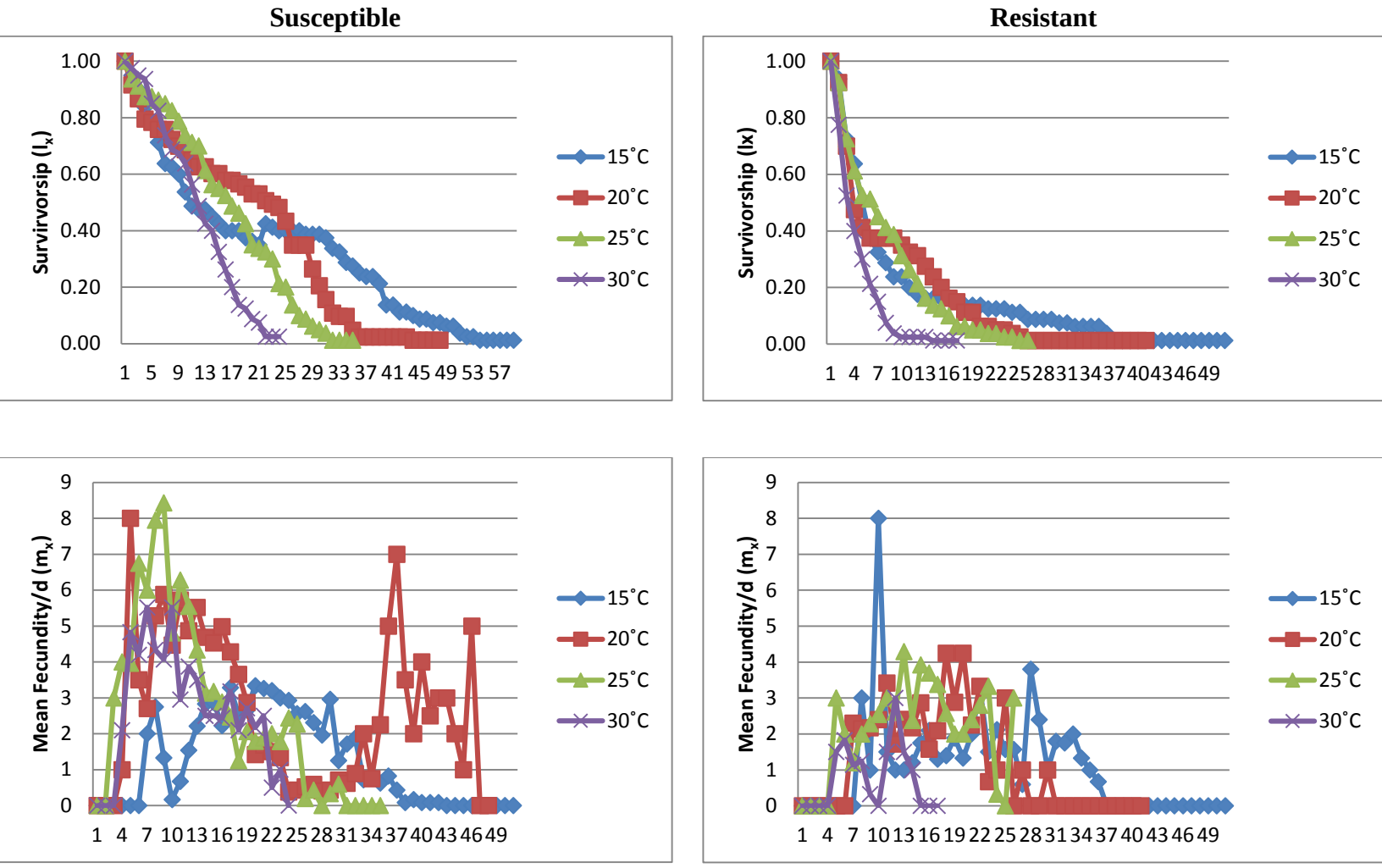

Figure 3.7 Survivorship and mean fecundity as a function of calendar age for soybean aphids on susceptible or *Rag1-r*esistant plants at either 15, 20, 25, or 30˚C.

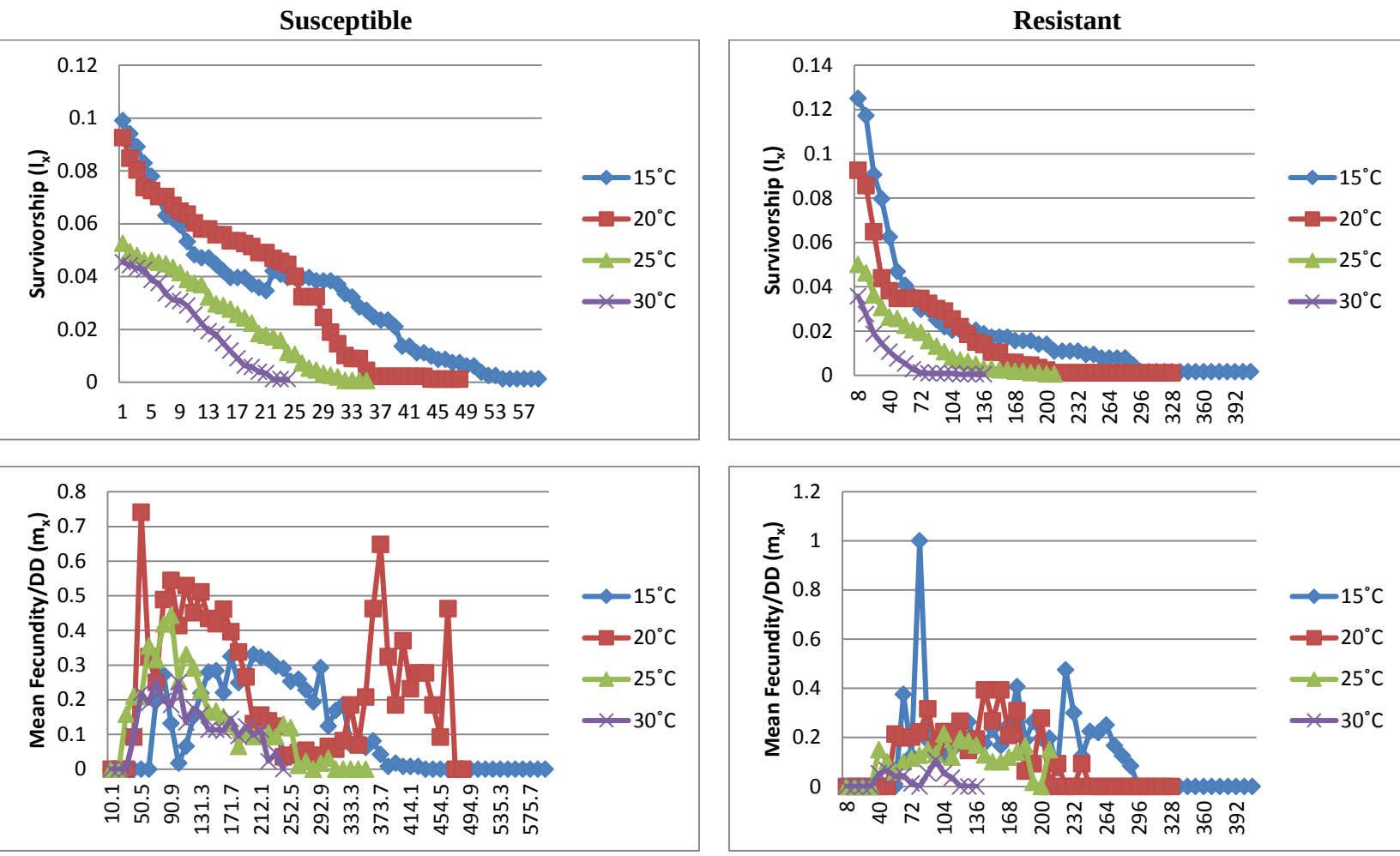

Figure 3.8 Survivorship and mean fecundity as a function of degree-days for soybean aphids on susceptible or *Rag1*-resistant plants at either 15˚C, 20˚C, 25˚C, or 30˚C.

Development Time (days)

Temperature	Susceptible	Resistant	Difference
15	11.6	12.3	-0.70
20	7.14	8.03	-0.89
25	5.22	6.52	-1.30
30	4.66	5.17	-0.51

Percent Survival

Temperature	Susceptible	Resistant	Difference
15	0.475	0.175	0.3
20	0.6988	0.35	0.3488
25	0.8625	0.45	0.4125
30	0.85	0.15	0.7

Number of Progeny

Temperature	Susceptible	Resistant	Difference
15	41.6	16.9	24.7
20	60	18.4	41.6
25	60.4	16.3	44.1
30	30.7	2.14	28.56

Longevity (days)

Temperature	Susceptible	Resistant	Difference
15	20.7	13.6	7.1
20	18.9	8.29	10.61
25	13.4	6.4	7
30	7.9	2.5	5.4

Finite Rate of Population Increase

Temperature	Susceptible	Resistant	Difference
15	1.26	1.17	0.09
20	1.63	1.21	0.42
25	1.91	1.31	0.6
0	1.66	1.04	0.62

Table 3.1 Differences in SBA responses between susceptible and resistant soybean seedlings over a range of temperatures (15 to 30°C). Consistent differences (S-R) indicate no effect of temperature on host plant resistance. Larger or smaller differences at extreme temperatures suggest level of resistance is affected.

Development				
	P-Value	**F Statistic**	**df_N**	**df_D**
Temperature	<.0001	105.2	3	6
Plant Type	0.0123	8.67	1	12
Temp X Plant Type	0.752	0.41	3	12
Development Rate				
	P-Value	**F Statistic**	**df_N**	**df_D**
Temperature	0.0009	24.22	3	6
Plant Type	0.0005	21.87	1	12
Temp X Plant Type	0.1524	2.11	3	12
Survival				
	P-Value	**F Statistic**	**df_N**	**df_D**
Temperature	0.0054	12.51	3	6
Plant Type	<.0001	117.91	1	12
Temp X Plant Type	0.0151	5.26	3	12
Progeny				
	P-Value	**F Statistic**	**df_N**	**df_D**
Temperature	0.0299	6.08	3	6
Plant Type	<.0001	140.21	1	12
Temp X Plant Type	0.0957	2.66	3	12
Longevity				
	P-Value	**F Statistic**	**df_N**	**df_D**
Temperature	0.0127	8.84	3	6
Plant Type	<.0001	45.39	1	12
Temp X Plant Type	0.4155	1.03	3	12

Table 3.2 Main effects, interactions, and statistical output for soybean aphids on susceptible or *Rag1*-resistant soybean seedlings under 15, 20, 25, or 30˚C.

Survival				
Temperature	**P-Value**	**F Statistic**	**df_N**	**df_D**
15˚C	0.002	15.39	1	12
20˚C	0.0009	19.02	1	12
25˚C	0.0002	26.61	1	12
30˚C	<.0001	61.38	1	12

Table 3.3 Statistics associated with lsmeans test for differences in plant type (resistant vs. susceptible) on soybean aphid survival at 15, 20, 25, or 30˚C

Chapter 4 - Thesis Summary, Implications for Pest Management, and Directions for Future Research

Temperature is one of the most influential abiotic factors affecting life on earth (Precht et al., 1973). It is particularly important for poikilothermic organisms such as insects, where life histories and population dynamics are strongly impacted by temperature-dependent development (Logan et al., 1976). In addition, temperature extremes reduce insect populations by lowering survival. Plants are also poikilotherms, which means that temperature has profound effects on their growth and development, including the balance of primary and secondary chemicals (Went, 1953; Precht et al., 1973). Consequently, temperature-related changes in plant quality and productivity have important indirect effects on herbivorous insect populations. However, separating direct from indirect temperature effects is difficult, especially for hemipteran species which feed continuously on host plants.

My thesis quantified direct and indirect (plant-mediated) effects of temperature on selected demographic traits of the SBA as well as on population growth parameters. One of the key questions I asked was: do different temperatures elicit different responses in soybeans that change the level of host plant resistance against SBA? Based on my results I concluded that exposure to lower temperatures strengthened host plant resistance, and that length of exposure was directly related to plant resistance within the limited range tested. In addition, higher temperature had a direct negative effect on SBA population growth, but it was not possible to determine whether higher temperatures influenced plant resistance. My research expands on what was already known about temperature effects on soybean resistance to SBA (Richardson, 2011; Chirumamilla et al., 2014) as well as how temperature influences SBA growth, development and survival (Hirano et al., 1996; McCornack et al., 2004).

In Chapter 2, I employed a technique whereby resistant soybean seedlings were conditioned for different durations to a lower or higher temperature than the propagation temperature before aphids were allowed to infest. I showed that temperature directly affects the expression of *Rag1* resistance, that the duration of plant exposure influences the strength of resistance, and that a decrease in temperature within a moderately low range may be important. A comparison of my findings with those of previous researchers suggests that soybeans respond to temperature in different ways over different ranges, and that changes in temperature and duration of exposure are important determinants of how host plant resistance traits are expressed. For example, my study showed that host plant resistance became stronger with a decrease in temperature between 25 and 20˚C. In contrast, Richardson (2011) and Chirumamilla et al. (2014) found that resistance became weaker when plants were exposed to a temperature lower than what I tested (14˚C). These findings are consistent with the highly variable results others have found for different insect-plant resistance examples. Therefore, future work with SBA should focus on examining the effects of temperature – both constant and changing – on host plant resistance, with emphasis on temperatures between 14 and 25˚C. More work is needed to elucidate if higher temperature either strengthens or weakens the expression of resistance. A more comprehensive knowledge of how temperature affects resistance to SBA would allow producers and pest managers to better understand under what temperature conditions plant resistance complements or offsets SBA population growth.

In Chapter 3, I quantified the relationship between temperature and SBA development on both susceptible and resistant plants and also compared other life history responses, including SBA survival, over a range of temperatures. Previous research (Hirano et al., 1996; McCornack et al., 2004) had focused on susceptible soybean varieties, but my work is the first to show how

temperature affects SBA on resistant soybeans and to compare SBA performance on susceptible and resistant plants. I predicted that both temperature and plant type (resistant vs. susceptible) would affect SBA responses and population growth parameters, and that there would be a dynamic interaction between the biotic (plant) and abiotic (temperature) factors. Results confirmed the prediction in that changes in some SBA responses for one variable were influenced by the other variable, but in a non-uniform way. I made a specific prediction that the thermal constants that allow estimates of SBA seasonal occurrence and population growth not only would be different for SBA on resistant versus susceptible plants, but that differences in either the degree-day constant and/or the lower thermal threshold would result from non-uniform changes in developmental rate between plant types over the range of temperatures tested. Results showed that temperature-developmental rate relationships were not the same for SBA on resistant and susceptible plants, confirming my prediction. Specifically, they diverged at the two higher temperatures with development lagging on resistant plants (Chapter 3, Figure 3.1). This finding suggests that effects of *Rag1* resistance on SBA development were expressed to a greater degree at higher temperatures, or that slower development at low temperatures due to insufficient heat energy obscured effects related to plant resistance.

Practical Applications for Soybean Pest Management

My thesis made several contributions that may allow soybean producers to manage SBA more efficiently and effectively.

Predictive Models

The ability to distinguish indirect plant-mediated effects of temperature from direct effects on pests will be important for generating useful predictive models for SBA. Both types of effects must be quantified to produce a robust model. The information gained from my research

contributes to a broader understanding of how temperature affects host plant resistance for SBA as well as how temperature directly impacts this pest. However, because soybean plants and SBA respond differently to different temperatures, and because responses may vary across a narrow range of temperature, their combined impact on SBA can only be understood by measuring responses over a wider range of temperatures than was possible in my thesis investigation.

An additional consideration is temperature variation. Because experimental temperatures were not allowed to fluctuate as they would in the field, effects on host plant resistance may be quite different. An important question to resolve will be how quickly plant resistance changes occur in response to increasing or decreasing temperature, especially within ranges where strong responses occur. The literature shows that increases or decreases in plant response can occur fairly quickly, and also that longer exposure may influence the strength of the temperature effect. An adequate understanding of the dynamic changes in plant quality under fluctuating temperature could, and should, be incorporated into a sophisticated computer model using real-time temperature data. Farmers already use degree-day models in conjunction with weather reports and temperature data. With the current technology of uploading local data to computers (e.g., HOBOware [Onset Computer Corp., Bourne, MA] and Watchdog loggers [Spectrum Technologies, Inc., Aurora, IL]), the task has become simpler and more attainable. Ultimately, it will be important to determine whether the magnitude and duration of changes to levels of host plant resistance are sufficient to warrant modeling effects of temperature on plant resistance into predictive models. However, if temperature conditions that occur frequently in the field can be shown to significantly reduce host plant resistance, then it may not be worth it to producers to spend money on *Rag1* cultivars to protect soybeans from SBA. However, until or unless that is established, future experimental studies with resistant soybeans should also include susceptible

plants as a reference to measure the dynamic interaction between temperature and plant resistance on SBA.

Compatibility Between Host Plant Resistance and Biological Control

If producers place greater reliance on host plant resistance in the future to manage SBA, this may result in positive environmental as well as financial benefits. For example, it is not uncommon for farmers to spray more insecticides "just in case" because they believe it will save their crop. But this practice puts more chemicals into the environment which, among other negative outcomes, can harm natural enemies and pollinators that protect and pollinate crops. On the other hand, the use of plant resistance can have direct and indirect negative effects on beneficial insects, including biological control agents (Price et al., 1980). With respect to the SBA, some research has been conducted to test the compatibility of plant resistance and biological control. For example, *Aphidius colemani* (Hymenoptera: Braconidae), a parasitoid that was imported to control the Russian wheat aphid *Diuraphis noxia*, attacks SBA on both susceptible and *Rag1* resistant soybeans. On susceptible soybeans, SBA grow to a larger size, and *A. colemani* parasitizes the healthier aphids since their offspring have a higher chance of reaching adulthood. But when *Aphidius colemani* parasitizes aphids feeding on resistant plants, body weight of the progeny declines as well as survivorship (Ode and Crompton, 2013). This does not automatically mean the effect of *Rag1*-resistant soybeans on natural enemies outweighs its benefits. *A. colemani* is a generalist, so they are not completely dependent on SBA to survive. The same goes for another generalist, the lady beetle *Harmonia axyridis*. Lundgren et al. (2009) demonstrated that *Rag1*-resistant soybeans reduced adult longevity of *H. axyridis*, presumably due to exposure to plant toxins ingested by SBA who fed on resistant leaves. Assuming levels of soybean resistance fluctuate temporally under varying temperature conditions, it would be

interesting to know how this affects levels of biological control, especially during periods when thermal conditions when plant resistance is weaker.

Predictions Related to Changing Regional Temperatures (Global Climate Change)

As the climate continues to change, effects on plant and insect communities will likewise undergo changes, which may have a net positive or negative impact on crop plants such as soybeans. Because SBA is a cool-adapted pest (McCornack et al., 2004), regions that undergo increases in average temperature may experience fewer infestations and lower pest densities from SBA. However, because of the conflicting data regarding how lower temperatures affect *Rag1* resistance in soybeans, how global climate change affects the frequency of temperatures that may increase or decrease plant resistance remains to be seen.

Recommendations for Future Research

My research provides at least partial answers to questions I raised. However, additional research is needed in some areas for SBA and for insect-plant systems in general. There are also some advantages to the approach I took which I would recommend to others who are studying the interaction between temperature and host plant resistance. Discussion on each of these topics follows.

Conditioning Method, Comparative Approach, and Criteria

Conditioning soybeans to temperature for different time intervals before inoculation with SBA was a somewhat unique approach that allowed me to test for indirect (plant-mediated) effects of temperature on this particular pest. Specifically, I wanted to separate effects of temperature on the expression of host plant resistance separately from direct effects of temperature on SBA fitness. In previous studies aimed at looking for temperature effects on

plant resistance for SBA (Richardson, 2011; Chirumamilla et al., 2014) and several other pests (Schweissing and Wilde, 1979; Salim and Saxena, 1991), investigators used a comparative approach whereby differences in pest responses on resistant versus susceptible plants were compared over a range of temperatures. In cases where the difference in responses either decreased or increased (usually at upper or lower temperatures), the relative differences were given as evidence for diminishing or enhanced resistance, respectively. While the comparative approach is a valid way of addressing this question, the conditioning approach is more definitive. In addition, it may be more realistic to a field setting since temperatures fluctuate which means that plants are subjected to temperature that induce changes for different amounts of time (see 'Field evaluation' below).

Evaluating and comparing several SBA responses (preimaginal development, survival, progeny, and adult longevity) over a range of temperatures gave a more complete assessment of how temperature affected the SBA than if I had chosen only one trait as has often been done in the literature. One reason why I wanted to look at multiple responses, is because the effects of temperature on plant resistance are not always expressed for all demographic traits, and also because the direction and/or magnitude of the effect may be different depending on which trait one examines. Specifically, the effect of temperature may be positive, negative, or none. For example, in my conditioning experiment (Chapter 2), SBA survival was low under the higher temperature, but development was faster which would have an offsetting effect on overall population growth. By using a life table approach, I was also able to compare the combined effects of temperature on demographic responses of the SBA. Therefore, I strongly advocate that future investigators evaluate all of the individual responses that contribute to population growth, and then follow that up by calculating and comparing life table parameters.

Field vs. Laboratory Evaluations of Host Plant Resistance

As noted in the 'Practical Applications for Soybean Pest Management' section, there are limitations to using laboratory-based experiments to predict insect and plant responses in the field, including effects of temperature on plant resistance. This follows because temperature and other abiotic factors change dynamically in amount, duration, and direction of change under field conditions. My conditioning experiment assessed the effect of time plants were exposed to low or high temperature on the expression of resistance. My experimental design, although unintentional, also involved an increase and decrease in temperature. Previous studies have that shown that changes in temperature can cause plant resistance break down or become enhanced, and that effects can be reversed (Sosa, 1979; Harvey et al. 1994). Therefore, an important question for future studies is how long will any given level of temperature-induced resistance be present in field-grown plants, especially if a reversal in temperature has the reverse effect on the expression of resistance? In general, future laboratory experiments should consider not only fixed temperatures, but also changes in direction and duration of exposure to temperatures. If one could link specific plant chemistry to resistance traits (e.g., antibiosis), it also may be possible to sample plants in the field under different conditions to determine if/how changing environmental conditions affect the expression and permanence of resistance. Bansal et al. (2014) found toxic secondary metabolites to be a mediator of antibiotic resistance to SBA in a lab-based experiment, so it appears to be feasible to test these levels in the field in order to evaluate the effect of abiotic factors on *Rag1* resistant soybean.

Factors other than temperature (e.g., wind, drought, etc.), may can add to temperature effects. Drought stress has been found to reduce feeding by herbivorous insects (Grinnan et al., 2013), as well as development (Dardeau et al., 2015) and population growth (Mody et al., 2009)

of phloem-feeding insects. Host plant resistance to phloem-feeding insects can also be modified by drought and sometimes the pest will actually perform better on the resistant variety (Verdugo et al., 2015). CO_2 levels also influence host plant resistance; increased levels of CO_2 have been known to boost population levels of the large raspberry aphid *Amphorophora idaei* (Martin and Johnson, 2010; Hentley et al., 2014). With a growing human population relying on the use of fossil fuels, the daily average concentration of CO_2 continues to rise, along with its alteration of the climate. In 2013, CO_2 levels reached 400 ppm. To put this in perspective, CO_2 levels did not surpass 300 ppm in the 800,000 years prior to the early 1900's (Blunden, 2013). It is not farfetched to say multiple abiotic factors are capable of affecting plants and insects in the field, thus, modeling and predicting effects of environment on plant resistance requires experimental evaluation of abiotic factors other than just temperature. In essence, evaluation of the dynamic changes to resistance is on a case by case basis.

Sources of Plant Resistance and Interactions with SBA Biotypes

In regards to materials, I only used one resistant soybean cultivar, but there are other *Rag1* varieties a scientist could include within their experiment. There are also other SBA resistance genes available, including: *Rag1, rag1c, Rag2, Rag3, rag4,* and *Rag5*; all of these genes were found in the field and isolated through gene mapping (Hill, et al. 2012). We know *Rag1* is successful in keeping biotype 1 and 3 at bay. As for the rest of the genes, *Rag2*, *rag1c*, *Rag3*, *rag4*, and *Rag5* (proposed) are effective against biotype 1 and 2 (Bansal et al., 2013). What we have yet to find out is how the interaction between all of the resistance genes and biotypes listed will change when exposed to a variety of temperatures, but Chirumamilla et al. (2014) has already demonstrated that *Rag2* resistance becomes enhanced at a higher temperature, but there was no change for a cultivar containing both *rag1c* and *rag4*. As evidenced by both my

work and that of other scientists, the impact of these resistance genes on SBA has the potential to change outside of optimal temperatures, along with any SBA resistance genes discovered in the future.

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