INTERACTIONS BETWEEN ULTRAVIOLET LIGHT AND SOYBEAN APHID, *APHIS GLYCINES* MATSUMURA (HEMIPTERA: APHIDIDAE)

A Thesis
Submitted to the Graduate Faculty
of the
North Dakota State University
of Agriculture and Applied Science

By

Steven Curtis Burdick

In Partial Fulfillment of the Requirements
for the Degree of
MASTER OF SCIENCE

Major Program:
Environmental and Conservation Sciences

May 2013

Fargo, North Dakota

North Dakota State University
Graduate School

Title

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MASTER OF SCIENCE

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ABSTRACT

Global increases in ultraviolet (UV) radiation have led to greater interest in its current and potential effects on organisms, including insects and plants. Here we report the short-term effects of UV on soybean aphids (*Aphis glycines* Matsumura), a common phytophagous pest of soybeans. We examined how modified amounts of UV radiation affect soybean aphids by focusing on changes in 1) soybean aphid densities and 2) within plant distribution. In a laboratory experiment artificially adding UV decreased soybean densities compared to a low UV control. In a field experiment blocking UV had minimal effects on soybean aphid densities. Further observations suggest that soybean aphid location could mediate UV effects; the soybean leaf may shield aphids from some direct harmful effects of UV. Our results demonstrate the potential importance of UV to insect herbivores and how insect behavior may mitigate negative effects.

ACKNOWLEDGEMENTS

I would like to thank my advisors, Dr. Jason Harmon and Dr. Deirdre Prischmann-Voldseth for their unyielding support, guidance, and patience during my time at NDSU. I would also like to thank Don Carey, Gene Schmidt, Jaclyn Eichele, and Sadie Fliegel for their tireless efforts assisting in any experiments I conducted. I'm grateful for my committee members, Dr. Marion Harris and Dr. Qi Zhang, and the entire faculty and staff of the entomology department.

Finally, I would like to thank Natalie Verworn and Curt and Linda Burdick for their continued support ensuring my success and NDSU.

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Introduction

Ultraviolet (UV) radiation, like many other abiotic factors (e.g. temperature, air, and water), can play an important role in ecosystems by potentially harming or helping most of the earth's organisms (Paul and Gwynn-Jones 2003, Caldwell et al. 2007). The intensity of UV radiation reaching the organism and the organism's tolerance to UV are two important factors determining how the organism is affected by UV. In this literature review I will first provide an introduction to UV radiation and how it's studied. Then I will review the general ecological effects of increasing UV radiation. Lastly, I will introduce my study system (soybeans and soybean aphids) and use the literature to speculate about some of the potential effects of UV radiation in that system.

What is UV?

The sun produces radiation, which is categorized based on its wavelength, frequency, and energy (Madronich et al. 1998). The most well-known classifications of the sun's radiation are visible light, infrared radiation, and UV radiation (Figure 1, Lean 1997). Visible light can be seen by humans as light levels and colors, infrared light is felt as heat, and UV radiation is neither seen nor felt by humans, but can potentially be important to humans and other organisms. As mentioned, these categories differ in the energy levels they produce; the shorter the wavelength the more energy is radiated (Madronich et al. 1998). UV radiation (characterized by wavelengths from 100-400nm) has the shortest wavelengths and the most energy of these three regions (Caldwell et al. 1998a,b, Madronich et al. 1998).

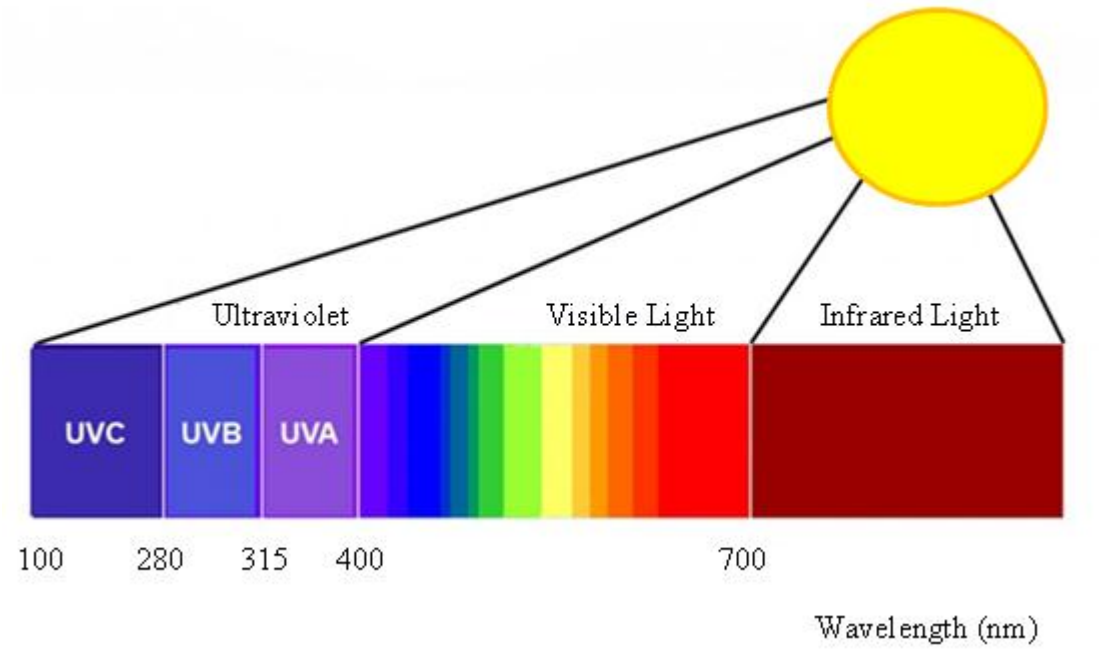

Figure 1. Relationship between the most abundant sections of the sun's radiation: ultraviolet radiation, visible light, and infrared radiation (adapted from Berg 2011).

The UV section can be separated into subtypes UV-A, UV-B, and UV-C, which have different characteristics and different effects on organisms (Caldwell et al. 2007, Paul and Gwynn-Jones 2003). Radiation from 100-280nm is classified as UV-C; UV-B is from 280-315nm; and UV-A is from 315-400nm (Madronich et al. 1998, Herman 2010). The ozone layer filters all UV-C, the most damaging UV subtype, before it reaches the earth's surface (Paul and Gwynn-Jones 2003). However, artificial UV-C is commonly used in medical and laboratory settings for sterilization and reducing surface bacteria (Andersen et al. 2006). Both UV-A and UV-B subtypes reach the earth's surface, although not in equal amounts (Paul and Gwynn-Jones 2003). Due to the longer wavelength of UV-A compared to UV-B, more UV-A is able to penetrate the ozone layer and reach the earth's surface (Paul and Gwynn Jones 2003). The intensity of these sections can change throughout time, which is important to consider when investigating the effects of UV on organisms.

Intensity of UV

Determining UV

There are two main factors that can determine if the organism is affected by UV radiation. First is the intensity of the UV radiation, which I will discuss now, and second is the organism's ability to tolerate/mitigate UV radiation, which I will discuss later in this review. The intensity of UV radiation is a measure of energy received per unit time. Humans have recently become more familiar with this idea of UV intensity through reporting on the UV-index in weather forecasts (Vanicek et al. 2000). The UV-index is a prediction of how intense UV radiation will be in a given location. People can use it to determine possible risks of UV exposure, which helps us avoid potential damaging effects of intense UV radiation. The UV-index is a helpful tool because the intensity of UV radiation that we experience is not consistent, varying from day to day, being influenced by the time of day, geographic location, ozone, cloud cover, and other small scale factors.

Time & location. The earth's tilt and rotations cause the intensity of radiation hitting a specific location to change over time (Parisi and Kimlin 1997, Herman 2010). Thus UV intensity is affected by both time of day or year, as well as geographic location. The earth rotates around its axis once every 24 hours (creating night and day) which makes the intensity of UV radiation in a given spot fluctuate over the course of a day (Figure 2). The highest intensities during a day occur when the sun is directly overhead (solar noon) (Parisi and Kimlin 1997, Herman 2010). During this time the sun's radiation has the shortest distance to travel before it reaches the earth. The mornings and evening have lower intensities because the sun penetrates the atmosphere at an angle having a greater distance for the rays to travel before they reach the earth's surface (Paul and Gwynn-Jones 2003).

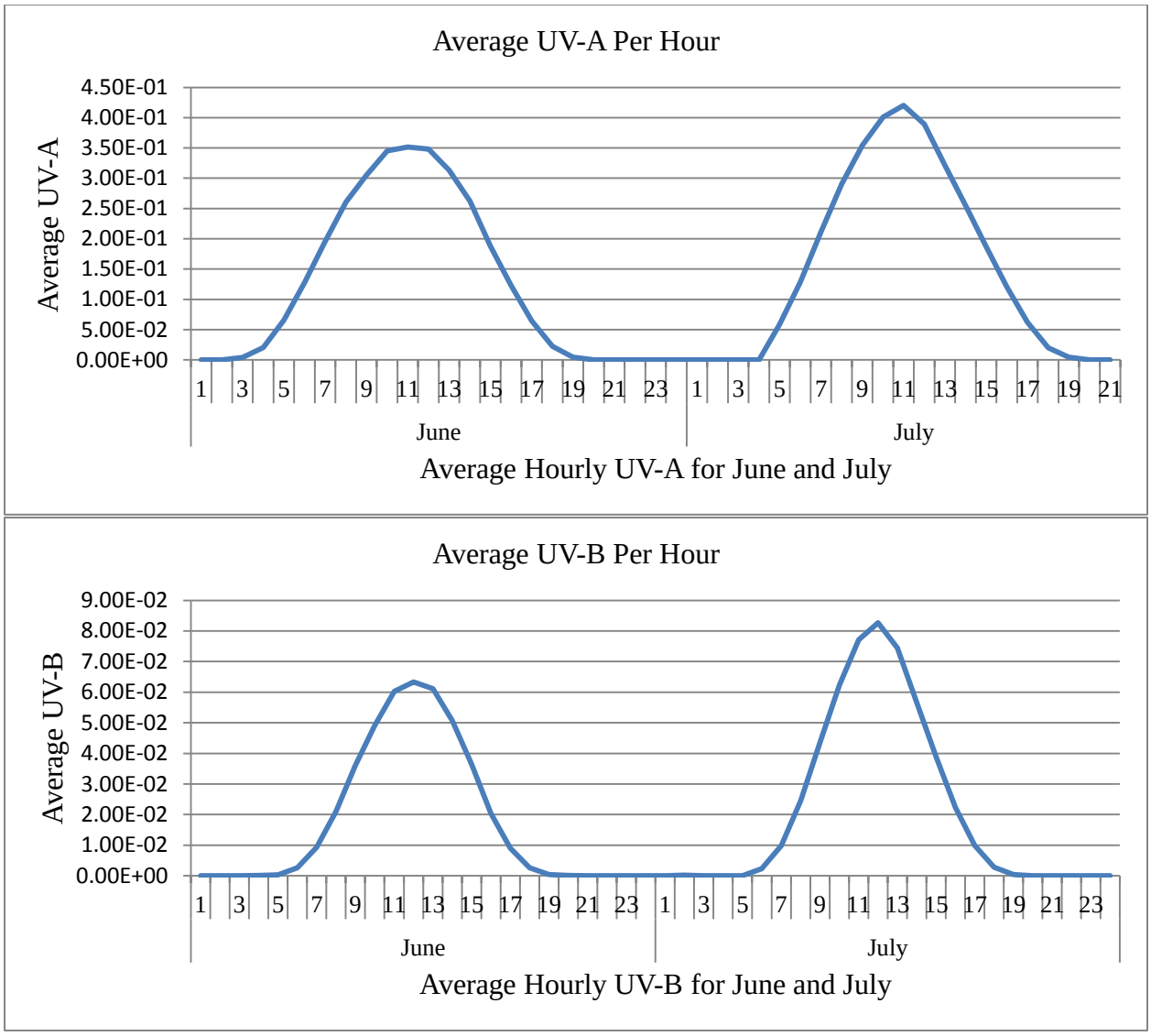

Figure 2. The average UV irradiance (watts per square meter (W/m^2)) measured in a given hour of the day averaged over all days in June or July of 2011. Data is from Fargo, ND, North Dakota Agricultural Weather Network (NDSU/NDAWN 2011).

The earth is also rotating around the sun at one rotation per year, creating seasons. As the earth rotates around the sun, locations receive a different amount of UV radiation throughout the course of a year (Parisi and Kimlin 1997, Figure 3).

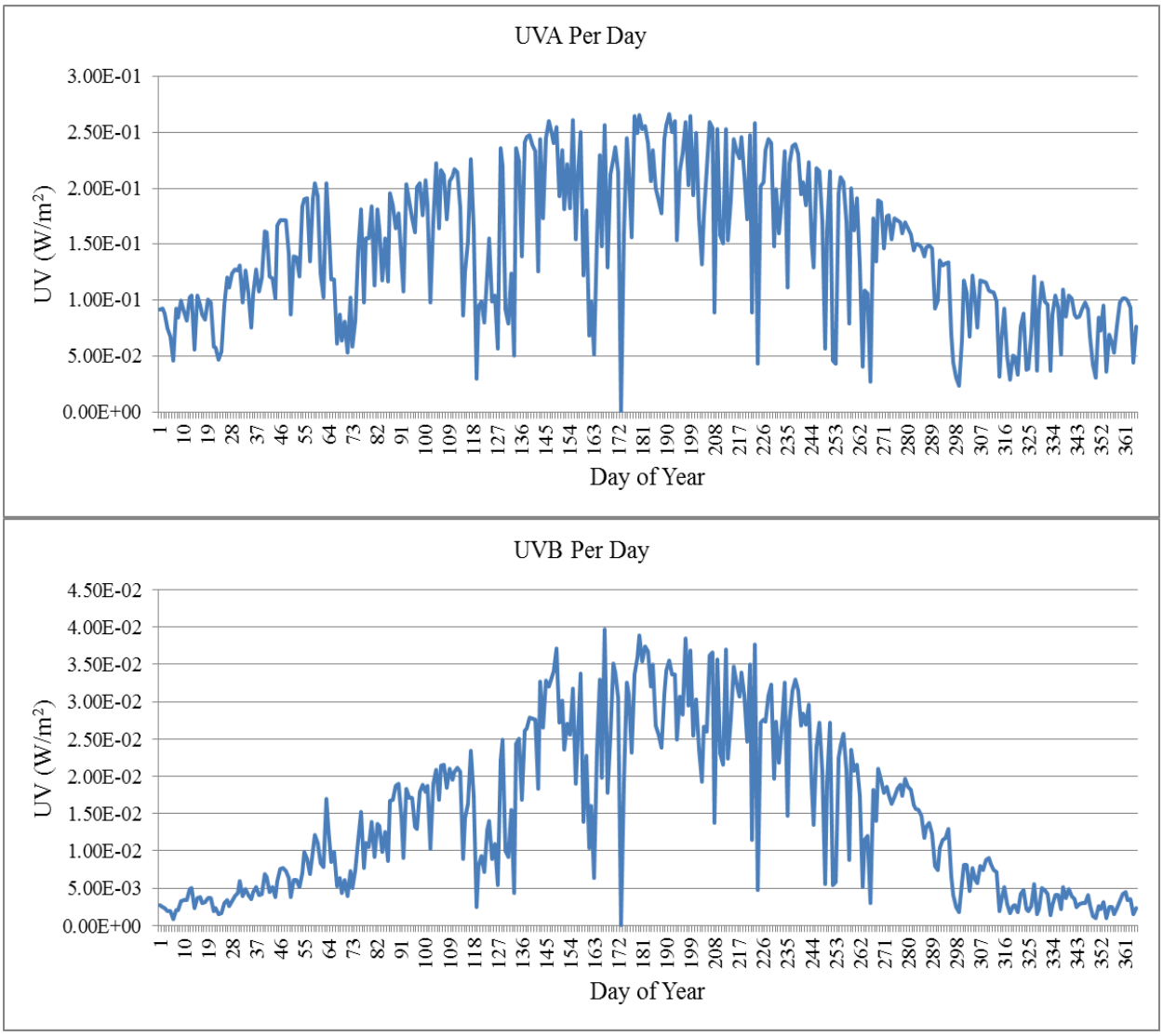

Figure 3. Average UV irradiance (watts per square meter (W/m^2)) per day for UV-A and UV-B recorded in Fargo, ND over the course of 2010.
Data is from Fargo, ND, North Dakota Agricultural Weather Network (NDSU/NDAWN 2010).

This variation in intensity over the course of a year changes with geographic location; the farther away from the equator the relatively greater intensity a location receives in their summer compared to their winter (Parisi and Kimlin 1997). Elevation also affects UV radiation intensities (Paul and Gwynn-Jones 2003, Herman 2010). The higher the altitude, the shorter distance UV has to travel, creating higher intensities at higher elevations.

Ozone. The intensity of UV radiation an area receives is also influenced by a number of natural filters. As the sun's rays shine down on the earth they encounter their first filter, the ozone layer. The ozone layer, much like a blanket over the earth, filters and absorbs much of the UV radiation before it reaches the earth's surface (Madronich et al. 1998, Gwynn-Jones 1999, Paul and Gwynn-Jones 2003, Herman 2010). All UV-C, some UV-B, and a little UV-A are filtered as the radiation passes through this protective layer (Paul and Gwynn-Jones 2003). The thinning and depletion of the ozone layer can cause an increase in UV reaching the earth's surface (Rousseax et al. 1998, Ballare et al. 2001), and is one of the primary reasons for long term changes in UV intensity.

Cloud cover. Clouds are primarily made up of water molecules, and since water is a good reflector of UV radiation, heavy cloud cover can scatter or reflect UV radiation back up to the atmosphere (Paul and Gwynn-Jones 2003, Herman 2010), making cloud cover a second natural filter influencing UV intensity. The amount of clouds in the atmosphere influences the amount of radiation the earth's surface receives (Paul and Gwynn-Jones 2003). Thin cloud cover can reduce infrared radiation reaching the earth (reducing the amount of heat felt), giving a false sense of reduced UV intensities. UV radiation can penetrate through these thin or scattered clouds, but as clouds become thicker, less UV intensities reach the earth. Cloud cover may not be consistent throughout a day, passing overhead and shading areas, thereby reducing UV for short periods of time.

Small-scale factors. There are multiple small-scale factors that also influence the amount of UV reaching terrestrial organisms. The type of surface that radiation reaches can reflect or absorb different amounts of UV (Paul and Gwynn-Jones 2003). For example, snow can reflect large amounts of UV radiation, thus creating high intensity microhabitats (Paul and Gwynn-

Jones 2003). In addition, plant canopies and shading can substantially reduce UV radiation in localized habitats (Gold and Caldwell 1983, Barnes et al. 1990).

Changing UV Intensity

Measurements of total UV intensities in the temperate latitudes have been increasing since people began recording UV data (Zerefos et al. 1995, Harris et al. 1997, McKenzie et al. 1999, Taalas et al. 2000a,b, Bassman 2004, Herman 2010). One of the primary reasons for this is the depletion of ozone, which results in less UV being filtered and a greater intensity of UV radiation reaching the ground (Caldwell and Flint 1994), particularly UV-B (Rousseax et al. 1998, Ballare et al. 2001). Most UV-A currently penetrates the ozone layer reaching the earth, thus UV-A sees minimal increases with ozone depletion (Madronich et al. 1998). UV-C does not have the ability to penetrate the atmosphere because it is heavily absorbed by both ozone and oxygen. Therefore even with a large amount of ozone loss (up to 90%), UV-C is expected to continue to be filtered (Ziska et al. 1992). UV-B intensities, which are only partially absorbed by the ozone, will therefore show the greatest increase as the ozone thins (Madronich et al. 1998, Gwynn-Jones 1999, Paul and Gwynn-Jones 2003), and researchers have documented increases in UV-B levels along with decreases in stratospheric ozone levels (Madronich et al. 1998). In future years, it is likely that many terrestrial organisms will experience an increase in UV-B radiation compared to current intensities (Taalas et al. 2000a). Therefore research has focused on effects of this subtype on terrestrial organisms (Rozema 1999, Paul and Gwynn-Jones 2003). However, not all research disentangles effects of UV-A from UV-B.

How UV is Studied

How an organism is affected by UV radiation will depend on the intensity of UV reaching the organism and the ability of the organism to tolerate or mitigate UV. To examine the effects of UV radiation, research is occasionally conducted by studying organisms that have undergone a change in their tolerance (tolerance reviewed in section *Innate and Induced Responses*), such as a behavioral change that makes them more susceptible to UV radiation (Blaustein et al. 1994). However, more often the effects of UV radiation are studied by manipulating the intensity of UV (Caldwell et al. 1998a, Bassman 2004), allowing researchers to examine effects of enhanced intensities that are similar to what organisms may experience in the future.

Controlled experiments with UV radiation are generally performed by manipulating UV intensities by either blocking UV radiation through filters or supplementing UV radiation via artificial lighting (Caldwell and Flint 1994, Rousseaux et al. 2001). Both methods allow researchers to provide multiple levels of UV to empirically discover their effects, and both methods have potential benefits and difficulties.

Filtering UV Radiation

Filtering UV radiation is when a material, usually a plastic film, is used to filter sunlight to block a specific range of wavelengths. Experiments that filter UV tend to be less expensive and require less equipment than experiments supplementing UV radiation (Caldwell and Flint 1994). Plastics are available as a clear plastic (allowing UV to penetrate) or a UV blocking plastic (filtering UV radiation). Plastic films can filter out either all UV radiation or only one specific UV subtype (UV-A or UV-B) (Ballare et al. 1996, Mazza et al. 2000, Rousseaux et al. 2001, Zavala et al. 2001, Costa et al. 2002, Chyzik et al. 2003, Antignus and Ben-Yakir 2004,

Diaz and Fereres 2007, Legarrea et al. 2012). The technique of separating UV subtypes can often provide convincing evidence that UV-B is influential (Caldwell and Flint 1994).

Using plastic filters gives researchers the ability to manipulate UV while keeping other light intensities consistent. Different filters vary in the amount and type of radiation that passes through, including both UV and photosynthetically active radiation (PAR). PAR (400-700nm) is the light plants are able to use for photosynthesis (Teramura 1980), so it is important to use filters that allow equivalent amounts of PAR to reach study organisms. Filtering out UV also allows visible light intensity to be more consistent with natural levels, and is often considered a benefit over supplementing UV radiation, which sometimes uses UV intensities outside natural ranges (Caldwell and Flint 1994). With two plastic filters that only vary in what UV radiation is filtered, researchers can minimize variation in humidity, temperature, and wind changes, thereby keeping microclimates similar across treatments (Caldwell and Flint 1994). Essentially, filtering naturally occurring UV allows researchers to inexpensively examine current levels of UV radiation with minimal disturbance to the microenvironment (Paul et al. 1997).

Using the approach of filtering UV radiation has some drawbacks and limitations that should be considered when determining the appropriate technique. While microclimates may be equal among treatments, filters can potentially alter the natural abiotic and biotic plant interactions beneath the plastic compared to situations that are open to all the elements (Caldwell and Flint 1994). These difficulties tend to become more problematic when plastics are kept over plants for long periods of time (Caldwell and Flint 1994). Another restriction for this technique is that it can only reduce current UV intensities, making it difficult to replicate experimental UV levels consistently through time and generate predictions related to enhanced levels of UV that

may occur in the future (Caldwell and Flint 1994, Caldwell et al. 1998a). Filtering UV is only one technique of UV manipulation; the other approach is the addition of UV radiation.

Adding UV Radiation

Adding UV radiation via high output lights provides the opportunity to control both the intensity and duration of the desired UV subtype (UV-A or UV-B). UV producing bulbs that provide UV radiation are typically used in lab or greenhouse experiments (Ziska et al. 1992, Gwynn-Jones et al. 1997, Caldwell et al. 1998a, McCloud and Berenbaum 1999, Warren et al. 2002). These UV producing bulbs emit either a UV-A or UV-B subtype to create different treatments.

Controlling the intensities and duration of UV exposure differs from using natural UV radiation because natural UV intensities are not consistent through time (i.e. daily and seasonal variation) and you can increase UV intensities beyond what occurs under field conditions. Inconsistent natural intensities through time make it difficult to replicate experiments using natural UV radiation. Being able to conduct UV experiments in a lab allows more control over environmental factors (i.e. temperature, humidity, lighting, etc.) among experiments. In addition, lab experiments allow one to conduct experiments year round.

However, there are some constraints associated with experimentally manipulating UV levels via UV bulbs. There has been some skepticism as to how accurately artificial UV addition experiments represent natural UV radiation wavelengths and intensities (Edwards 1992, Adamse et al. 1997, Ryan and Ireland 1997). Adding UV is generally more expensive than filtering UV light because it requires specific UV bulbs, lighting fixtures, and timers. Moreover, heat radiation and temperature should be monitored when adding UV to make sure they do not become a confounding factor (Caldwell and Flint 1994).

Effects of UV

UV has the potential to affect most organisms; however, it is almost impossible to say that UV has one specific effect on any organism. Certain groups of organisms are consistently shown to be affected by UV in a negative way, but even within these groups the severity of the effect varies greatly across individual organisms and specific studies (Bancroft et al. 2007). This may potentially be caused by some sort of dose response to UV, with the intensities varying between systems. In other cases UV actually benefits organisms (reviewed below). In reality, there is a spectrum of possible effects of UV on terrestrial organisms, ranging from beneficial, to negligible, to detrimental, which I will subsequently discuss.

When considering the severity of the effect, UV subsections (UVA and UVB) may have the potential to affect the tissues of organisms differently. UVB is a shorter wavelength and therefore is absorbed by the first layer of tissue, unable to penetrate deeper. UVA is a longer wavelength and has the potential to penetrate tissues and skin, affecting the organism differently than UVB. UVB may cause sunburns and quick damage; whereas UVA may cause long term damage including sun spots, cancers, and deeper tissue damage (Mead, 2008).

In the following sections I am specifically interested in the net phenomenological effect of both UVA and UVB, but not necessarily why or how that effect occurs (that will be covered in the section *How UV influences herbivore density*). Within each section I begin with some general examples across biological systems. Then I illustrate specific examples for herbivorous insects, primarily aphids. I give special attention to the latter group as it is the most closely associated with my specific research questions (Chapter 2), and recent studies have shown that aphids exhibit a variety of responses when exposed to UV radiation (Table 1, Antignus et al. 1996, 1998, Antignus 2000, Costa et al. 2002, Chyzik et al. 2003, Kulmann and Muller 2009).

Beneficial Effects of UV

General beneficial examples. Some organisms, including humans, utilize UV radiation in beneficial ways. Exposure to proper amounts of UV can provide health benefits to humans, including increases in body metabolism and the acquisition of adequate vitamin D, which has been linked to strengthening bones and the immune system (Mead 2008).

UV can also influence many behavior activities that are associated with communication. This can encompass a wide breadth of effects; for example, UV has been shown to influence agonistic behavior (behavior that is associated with actual aggression or displays of aggression), reproduction/mating behaviors, and social communication (Alberts, 1989; Fleishman et al. 1993, Gehrmann 1994).

Perception of UV. Some animals, including certain birds, insects, and reptiles benefit from UV radiation by being able to see reflective UV radiation on plants and other animals (Gwynn-Jones 1999). Insects are able to perceive UV through photoreceptors which have a peak of sensitivity in the UVA region, around 360nm; this sensitivity also extends into the UVB region, decreasing significantly below 300nm (Mazza et al. 2010). Sensitivity to UV is important for many daily activities including navigation, ability to find host plants, and forage effectively (Paul and Gwynn-Jones 2003). Using UV in vision is also important in prey location, mimicry, cryptic coloration, mate selection and trophic associations between plants and animals (Hunt et al. 2001, Kevan et al. 2001). The ability of insects to utilize UV radiation for vision, especially UV-A, is a trait that dates back to the Paleozoic (> 350mya), suggesting a strong evolutionary significance for having the ability to use UV for vision (Briscoe and Chittka 2001).

Table 1. Overview of various characteristics in response to UV radiation that affect aphid populations.

UV Induced Changes that Affect Aphid Populations		
Positive		
green peach aphid (*Myzus persicae*)	cages covered in UV transparent films had a 1.5-2.0 times greater propagation rate than cages covered with UV blocking films	Chyzik et al. 2003
potato aphid (*Macrosiphum euphorbiae*)	density reduced under UV blocking net compaired to UV transparent	Legarrea et al. 2012
cotton aphid (*Aphis gossypii*)	population was lower on tomatoes grown under UV blocking plastic	Nakagaki et al. 1982
Neutral		
green peach aphid (*Myzus persicae*)	blocking of all UV had no effect on the aphid development time	Chyzik et al. 2003
	population growth did not differ on broccoli plants under high UV-B	Kuhlmann and Muller 2010
potato aphid (*Macrosiphum euphorbiae*)	no effects of UV-B on fecundity	Guay et al. 2009
	no significant effect of daily UV-B exposure on movement, development, or fecundity	Nguyen et al. 2009
	reproduction was not directly affected by UV-B exposure	
Negative		
pea aphid (*Acyrthosiphon pisum*)	UV-B radiation adversely affected aphid fecundity (although much less than heat)	Guay et al. 2009
cabbage aphid (*Brevicoryne brassicae*)	population growth was significantly reduced on broccoli plants under high-UV-B	Kuhlmann and Muller 2010

Beneficial effects on herbivorous insects. Beneficial effects of UV on insect herbivores are primarily related to flight and the ability to successfully locate host plants, although some studies indicate insect population growth is higher on plants exposed to ambient levels of UV. In 2003, Chyzik et al. reported that both the flight activity and population density of *Myzus persicae* were suppressed on peppers under a plastic film that blocked UV radiation, suggesting that the aphids did better on plants receiving more UV. Specifically, they found that aphids on plants

covered by UV-transparent films (i.e. allowed UV natural, higher levels of UV radiation) had a significantly greater propagation rate than those covered by UV-absorbing films (lower UV treatment) (Chyzik et al. 2003). Chyzik et al. (2003) believed this was the first report of inhibitory effects of UV elimination on aphid propagation.

In 2002, Costa and colleagues experimented with suspending different greenhouse plastics over two genera of plants that are common hosts for aphids (*Chrysanthemum* sp. and *Solidago* sp.). They found that aphid numbers were lower under the UV blocking plastics and concluded that certain plastics could be useful in integrated pest management (IPM) programs (Costa et al. 2002). When trapping alate aphids, Chyzik et al. (2003) found fifty times more alates were trapped under UV-transparent film compared to UV-absorbing film. Counts of herbivorous insect pests such as whitefly, aphids, and thrips have been greater in UV-transparent tunnels (higher UV) compared to UV-absorbing plastic tunnels (lower UV) (Antignus et al. 1996, 1998, Antignus 2000), which may be due to altered visual cues or insect behavioral responses (Antignus 2000).

Negative Effects of UV

General negative examples. There are several negative consequences of short and long term exposure to UV on humans and other animals, but the most prominent is acute and chronic damage to skin and eyes (Gwynn-Jones 1999, Paul and Gwynn-Jones 2003). From a human prospective, both UV-A and UV-B can cause skin damage and cancer, however, they damage skin differently (Ichihashi et al. 2003, Mead 2008). UV-A penetrates deeper into the skin causing wrinkling, loss of elasticity, and premature aging (Mead 2008). UV-B is absorbed by the first layer of the skin (epidermis) and causes visible sunburns and redness (Mead 2008). UV can also cause navigation difficulties and behavioral changes in animals (Gwynn-Jones 1999,

Paul and Gwynn-Jones 2003) that can contribute to population declines and in extreme cases, extinction (Blaustein et al. 1994).

Negative effects on herbivorous insect populations. Exposure to UV radiation has been shown to negatively impact the density and reproduction of some insect species. Mazza et al. (1999b) found that when UV-B was filtered using UV blocking plastics thrips densities increase by 3-5 fold and concluded that there was an inverse dose response relationship between UV-B levels and thrips density. Kuhlmann and Muller (2010) found that *Brevicoryne brassicae* populations (Hemiptera: Aphididae) were significantly reduced on broccoli grown under high UV-B radiation compared to low UV-B radiation. Similarly, Guay et al. (2009) found that UV-B adversely affected pea aphid (*Acyrthosiphon pisum*) fecundity while on broad bean leaf discs (*Vicia faba*) in petri dishes. UV can also affect insect behavior (which I will discuss in the section *Insect behavior changes in response to UV radiation)*, which can indirectly impact herbivore fitness and reproduction (Mazza et al. 1999b).

Neutral Effects of UV

Some organisms are not affected by UV radiation, either because they can tolerate UV or they are mitigating the effects of UV radiation. One example is the aphid *M. persicae*, which showed no significant difference in fecundity and overall population density between UV present and UV exclusion treatments while on broccoli (Kuhlmann and Muller 2010). Kuhlman and Muller (2010) noted that aphids generally moved to the underside of the leaf, providing an intriguing hypothesis about the potential for a behavioral change, in this case movement, to mitigate effects of UV radiation.

How UV Influences Herbivore Densities

Given the broad range of effects UV can have on organisms, it is helpful to consider exactly how UV influences organisms and how those mechanisms could ultimately influence herbivore densities. This may provide insights on when to expect positive, negative, or neutral effects of UV in a given system. To disentangle some of these potential mechanisms, I will discuss differences between direct effects of UV on herbivorous insects and indirect effects of UV that occur because the UV alters the plant, which subsequently affects the herbivore (Figure 4).

Direct vs. Indirect Effects of UV on Herbivorous Insects

The majority of studies investigating effects of UV on herbivores assess herbivores while feeding on the host plant. This means that any observed effect on the insect could be due to direct effects of UV on the insect or because of indirect effects mediated by a change in the host plant (Figure 4, Caldwell et al. 1998a). Direct and indirect effects of UV could affect insects in very different ways. A direct effect is one where nothing else is necessary for the effect to occur. In this system, for example, UV exposure could directly increase the mortality of an insect alone in a petri dish. An indirect effect comes about because the UV exposure alters the host plant in some way, which ends up affecting the herbivorous insect that is feeding upon it. A hypothetical example would be if UV drastically decreased the nutrient content of the plant, making it a poor food for the insect feeding on it.

In this case, the UV isn't directly doing anything to the insect, but it is indirectly affecting it by changing the quality of its food. Similar UV induced changes in plant chemistry, morphology, and physiology can affect the plant and consequently the herbivore that feeds on them (Lindroth et al. 1993, McCloud and Berenbaum 1994, Ballare et al. 1996, Caldwell et al.

2007). Despite the fact that most studies do not separate direct and indirect effects of UV on insects, we can look for potential indirect effects by reviewing the direct effects UV have on plants and then using the literature to suggest how such changes could ultimately influence the herbivore. Considering such direct and indirect pathways can be important for better understanding how plant-insect interactions are altered under changing environmental conditions (Massad 2010). To better understand potential indirect effects of UV on herbivorous insects mediated by the host plant, I first review some of the direct effects of UV on to plants (Table 2) and then speculate on how such changes can influence the herbivore.

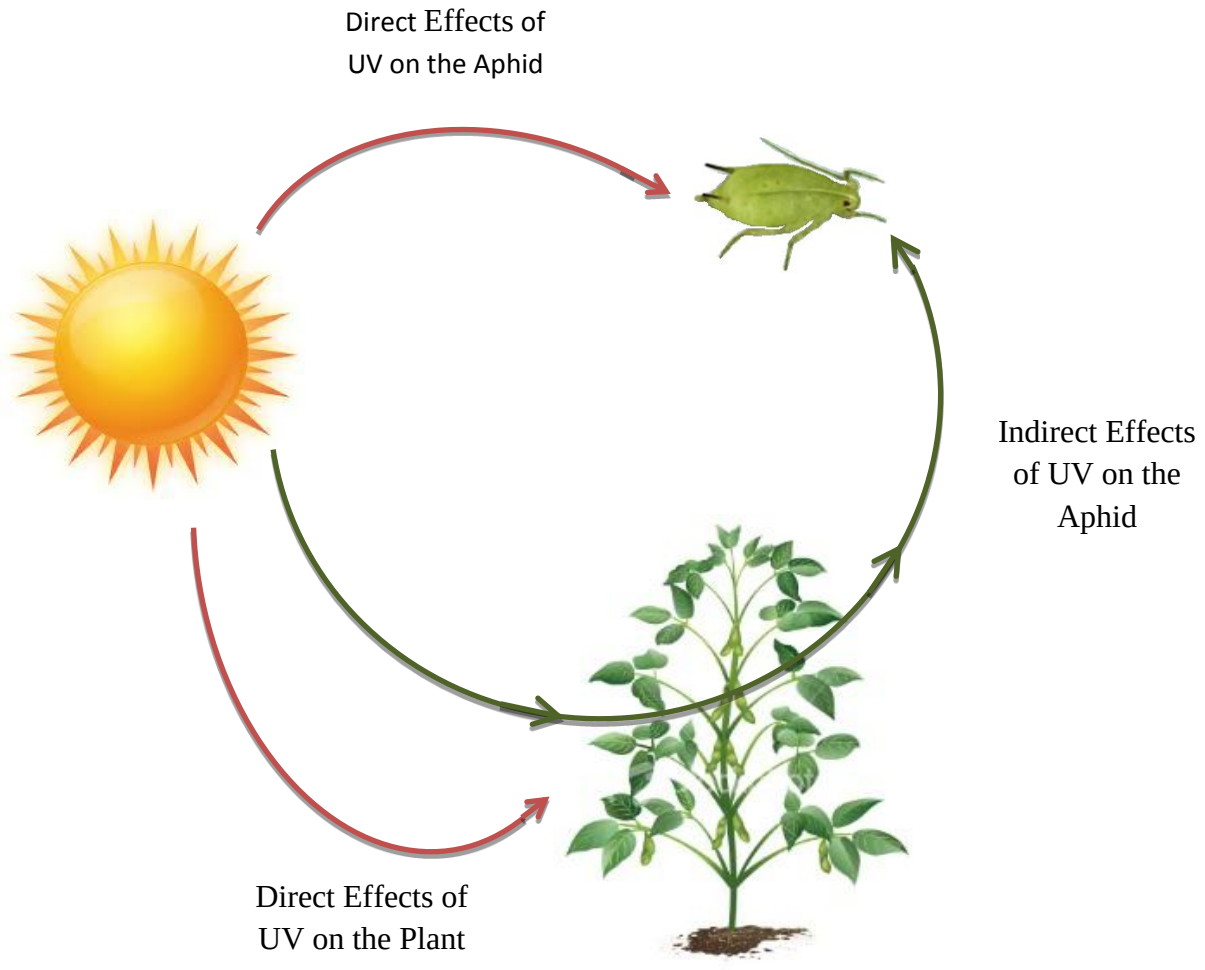

Figure 4. Diagram illustrating a direct effect of UV on an aphid vs. an indirect effect of UV on the aphid mediated by UV altering the aphid's host plant.

Physical plant effects. Increases in the intensity of UV-B radiation are often, but not always, detrimental to plant growth and development (Sisson and Caldwell 1976, Kossuth and Biggs 1981, Teramura and Murali 1986, Strid et al. 1994, Caldwell et al. 1998a,b, Tevini 2004, Tevini and Teramura 1989). Exposure to UV radiation can result in thicker leaves with more wax (Barnes et al. 1990, Garcia et al. 1997, Liakoura et al. 1999). The cuticle wax layer plays a major role in plant protection, as it is the first layer of protection between plants and their environment (Muller and Riederer 2005) and can protect them from abiotic factors such as UV radiation (Long et al. 2003, Pfundel et al. 2006).

Other plants have been reported to have slower leaf elongation, smaller leaf areas, and reductions in leaf expansion due to UV-B exposure; potentially either because it's a negative consequence of UV exposure or as a mitigation effort, as smaller leaf surface can reduce the amount of UV the plant is exposed to (Dickson and Caldwell 1978; Teramura and Caldwell 1981, Searles et al. 1995, Ballare et al. 1996, 2001, Krizek et al. 1997, 1998, Mazza et al. 1999a, Xiong and Day 2001, Day 2001). These changes can have unanticipated knock-off effects. For example, changes in plant height and leaf area can change the canopy cover and ultimately the competitive balance between species for visible light (Gold and Caldwell 1983, Caldwell et al. 1998a, Barnes et al. 1990). In agriculture crops, reduced growth and height can correlate with reduced yields (Yin et al. 2011) creating economic impacts.

Chemical plant effects. Similar to how UV affects plants physically, UV can also affect many chemical processes within the plant changing plant chemistry and nutrient levels that are involved in development and acclimation efforts (Jansen et al. 1998). For example, some plants can mitigate UV by accumulating phenolic compounds (e.g., flavonoids), in the epidermal layer, which acts as a "sunscreen" absorbing UV-B radiation while allowing quantum (PAR) light to

penetrate (Lake et al. 2009, Mazid et al. 2011). Increased UV-B radiation can increase flavonoid production, providing further evidence these compounds provide protection from UV-B radiation (Caldwell et al. 1983, Saviranta et al. 2010).

Table 2. Overview of both physical and chemical plant responses to UV radiation.

How UV Radiation Affects Plants	
Physical Plant Changes Induced From UV Radiation	
Changes in the Leaf	
reductions in leaf expansion	Dickson and Caldwell 1978, Teramura and Caldwell 1981, Teramura et al. 1983
reduced total wax coverage on leaves	Kuhlmann and Muller 2009
increased leaf thickness and cuticle thickness	Garcia et al. 1997, Liakoura et al. 1999
reductions in leaf area	Kadur et al. 2007
Total Plant Changes	
reduces total plant growth	Tevini and Teramura 1989, Strid et al. 1994, Tevini 2004, unpublished Kuhlmann
reduction in biomass allocation	Teramura 1980
inhibited stem elongation	Mazza et al. 1999a,b
reduction in dry weght and freash weight	Kadur et al. 2007
delayed seedling emergence	Kadur et al. 2007
Chemical Plant Changes Induced From UV Radiation	
Changes in Amount of Chemicals	
accumulation of methanol-soluble phenolics	Mazza et al. 1999a
acummulation of flavonoids	Harborne 1988
increase lignin content	Rozema et al. 1997
increases in total leaf nitrogen	Hatcher and Paul 1994, Lindroth et al. 2000
changes in carbohydrates and fibers	Lindroth et al. 2000
increased amino acids	Salt et al. 1998
increases in defense related phylpropanoid derivatives	McCloud and Berenbaum 1994, Grant-Petersson and Renwick 1996, Lavola et al. 1998
changes in proteinase inhibitors	Stratmann et al. 2000, Ryan 1990, McManus and Burdess 1995
Changes in Chemical Processes	
inhibits net photosynthesis	Brandle et al. 1977, Ziska et al. 1992, Teramura and Sullivan 1994, Ambasht and Agarwal 1998
reduces transpiration	Teramura 1980, Caldwell 1977
changes dark respiration	Teramura 1980, Sisson and Caldwell 1976
modifications in gene expression	Savenstrand et al. 2002, Brosche and Strid 2003
changes in secondary metabolism	Feucht et al. 1996, Picman et al. 1995, Glassgen et al. 1998, Norton 1999
suppression of chlorophyll synthesis	Kulandaivelu et al. 1991
inhibits electron transport	Noorudeen and Kulandaivelu 1982, Niyogi 1999
influence cyanogenic activity	Lindroth et al. 2000

In addition to UV absorbing sunscreens, high UV intensities can also cause reductions in nitrogen levels within the plant (Hatcher and Paul 1994, Lindroth et al. 2000). For legume species, nodules aid in nitrogen uptake and consequently affect the sensitivity of the plant to UV-B radiation. The effect of UV-B on nodulation activity has varied results, with some studies stimulating nodulation (Pinto et al. 2002) and others decreasing nodulation (Rajendiran and Ramanujam 2006).

Although we know that plants experience chemical changes in response to UV, sometimes the consequences of these changes are unknown. It's known that plants change chemically, but it's unknown if these changes confer any resistance. For example, UV radiation has been shown to affect numerous chemical processes, which include inhibition of electron transport (Noorudeen and Kulandaivelu 1982, Niyogi 1999), suppression of chlorophyll synthesis (Kulandaivelu et al. 1991), and impaired photosynthetic processes (Allen et al. 1998). In addition, UV can change levels of carbohydrates, fibers (Lindroth et al. 2000), and amino acids (Salt et al. 1998). It is unclear if these chemical changes affect UV resistance; however, it is known these changes may alter the attractiveness of the plant to the herbivore (Caldwell et al. 2007). Understanding these plant changes can provide predictions as to how insect herbivores are indirectly affected.

How plant changes affect insect herbivores. Changes within the plant in response to UV radiation, as discussed above, can indirectly affect the herbivorous insects that feed on them. UV induced plant changes (both physically and chemically) can alter the susceptibility and attractiveness of the plant to the herbivore (Lindroth et al. 1993, McCloud and Berenbaum 1994, Ballare et al. 1996). For example, increases in wax thickness can also facilitate increased resistance against herbivorous insects (Muller 2008). UV-induced changes in total leaf nitrogen

and increases in defense related compounds can directly influence the attractiveness of the plant to the herbivorous insects (Caputo et al. 2006).

Secondary defense related compounds are a main consideration when looking at plant-insect interactions. These secondary compounds help increase the plant's resistance to abiotic (UV) and biotic (herbivore) stressors without having any direct influence on normal plant growth, development, or reproduction (Rosenthal 1991, Wink 1999). However, these secondary compounds are thought to be costly to produce, which can indirectly reduce plant growth and reproduction providing evidence that their role in plants facilitates a higher resistance to biotic and abiotic factors (Simms 1992, Karban and Baldwin 1997, Harvell and Tollrian 1999, Siemens et al. 2002). UV radiation can influence these defense related compounds (McCloud and Berenbaum 1994, Grant-Petersson and Renwick 1996, Lavola 1998, Reifenrath and Muller 2007, Kuhlmann and Muller 2010), which can indirectly affect herbivorous insects (Fuhrer 2003, Bidart-Bouzat and Imeh-Nathaniel 2008).

Innate and Induced Responses

Given that UV can potentially have a variety of effects across different organisms and even when considering the same organism in different situations, an important question becomes what factors help determine what effect UV will have. I have already briefly mentioned that the intensity of UV can be variable, and this can play a big role in what happens to the organism. However, there are two additional factors related to the organism itself that can influence the effect of UV: 1) innate tolerance mechanisms the organism has and 2) induced factors that can help the organism mitigate the effect of UV. These two factors differ in that an organism either has or doesn't have the specific tolerance mechanism, whereas mitigation arises when an induced

response from UV exposure offers protection. Mitigation responses change the susceptibility of the organism to the effect, which in turn can change the effect size.

The degree to which organisms are affected by UV radiation can be modified because of physical, chemical, or behavioral adaptations that allow them to tolerate UV radiation (Ohtsuka and Osakabe 2009, Paul and Gwynn-Jones 2003). Tolerance can be either an innate tolerance (naturally shows no effect to UV) or an induced tolerance (induced response to provide tolerance). Terrestrial organisms have become well adapted over the years, protecting themselves from UV radiation by using exoskeletons, fur, or plumage (Ohtsuka and Osakabe 2009, Paul and Gwynn-Jones 2003). In other examples involving plants, we find that some plant species are more sensitive or tolerant to UV than others and this tolerance is based on innate differences between the plants such as physical or chemical barriers (previously reviewed).

Organisms can also have behavioral strategies that help tolerate and mediate the effects of UV exposure. Organisms can be located or can actively move to locations that are shielded from UV radiation (Barcelo 1981, Mazza et al. 1999b, 2002). For example, poison dart frogs avoid areas of high UV-B by moving and taking refuge in low UV-B areas (Han et al. 2007). This example illustrates how organisms can actively induce a change in their behavior in response to UV, which provides protection from the effects of UV radiation. Many other examples are found in the plant literature where the presence of certain amounts of UV radiation invokes a change in the plant, which makes it more tolerant of the UV radiation (reviewed above).

Insect Behavioral Changes in Response to UV Radiation

One of the primary ways that UV may ultimately affect insect population dynamics is by changing that insect's behavior. There is a fairly wide range of potential effects already demonstrated by UV (Table 3), including some that could be involved in mitigating potential negative effects of UV exposure.

Various insects detect and rely on UV radiation for numerous crucial behaviors including orientation, navigation (reviewed by Kuhlmann and Muller 2010), overall flight activity (Chyzik et al. 2003), foraging, and reproduction (reviewed by Costa 2002, Antignus et al. 2004, Hastad et al. 2005) (see Table 3). When insects redistribute themselves on their host plant they subsequently spend less time feeding and may be sacrificing a nutritionally optimal feeding site for an inferior one that has less UV exposure. Field experiments in a wide range of ecosystems report the intensity of insect herbivory often increased when UV-B was blocked using filters, potentially suggesting that these insects spent more time feeding and less time moving to mitigate UV radiation (Bothwell et al. 1994, Ballaré et al. 1996, Rousseaux et al. 1998, Mazza et al. 1999b, Zavala et al. 2001).

Changes in UV intensities can also potentially change the way insects perceive UV radiation, changing their behavior. Predicted increases in UV levels can alter these crucial behaviors creating potential unanticipated effects such as insects losing the ability to recognize host plants (reviewed by Costa 2002, Antignus et al. 2004). This could have huge consequences for pollinator species that play a critical role in plant pollination and reproduction. Although not always the case, bumblebee activities have been reported to be 94% greater under clear films compared to UV-B blocking films (Morandin et al. 2001) providing evidence UV-B aids in flight activity.

UV radiation may induce rapid changes in behavior, some of which may provide mitigation from UV radiation. Insects may avoid abiotic stressors by moving to protected microhabitats, such as below a leaf, which can lessen the negative effects of some abiotic stressors (Pincebourde et al. 2007, Barton and Schmitz 2009, Kearney et al. 2009).

Since the transmission of UV-B through the leaf's surface is minimal (due to UV-B absorbing compounds found in the epidermis as discussed earlier) insects on the lower leaf surface should also have reduced UV exposure.

Table 3. Overview of how UV can alter the movement of various aphid species.

How UV Radiation Influences Aphid Movement		
Alate Aphids		
green peach aphid (*Myzus persicae*)	UV absorbing film reduced flight activity and overall density of alate aphids	Chyzik et al. 2003
unidentified aphid species	Coverd greenhouses with UV blocking film that blocked UV <380 +IR had reduced numbers of alate aphids captured on yellow sticky traps compared to a standard <360 plastic	Costa et al. 2002
cotton aphid (*Aphis gossypii*)	Fewer aphids entered and fewer alate were recorded in UV blocked greenhouses vs. one having more UV	Kumar and Poehling 2006
All Aphids		
potato aphid (*Macrosophum euphorbiae*)	Aphids exposed to UV-B frequently settled on the least exposed surface of the leaf discs in petri dishes	Nguyen et al. 2009
green peach aphid (*Myzus persicae*)	Infestation rate in tunnels covered in UV transparent film was higher compared to tunnels covered in UV-absorbing films (spring followed same trend)	Chyzik et al. 2003
potato aphid (*Macrosiphum euphorbiae*) and lettuce aphid (*Acyrthosiphum lactucae*)	Reduction in aphid abundanceand delay in colinization in tunnels with UV blocking films	Diaz and Fereres 2006

Movement to areas that are protected may be a behavioral adaptation to UV-B radiation. Ohtsuka and Osakabe (2009) conducted an experiment to determine if the deleterious effects of UV-B can be avoided through herbivore location (i.e. the herbivore being in a location protected from UV radiation). They reported that the survivorship and egg production of spider mite,

T. urticae, was strongly decreased when exposed to UV-B radiation (Ohtsuka and Osakabe 2009). They concluded that it is an essential behavior for *T. urticae* to be located on habitats that are protected from UV-B radiation, such as the lower leaf surface, where survival rates increased. This type of avoidance behavior in response to UV-B has been reported in various species, some of which include: a number of insect species (Mazza et al. 1999b, Kuhlmann and Muller 2010), mites (Barcelo 1981, Ohtsuka and Masahiro 2009), and frogs (Han et al. 2007, Van de Mortel and Buttemer 1998). A study looking at the effects of UV-B on aphids casually mentioned that aphids, presumably from their experiment, usually investigate the adaxial leaf surface that is exposed to UV-B before settling on the abaxial side (Kuhlmann and Muller, 2010). This provides a tantalizing suggestion that aphids may receive reduced UV exposure by moving to the abaxial side of leaves which receives less UV-B radiation.

Overview of Soybean Aphid

Why Study Soybean Aphids?

Soybean aphid (*Aphis glycines* Matsumura) is an invasive species that was first discovered in North America in 2000, and since then has received a tremendous amount of attention in order to elucidate more of its biology and ecology. Since the soybean aphid reproduces parthenogenetically (has the ability to produce only females asexually) on soybeans, researchers can easily isolate single aphids and thus conduct experiments with genetic clones and single lines. The small size and limited needs of soybean aphids makes laboratory rearing simple usually only requiring florescent lights and soybean plants. A large amount of previous research has been done since the soybean aphid's arrival including general biology studies, ecology studies, and natural enemy studies (Ragsdale et al. 2011). These reproductive characteristics coupled with an immense amount of background information available for the soybean aphid has

turned the soybean aphid into a common study species. I am interested in whether insects such as the soybean aphid are affected by changing environmental conditions, particularly UV radiation. To address that question I will first provide background information describing the biology of the soybean aphid.

Arrival in North America

The soybean aphid is an invasive pest (native to eastern Asia), which arrived in the United States and spread rapidly across the Midwest (Ragsdale et al. 2011). They were officially documented in North America in Wisconsin in July of 2000, but probably arrived years earlier (Venette and Ragsdale 2004, McCornack et al. 2004, 2005, Ragsdale et al. 2004). By 2004, the soybean aphid had spread to 21 states and 3 provinces in Canada infesting 80% of U.S. soybean fields, making itself an important economic pest (Venette and Ragsdale 2004, McCornack et al. 2004, Mignault et al. 2006). The unique life cycle and reproductive characteristics of the soybean aphid adds to the difficulty of managing these insect pests.

Life Cycle

Soybean aphids have a complex life cycle, alternating host plants to complete a reproduction cycle. This life cycle strategy is known as a heteroecious holocyclic life cycle (Dixon 1998, Wu et al. 2004). This means they have a host alternating life cycle (with the primary host being European Buckthorn (*Rhamnus* spp.) and the secondary host being soybeans in the spring and summer. Sexual morphs produce eggs on wintering host. Soybean aphids also parthenogentically reproduce in the spring and summer, which gives them the ability to reproduce extremely quickly (Ragsdale et al. 2004, Wu et al. 2004). The soybean aphid overwinters on buckthorn, *Rhamnus* spp., as an egg (Ragsdale et al. 2004, McCornack 2005) and spends the summer months feeding on soybeans where they will produce apterous (wingless)

females with each generation ranging from 2-16 days in length (Wang et al. 1962, Ragsdale et al. 2004). Soybean aphids commonly are found foraging on the stem and the bottom of young trifoliates of soybean plants but as populations grow they are often found throughout the entire plant. The soybean aphid's life cycle coupled with its ability to reproduce quickly creates problems when trying to manage populations. In addition to their life cycle, abiotic factors can greatly influence soybean aphid abundance on host plants.

Host Plant

The soybean (*Glycine max* (L.) Merr.) is a legume species native to Eastern Asia that arrived in North America in the mid-1760s (Hymowitz and Harlan 1983). Soybeans today are the second highest crop in cash sales and the number one agricultural export being used for oils, domestic feed, and human consumption (Gibson and Benson 2005). Research on soybeans began in the late 1800s and has continued since, developing soybeans into a genetically modified biotech crop (Gibson and Benson 2005). Soybeans are the host plant for the soybean aphid, which is an invasive pest also from Asia creating significant yield loss (Fehr and Caviness 1977, Ragsdale et al. 2004). Before the arrival of soybean aphids, less than 2% of soybean fields were scouted (Ragsdale et al. 2011). Current scouting efforts have exceeded 75% of all fields (Ragsdale et al. 2011).

Soybean Aphid as a Pest

Soybean aphids are a pest of soybeans because of their large economic impact and ability to reproduce extremely rapidly (Ragsdale et al. 2011). Although many herbivores are occasionally associated with soybeans, (i.e. spider mites, Orthoptera, Coleoptera, Lepidoptera) the soybean aphid is the single most important arthropod pest of soybeans and can cause more than a 40-50% reduction in yield if left untreated (Halbert et al. 1986, Wang et al. 1994,

Ragsdale et al. 2004, 2007, 2011). With the 75 million acres of soybeans planted in 2011 (USDA 2011) yield losses of 40-50% may have huge economic implications. Estimates through models predict the future cost of controlling this pest in the United States could reach over one billion dollars per year (Kim et al. 2008). The ability of soybean aphids to parthenogenetically reproduce can result in rapid population growth and dispersal. On soybeans, the soybean aphid can produce up to 18 generations in a single summer and double their population in as fast as 1.5 days making timely treatments with insecticide difficult (McCornack et al. 2004). Soybean aphids can survive summer temperature up to 35°C (Rice et al. 2005) and winter temperatures, as eggs, down to -34 °C (Crompton 2007, McCornack et al. 2005, Ragsdale et al. 2004) making them an extremely tolerant pest and able to inhabit many climates. All of these factors contribute to the pest status the soybean aphid has earned.

Soybean aphids affect soybean through negative changes within the plant, which as discussed previously, substantially reduce yields. While it is clear that the aphid presents a substantial economic risk to soybean growers (Kim et al. 2008, Ragsdale et al. 2011), precise damage estimates can be difficult to predict as soybean aphids can affect the soybeans in various ways. Soybean aphids are problematic for their host plant because unlike chewing insects they feed by piercing and sucking mouthparts in which they inject saliva and remove assimilates (Goggin 2007). Feeding by soybean aphids can cause multiple problems for the soybean including photosynthetic pathway interference (Macedo et al. 2003), reduction in seed protein content (Wu et al. 2004), direct consumption of plant nutrients, reduced plant height, reduced pod numbers, reduced total yields (Dai and Fan 1991, Lin et al. 1993, McCornack et al. 2004, Ostlie 2005, Wang et al. 1996), the vectoring of viruses (Iwaki et al. 1979, Hill et al 2001, Clark and Perry 2002, Grau et al. 2002, Wu et al. 2004, Davis and Radcliffe 2008) and changes in

glucosinolate concentrations (Kuhlmann 2009). In summary, the soybean aphid is economically damaging, negatively affects plant development, reduces yields, and is difficult to manage in agricultural ecosystems. Therefore, it is clear that understanding the soybean aphid's role as a pest is very important in successful soybean production.

Potential Effects of UV on Soybean Aphids

While we have yet to find any studies examining the effects of UV radiation on soybean aphids, we can try to speculate on potential effects given previous research in related systems. As reviewed above, different herbivore species have been found to be negatively or positive affected by UV. The same is true when looking just at other aphid species. For example, the cabbage aphid, *Brevicoryne brassicae*, had significantly reduced densities on broccoli grown under high UV-B conditions (Kulmann and Muller 2010). However, the green peach aphid, *Myzus persicae*, showed no significant difference between UV treatments on broccoli (Kulmann and Muller 2010). In contrast, another study with the green peach aphid, *M. persicae*, indicated that aphids on pepper plants covered by UV-transparent films (i.e. allowed UV radiation to pass through) had a 1.5-2.0 times greater propagation rate than those covered in UV-absorbing films (Chyzik et al. 2003).

We can consider the possibility of indirect effects of UV by considering previous work on UV effects on soybeans. Soybeans, similar to almost all other plants, are affected and influenced by UV radiation. Experiments researching UV usually study the effects of enhanced UV-B radiation; they have reported physical changes in both leaf area (Koti et al. 2007) and wax layer thickness (Kakani et al. 2004), both of which could potentially alter herbivore densities. Additionally chemical changes also take place within the plant. An example of this is when the plant is exposed to enhanced UV-B radiation; UV absorbing compounds are often found

accumulating in the epidermis (Grammatikopoulos et al. 1999). These compounds, as discussed earlier, accumulate in the epidermal layer absorbing UV radiation acting as a "sunscreen" (Lake et al. 2009, Mazid et al. 2011). However, it is unclear if these specific chemical changes would impact soybean aphids.

Conclusions

Ultraviolet (UV) radiation can be important to many of the earth's organisms (Caldwell et al. 2007, Paul and Gwynn-Jones 2003); however the type of effect it can have is extremely variable. What effect UV has on a given organism is influenced by the intensity of UV radiation, which has been increasing in association with the depletion of ozone (Kerr 1988, NASA 1988). The effect is also influenced by a number of mechanistic factors that influence an organism's tolerance or response to UV.

Our goal was to use the literature to develop predictions of how UV radiation may affect the soybean aphid. Previous research indicates that the density of other aphid species can be influenced by exposure to UV radiation (Chyzik et al. 2003, Kuhlmann and Muller 2010), however there is no clear cut expectation of whether UV should enhance or diminish aphid populations. Studies have also shown affects to host plants, which can alter the plants susceptibility and attractiveness to herbivores (Lindroth et al. 1993; McCloud and Berenbaum 1994, Ballare et al. 1996), including effects to soybeans. However, there were again no clear cut predictions about how UV radiation would directly or indirect affect soybean aphids. The lack of clear predictions about soybean aphid interactions with UV radiation provides a unique opportunity to examine if, like other aphid species, soybean aphids are affected by changes in UV radiation levels, and if so, what mechanisms may be important in determining the types of effects UV may have.

DENSITY AND DISTRIBUTION OF SOYBEAN APHID, *APHIS GLYCINES* MATSUMURA (HEMIPTERA: APHIDIDAE) IN RESPONSE TO UV RADIATION

Introduction

Terrestrial organisms exist in a dynamic environment and must constantly deal with multiple abiotic factors, including temperature, atmospheric gases, and light. Among those ultraviolet radiation (UV) has increasing relevance in today's world due to depletion of the ozone layer (NASA 1988, Houghton and Woodwell 1989, Adamse et al. 1990, Kerr 1988). The ozone layer blocks more UV-B (280-315 nm) than UV-A (315-400 nm). Thus the thinning of the ozone layer will allow more of the former to reach the Earth's surface (Madronich et al. 1998, Tallas 2000). Consequently, UV-B has been the main focus for research when examining future effects of UV radiation on terrestrial organisms (Rozema 1999, Gwynn-Jones 1999, Paul and Gwynn-Jones 2003), although not all research disentangles the effects of UV-A from UV-B.

Although UV can have beneficial effects on plants and animals, there are numerous instances where exposure has negative consequences (Kiesecker and Blaustein 1995, Blaustein et al. 1994, Gwynn-Jones 1999, Chyzik et al. 2003, Paul and Gwynn-Jones 2003, Caldwell et al. 2007, Ohtsuka and Osakabe 2009, Kulmann and Müller 2010). Effects of UV may be direct or indirect. For example, herbivores may be indirectly affected by UV radiation via alterations in their host plants (Fuhrer 2003, Bidart-Bouzat and Imeh-Nathaniel 2008). Many organisms possess innate or induced physical and chemical adaptations that help mitigate negative impacts of UV (Paul and Gwynn-Jones 2003). Some insects and other closely related arthropods may minimize their exposure to UV radiation by being located in relatively protected locations such as the undersurface (abaxial side) of leaves (Ohtsuka and Osakabe 2009; Kuhlmann and Muller 2010).

Soybean aphids (*Aphis glycines* Matsumura) are economically important herbivorous pests of soybeans (Kim et al. 2008, Ragsdale et al. 2011). Apterous individuals are capable of intraplant movement (Whalen and Harmon 2012). Like other aphid species, they are commonly found on abaxial leaf surfaces (Ragsdale 2007, Rice et al. 2005). Phloem-feeding insects may prefer the abaxial surface for several reasons, including accessibility to phloem tissue (Freeman et al. 2001). The microclimate of the abaxial surface may also be more suitable for the insect (Wiktelius 1987, Ma 2000, Ma and Ma 2007a,b), possibly due to protection from abiotic stressors such as UV.

The goal of this research was to examine the effects of UV radiation on soybean aphids; specifically the growth of small aphid populations and their within-plant distribution. Our primary hypothesis was that exposure to UV light would be detrimental to soybean aphid populations. A secondary hypothesis was that the location of aphids on the plant reduces the severity of negative effects associated with exposure to UV light. To test the effect of UV radiation we used two complementary methods. First we used UV producing bulbs in the laboratory to supplement UV levels. Next, we selectively blocked UV using specialized plastic films in the field. Experiments were conducted on a brief time frame (7 d) to focus on short-term effects of UV on aphids rather than long term effects where aphids could be influenced by UV-induced host plant changes to a greater degree. Based on differences in aphid distribution across the two experiments, we performed a follow up experiment that specifically tested whether aphid location in relation to the side of the leaf altered soybean aphid density.

Materials and Methods

Insect Colonies

Soybean aphids used in experiments were obtained from lab reared colonies established in 2008 from individuals collected on soybean at the North Dakota State University Agricultural Experimental Station near Prosper, ND. Field-collected aphids from the same general location were periodically added to colonies to contribute to maintenance of genetic diversity. Aphids were reared on a susceptible soybean variety (RG607RR, NDSU Research Foundation, Fargo, ND) in 47.5 x 47.5 x 47.5cm cages (#44545F, MegaView Science Co., Taichung, Taiwan) and transferred to clean plants every 5-7 d. Watering was done as needed and no fertilizer was added to the soil. Aphid colonies were maintained under high output fluorescent lights (Sunblaze T5, Sunlight Sully Incorporated, Vancouver, Washington) on a 16L:8D photoperiod at 25 ± 5°C, 60-80% RH. When the lights were on, typical levels (measurements taken approximately 30.48cm below the light) of photosynthetically active radiation (PAR, 400-700 nm) outside cages were 750 µmol/m2s (#3415FXSE, Spectrum Technologies, Plainfield, IL) and UV levels (280-400nm) were 4.0 µmol/m2s (#3414F, Spectrum Technologies, Plainfield, Illinois). Soybean aphids used in all experiments were late-stage nymphs and adults (based on size).

Effects of Adding UV radiation on Aphid Densities and Within-Plant Distribution in the Lab

In this experiment we explored how adding UV-A and UV-B light, alone and in combination, affected aphid population growth and within-plant distribution from the point of aphid infestation through the duration of the experiment. The experiment was carried out in a laboratory physically partitioned into four distinct areas using white foam boards (6.35mm thick foam board, partitioned areas were 1.27m long x 0.76m wide). There were four UV radiation

treatments, with each one randomly assigned to a treatment area: 1) no supplemental UV, 2) +UV-A, 3) +UV-B, 4) +UV-A+UV-B. To avoid confounding treatment effects from effects related to the physical location in the room, treatments were reassigned between blocks. In all treatments four high output fluorescent bulbs (T5 6500°K, Sunlight Supply Inc., Vancouver, WA) within a light fixture (Sunblaze T5, Sunlight Sully Incorporated, Vancouver, WA) were used to provide quantum (PAR) light on a 16:8 light:dark cycle. UV radiation was added to the appropriate treatment areas using special bulbs (1.2m T12 40 watt bulbs, Q Lab Corporation, Westlake, OH: UV-A 340, UV-B 313 EL) that were on for 3 h centered at solar noon (i.e. noon - 3pm). UV-B 313 EL bulbs emitted radiation simulating natural UV-B radiation (280-315nm) with the majority of intensity occurring from 290-315nm. UV-A 340 bulbs (310-400nm) provided a replication of natural UV-A radiation (315-400nm). The number of UV bulbs was standardized for each individual and combination treatment (i.e. 4 UV-A bulbs in the +UV-A treatment, 4 UV-B bulbs in the +UV-B treatment, and 2 UV-A and 2 UV-B bulbs in the +UV-A+UV-B treatment). Bulbs providing quantum light were positioned directly overhead of plants, whereas UV bulbs we located on each side of the quantum light fixture providing UV light from each side slightly angling towards the center.

UV radiation and quantum (PAR) light were accessed once during the experiment using light meters (UV meter, #3414F, Spectrum Technologies, Plainfield, IL; PAR meter, #3415FXSE, Spectrum Technologies, Plainfield, IL). A total of 240 separate readings were recorded during the experiment (UV, n=120; PAR, n=120). UV and PAR readings were taken when UV emitting bulbs were on (i.e. noon-3pm) within each of the four treatments (no UV, UV-A, UV-B, UV-A+UV-B) at three different locations within each treatment (for each meter: outside cage n=10, inside cage above leaf n=10, inside cage below leaf n=10). Readings were

taken with meters oriented straight up towards the light source. PAR readings were also taken at the same time and locations within each of the four treatments at the same three different locations on the plant (outside cage n=10, inside cage above leaf n=10, inside cage below leaf n=10).

Temperature was quantified throughout the experiment using HOBO data loggers (UA-001-08, Onset, HOBO data pedant, Pocasset, MA). One HOBO was placed on a stake 5cm above soil surface in a tube cage within each treatment area and it recorded temperature on an hourly basis for the duration of the experiment.

Soybean plants were a susceptible soybean variety (RG607RR, NDSU Research Foundation, Fargo, ND) and was grown individually in plastic pots (10.2 × 10.2cm, Tessman Seed Co, St. Paul, MN) filled with a commercial horticultural mix (Sunshine Mix LC1, Sun Gro Horticulture, Vancouver, BC) under standard lab conditions as per plants used for rearing insects (i.e. no UV radiation). At the V4 growth stage (four fully expanded trifoliates), soybean plants were transferred to treatment areas (no UV, +UV-A, +UV-B, +UV-A+UV-B). There were five plants per treatment area replicated twice across two temporal blocks.

Immediately after plants were moved into each treatment area, eight soybean aphids were transferred to the adaxial (upper) surface of a unifoliate leaf (one leaf blade per leaf stem) using a small paintbrush. Each plant was then placed in a larger round plastic pot (21.0cm diameter, 15.2cm height and covered with a plastic tube cage (40.6cm high X 20cm diameter) with a nylon organdy mesh top (19cm diameter) and two side panels (5.0 width X 6.9 height) to provide air movement while preventing aphids from moving between plants. Soybean aphids were allowed to freely distribute themselves on individual host plants and aphid densities and location (i.e. upper/lower leaf surface, stem) were recorded on days 1, 2, 4, and 7 after infestation. Effect of

UV radiation treatments on aphid performance was measured by analyzing adult survival, reproduction, and per capita population growth.

Effects of Blocking UV radiation on Aphid Densities and Within-Plant Distribution in the Field

In this experiment we examined how natural UV radiation affected soybean aphid population growth and within plant distribution in the field by selectively blocking UV radiation using specialized plastic films. The experiment was carried out on individually caged plants (RG607RR, NDSU Research Foundation, Fargo, ND) within a larger soybean field near Fargo, ND (one acre field, row spacing- 30", plant spacing- 1.5", RG607RR). Plants within each treatment cage were thinned to one plant per cage right before treatments were applied. Plants outside of cages remained in normal field spacing (including plants under plastic but outside cage). There were three UV radiation treatments randomly assigned to field plants: 1) no film control, 2) plastic film that selectively blocks UV radiation (Dura-Film Super 4; AT films, Edmonton, Alberta; blocks UV wavelengths below 380 nm but transmits PAR 400-700 nm), 3) plastic control film that does not block UV (Tufflite IV film; Berry Plastics, Monroe, LA; transmits UV wavelengths between 290-400 nm and PAR 400-700 nm). There were 30 replications of each experimental treatment established in two temporal blocks (15 replications per block) varying in plant age and the location of aphid infestation. Two replicates that had zero aphids after one day were removed from the analysis.

The first experimental run was established when plants were at the V4 growth stage (four fully expanded trifoliates) on 07/09/2012. Cages and plastic tents were set-up on 07/10/2012. Mesh cages were erected (#1451DC, 24 x 20 mesh, 24" x 24" x 54", Rancho Dominguez, CA) to prevent contamination of experimental plants by resident aphid populations and natural enemies.

Cages were randomly assigned throughout the field and plants were thinned to one per cage. Plants in both runs were thoroughly checked for resident aphids and natural enemies, which were removed from plants before aphids were added to treatments. For the first experimental run, an insecticide, PyGanic® (EC 1.4II, MGK, Minneapolis, MN) was sprayed on plants after treatments were applied and aphids were infested four days after.

For the first experimental run, eight adult soybean aphids were transferred to the adaxial surface of a unifoliate leaf using a small paintbrush. Two UV radiation treatments (UV blocking film, UV transparent film) were then imposed by placing tents (3.6m long × 2.1m wide × 0.6cm) made by stretching the specialized plastic film over a metal conduit frame (0.6m wide × 1.2m tall) over the mesh cages. Plastic tents were stretched across mesh cages (top of cages were slightly angled to allow for water runoff) and secured to the ground using metal stakes (see Figure 5). Control plants (no plastic film) were set up in the same way but did not have any plastic over the mesh cages.

Figure 5. Picture of field cage set up in treatments with plastic (UV blocking or UV transparent).

The second experimental run was established when plants were at the R2 growth stage (open flowers at one of the two uppermost nodes of the main stem) on 07/19/2012, and eight adult soybean aphids were placed on the adaxial surface of the newest expanded trifoliate. For this run we randomly selected new plants within the field and set up cages and plastic tents as outlined previously. Plants were thoroughly checked for any aphids or natural enemies right after the treatments of plastic films were applied and aphids were infested the same day.

Aphids were transferred onto detached soybean leaf pieces in the lab, which were placed into small plastic cups (44.4ml Solo cup co., Urbana, IL) with moist cotton and transferred to the field in coolers. Leaf pieces were then draped on the uppermost trifoliate of experimental plants. In both runs, aphids were allowed to freely distribute themselves on the individual host plants and aphid densities and location (i.e. upper/lower leaf surface, stem) were recorded on days 1, 2, 4, and 7 after infestation. Effects of treatments on aphid performance were measured by analyzing aphid survival, reproduction, and per capita population growth.

UV radiation and PAR light were accessed daily during the experiment using light meters (meters discussed previously). A total of 164 separate readings were recorded during the experiment (UV, n=82; PAR n=82). UV and PAR readings were taken once daily centered around solar noon (i.e. noon-3pm) within each of the three treatments (no film, UV blocking film, UV transparent film) at three different locations within each treatment (for each meter: outside cage n=6, inside cage above leaf n=11, inside cage below leaf n=11). PAR readings were also taken at the same time and locations within each of the 3 treatments at the same three different locations on the plant (outside cage n=6, inside cage above leaf n=11, inside cage below leaf n=11).

Temperature was quantified throughout the experiment using HOBO data loggers (discussed previously). One HOBO was placed 15cm above the soil surface next to the stem of an individual plant in each treatment and recorded temperature on a hourly basis for the duration of the experiment.

Effects of Leaf Orientation on Aphid Densities in the Field

In the first two experiments aphid movement was not restricted, therefore their exposure and response to UV radiation may have been dependent on within plant location, specifically the lower versus upper leaf surface. However, aphid performance may also have been affected by differential leaf surface characteristics between abaxial and adaxial leaf surfaces (e.g. waxes, trichomes, thickness) independent of UV radiation. In this experiment we explored how manipulating soybean leaf orientation and the leaf surface onto which aphids were placed affected their survival and population growth under natural field conditions. We used a factorial design, with aphids confined to one of the following four treatment locations: 1) adaxial, facing up, 2) adaxial, facing down, 3) abaxial, facing down, 4) abaxial, facing up. There were 10 replications per treatment. The experiment was carried out in a soybean field (as previously described) on randomly selected soybean plants.

Five adult soybean aphids were transferred using a small paintbrush to either the adaxial or abaxial surface of the newest fully expanded trifoliate and a clip cage placed over them to confine them to that leaf surface. Clip cages were constructed from clear celluloid tube (6 cm diameter × 1.9 cm high × 1.6 mm thick) and 1.6mm closed cell foam was used to form a tight seal where the clip cage comes together. The top and bottom of the clip cage was covered with a nylon organdy mesh top that was held in place using hot glue. Clip cages were held together by a 10.2 cm stainless steel hair clip. Steel rods (5.24 m × 0.63 cm), with a 17.8 cm, 16 gauge

copper wire, was sunk into the ground adjacent to an experimental plant, and an alligator clip was attached to the hair clip to keep the clip cage in place (Figure 6). Clip cages were used to keep aphids confined to a leaf side and assist in manipulating the soybean leaf to desired experimental position. Soybean aphid densities were recorded daily for six days after infestation. Treatment effects on aphid performance were assessed by measuring adult survival, reproduction, and per capita population growth.

Figure 6. Picture of clip cage set up in leaf orientation experiment.

Unlike previous experiments we did not manipulate UV radiation levels during this experiment, however, when aphids were located below the leaf, the leaf itself blocked some light. UV and temperature were assessed throughout the experiment for each treatment. Light measurements were taken through the nylon organdy mesh 1 cm away from the above/below leaf surface, similar to where the aphids were located for each treatment. A total of 40 separate UV (n=20) and PAR (n=20) readings were recorded during the experiment (meters discussed in previous experiments). UV readings were taken once daily centered around solar noon (i.e. noon-3pm) at either above the leaf (n=10) or below the leaf (n=10). PAR readings were also taken at the same time and locations (above leaf, n=10; below leaf, n=10).

Temperature was quantified throughout the experiment using HOBO data loggers (described previously). One HOBO was placed inside a clip cage in a mock treatment setup (one HOBO above leaf, one HOBO below leaf) and recorded temperature hourly throughout the duration of the experiment. We also quantified the surface temperature of both the bottom (n=20) and top of the leaf (n=20) using a laser temperature gun thermometer (Milwaukee Electric Tool Corp., Brookfield, WI, 53005).

Statistical Analysis

All statistical tests were run using JMP 9.0 statistical software (SAS Institute Inc. 2010). Our primary response variable was aphid population growth, specifically the log of aphid density on the last day of the experiment divided by the log of aphid density after one day. This measure corrects for differences in how successfully we transferred adult aphids on different plants. In the 3rd experiment (leaf orientation) three replicates had aphids after the first day but no aphids at the end of the experiment. We viewed these as still biologically significant, as it could have been a treatment effect, and therefore used an X+0.5 transformation for the last days count. For both field and laboratory experiments a temporal block was added to take into account that the experiment was repeated twice, and one term was added to differentiate the treatments. Temporal blocks between both lab and field experiments were not significantly different from each other. Even though in the 2nd experiment (blocking UV) there were slight methodological differences in where aphids were added to the plant, there was no differences in abiotic and population measurements between rounds. Pre-planned contrasts across treatments were performed using Tukey's multiple comparisons test at the α=0.05 level. The clip cage experiment included two independent variables (abaxial vs. adaxial side and face up vs. face down) plus their interaction.

For each experiment we recorded a number of environmental variables to make sure that treatments had the intended differences in UV and that there were no other abiotic factors confounding our treatments. Differences in these factors were determined using ANOVA with contrasts between treatments as needed. Transformations were used where needed to meet the standard assumptions of ANOVA. Since temperature measurements were taken every 60 minutes from a single HOBO per treatment we can only make qualitative comparisons of temperature differences, not any statistical tests.

In the laboratory and field experiments we were also interested in the location of aphids throughout the plant. To investigate this we recorded aphids as being in one of three locations: above the leaf, below the leaf, and other (primarily the stem) and calculated the proportion of aphids on the plant that were in each category. We used MANOVA to determine if the distribution of aphids in these categories differed among the treatments. Since the proportion of aphids in all three categories would sum to one, we used just above and below the leaf to ensure that each variable was an independent measure within the model (Cisneros and Rosenheim 1998).

Results

UV Radiation Addition Lab Experiment

The primary goal of our treatments was to modify UV exposure while keeping other abiotic conditions as similar as possible. Our measurements outside and inside the cage show a drastic reduction in UV levels in the no UV treatment (Table 4) and relatively similar total UV measurements in the other three treatments. Temperature and visible light was qualitatively similar among all treatments, though there were some statistical differences in the amount of visible light outside the cage and below the leaf inside the cage (Table 4). Because we were also

interested in the location of aphids, we took measurements of UV and PAR above and below the leaf in the cage. We found that the no UV treatment still had significantly less UV than the other treatments, but that all four treatments had substantially reduced UV under the leaf, which was lower than the amount of UV radiation received above the leaf in the no UV control experiment (Table 4).

The aphid population growth over the course of the experiment depended on the treatment that was applied to the plant (Figure 7; $F_{3,35}=5.78$, $p=0.003$). Contrasts indicate that aphid growth in the no-UV control treatment was greater than all three treatments with UV radiation added and that the three UV treatments had similar levels of aphid growth (Figure 7). At the end of the experiment aphids were distributed throughout the plant, including on the top of leaves, the bottom of leaves, and the stem (Figure 8). However, there was no difference in this distribution across the four treatments ($F_{8,68}=1.14$, $p=0.35$).

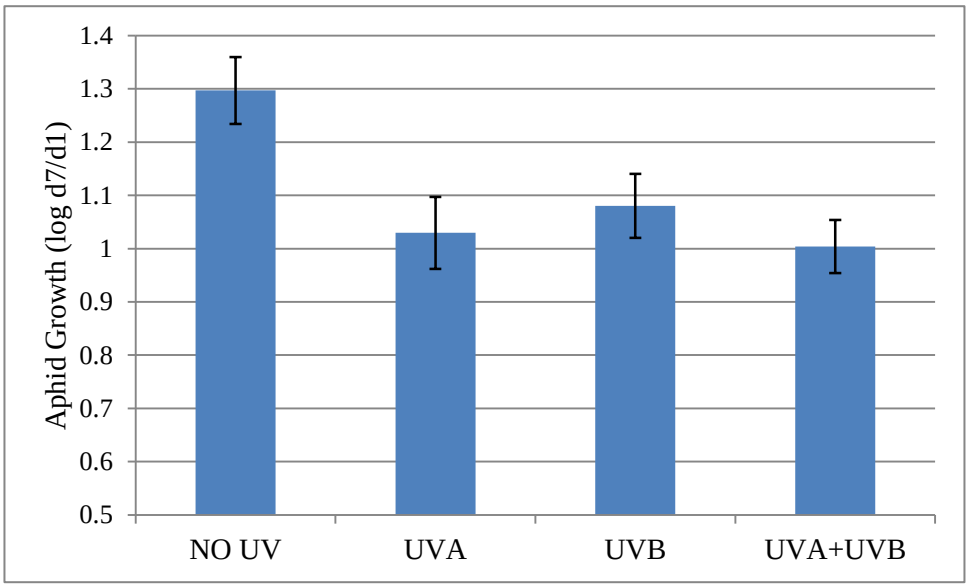

Figure 7. Log aphid growth (± 1 SE) per UV treatment in a laboratory experiment with addition of UV light.

Table 4. Average (± 1 SE) of individual light and temperature readings taken during solar noon (i.e. when UV bulbs were on) during the UV addition experiment.

	No UV	**UV-A**	**UV-B**	**UV-A+UV-B**	**p-value**
Temperature (°C)	27.2 ± 0.141	27.3 ± 0.147	27.2 ± 0.145	27.0 ± 0.149	n/a[1]
Outside Cage					
UV (µmol/m2s)	5.58 ± 0.29 a	29.2 ± 0.40 b	28.9 ± 0.58 b	29.1 ± 0.32 b	<0.0001
PAR (µmol/m2s)	733.6 ± 7.23 a	737 ± 5.97 a	756 ± 6.79 a	752.9 ± 2.97 a	0.033 [2]
Inside Cage – Above Leaf					
UV (µmol/m2s)	3.35 ± 0.10 a	24.79 ± 0.22 b	24.21 ± 0.20 b	24.66 ± 0.15 b	<0.0001
PAR (µmol/m2s)	394.2 ± 2.03 a	396.0 ± 2.48 a	403.8 ± 1.80 a	395.6 ± 6.30 a	0.48
Inside Cage – Below Leaf					
UV (µmol/m2s)	0.19 ± 0.03 a	1.54 ± 0.03 b	1.57 ± 0.06 b	1.50 ± 0.05 b	<0.0001
PAR (µmol/m2s)	45.7 ± 0.88 a	48.5 ± 1.98 a	42.0 ± 1.73 a	43.8 ± 1.36 a	0.053

[1]Temperature measurements were taken every 60 minutes from a single HOBO per treatment. Thus we can only make qualitative comparisons of temperature differences, not any statistical tests.

[2] Despite a statistically significant value for the ANOVA, comparisons among all treatments using Tukey's did not find a statistical difference between any of the two treatments (α=0.05).

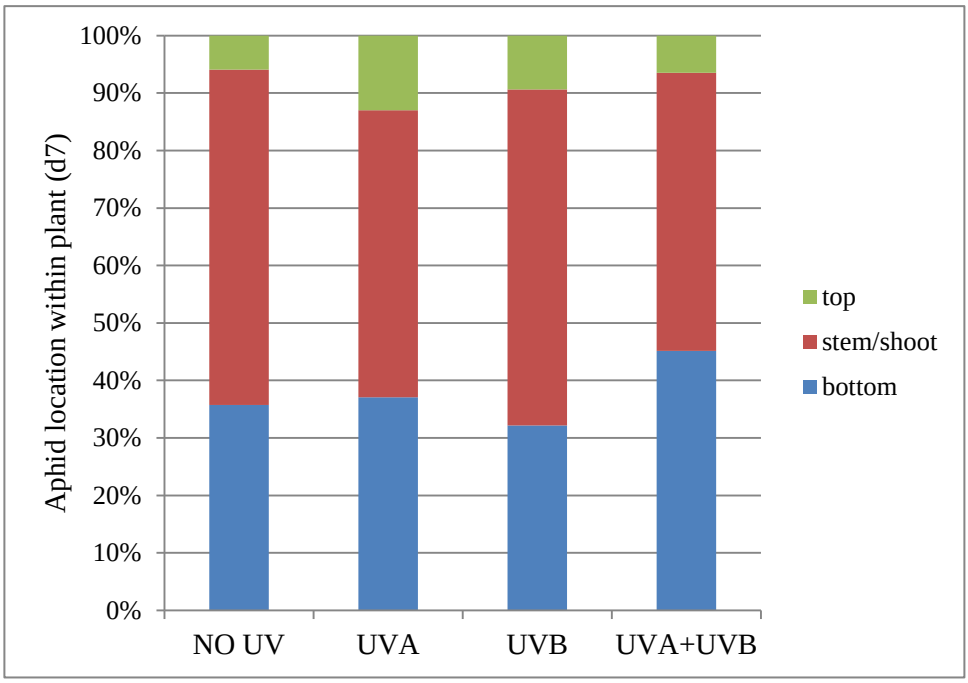

Figure 8. Proportion of the aphid population located on different parts of the plant on day 7 in a laboratory experiment with addition of UV light.

UV Radiation Blocking Field Experiment

As expected, UV radiation was substantially lower in the treatments with the UV blocking film compared to the UV transparent film (Table 5), and this was true whether under only the plastic or if under both the plastic and the cage material. Light conditions and temperature appeared comparable across treatments (Table 5). Since the two replicates of this experiment differed in where the original aphids placed on the plant, we did the same abiotic measurements toward the top and middle/bottom of the plant, but found no significant difference in UV or visible light ($p<0.05$). As with the laboratory experiment, UV radiation was substantially lower below the leaf compared to above the leaf. In all treatments the UV radiation below the leaf was lower than the UV radiation above the leaf in the UV blocking treatment.

Aphid population growth was similar across all three treatments (Figure 9; $F_{2,84}$=1.50, p=0.23). Across all three treatments there was a difference in the distribution of aphids (Figure 10; $F_{4,166}$=3.62, p=0.007). This difference was likely driven by the plants in the no film control having almost no aphids located on the stem (<2%) whereas in the blocking film (no UV) there was approximately 7% of aphids on the stem and in the transparent film plants over 10% of the aphids were on the stem. The same analysis performed with just the plants in the blocking and transparent films shows no difference in their aphids' distribution ($F_{2,56}$=1.28, p=0.29).

Table 5. Average (± 1 SE) of individual light and temperature readings taken during solar noon (i.e. noon-3pm) during the UV blocking experiment.

	No Film	**UV Blocking Film**	**UV Transparent Film**	p-value
Temperature	25.8 ± 0.357	26.4 ± 0.345	26.2 ± 0.363	n/a[1]
Outside Cage (but under plastic)				
UV (µmol/m2s)	149.0 ± 10.45 a	54.6 ± 3.48 b	126.7 ± 7.75 a	<0.0001
PAR (µmol/m2s)	1967 ± 16.33 a	1890 ± 36.44 a	1886 ± 43.04 a	0.26
Inside Cage – Above Leaf				
UV (µmol/m2s)	45.7 ± 7.65 a	17.4 ± 2.30 b	35.9 ± 3.94 a	<0.0006
PAR (µmol/m2s)	676.4 ± 136.8 a	547.5 ± 79.0 a	487.7 ± 70.41 a	0.65
Inside Cage – Below Leaf				
UV (µmol/m2s)	3.07 ± 0.34 a	1.29 ± 0.13 b	2.4 ± 0.16 a	<0.0001
PAR (µmol/m2s)	26.09 ± 2.89 a	31.90 ± 4.24 a	34.09 ± 3.68 a	0.27

[1]Temperature measurements were taken every 60 minutes from a single HOBO per treatment. Thus we can only make qualitative comparisons of temperature differences, not any statistical tests.

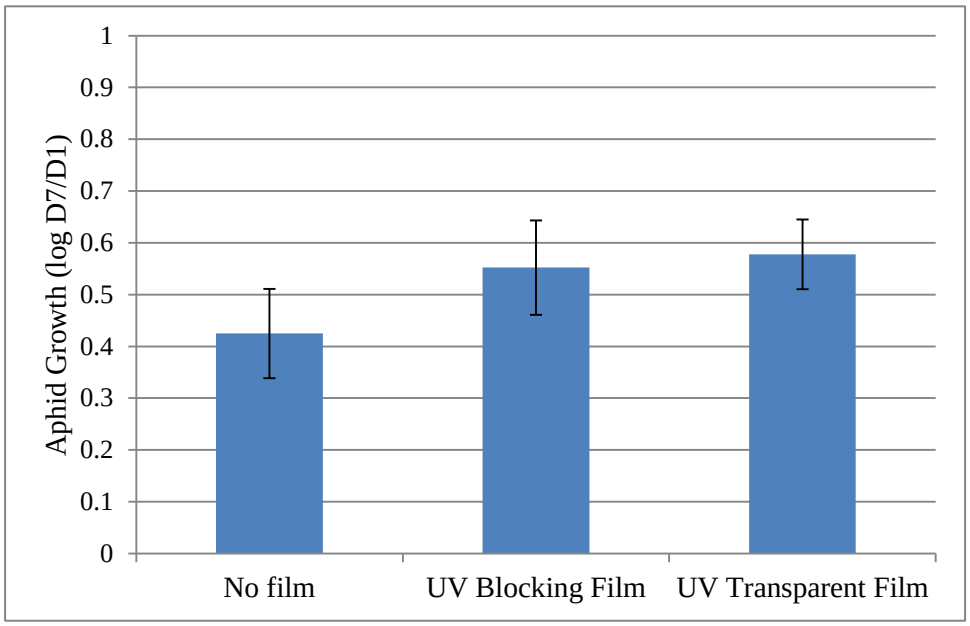

Figure 9. Log aphid growth (± 1 SE) per UV treatment in a field experiment with blocking of UV light.

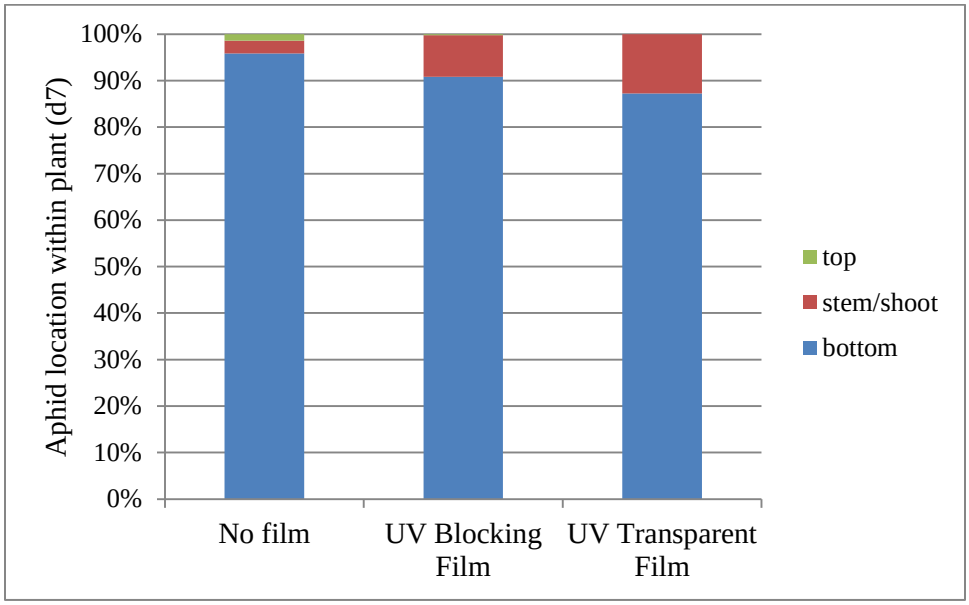

Figure 10. Proportion of the aphid population located on different parts of the plant on day 7 in a field experiment with blocking of UV light.

Leaf Orientation Field Experiment

Similar to the previous experiments, the amount of UV radiation aphids were exposed to when in clip cages above the leaf was substantially higher than when they were in clip cages underneath the leaf (Table 6). UV exposure was not the only difference in abiotic effects across treatments: levels of visible lights were significantly reduced under the leaf (Table 6).

Table 6. Average (± 1 SE) of individual light and temperature readings taken during solar noon (i.e. noon-3pm) during the leaf orientation experiment.

	Through mesh –Above Leaf	**Through mesh – Below Leaf**	p-value
Temperature (°C)	21.2 ± 0.81	19.5 ± 0.70	n/a[1]
Leaf Surface Temp. (°C)[2]	29.6 ± 0.40 a	29.2 ± 0.34 a	0.52
UV (µmol/m2s)	75.8 ± 5.99 a	1.25 ± 0.35 b	<0.0001
PAR (µmol/m2s)	1255 ± 52.64 a	88.0 ± 29.30 b	<0.0001

[1]Temperature measurements were taken every 60 minutes from a single HOBO per treatment. Thus we can only make qualitative comparisons of temperature differences, not any statistical tests.
[2]Leaf surface temperature measurements were taken on leaf without clip cage because the mesh on the clip cage obstructs the accuracy of the reading.

Growth of soybean aphids in clip cages were affected by both the side of the leaf aphids were confined to (abaxial vs. adaxial) and the orientation of the leaf (face up vs. face down), such that there was an interaction between leaf side and orientation (Figure 11; $F_{1,36}$=6.34, p=0.016). Across all data, aphids faced down (lower UV exposure) had a higher growth rate than aphids faced up (higher UV exposure) ($F_{1,36}$=8.61, p=0.0058), and aphids on the abaxial side had higher growth than aphids on the adaxial side ($F_{1,36}$=7.10, p=0.012). However, the interaction comes about because the aphids confined to the adaxial side had a much greater change in growth rate when faced up vs. faced down when compared to aphids on the abaxial side.

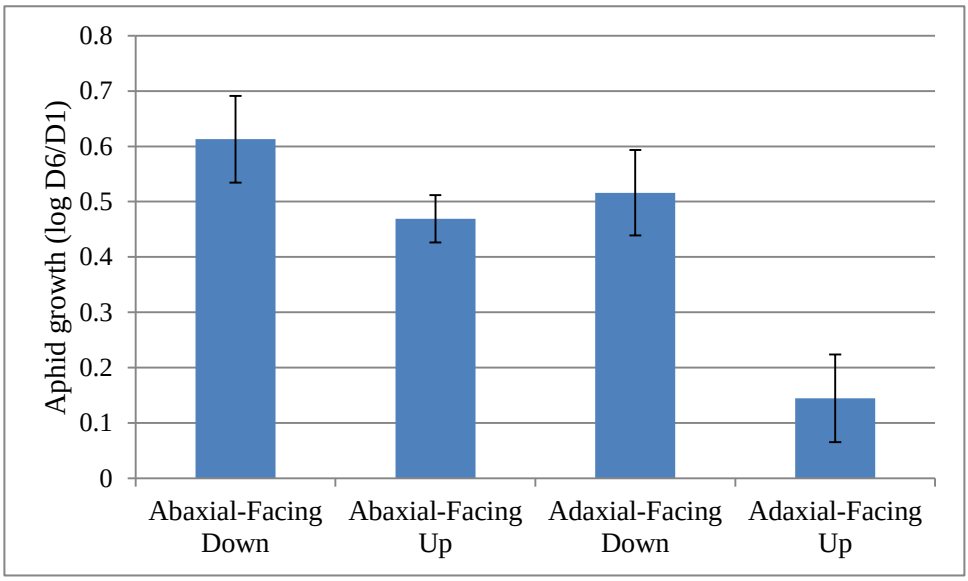

Figure 11. Log aphid growth (± 1 SE) per treatment in field experiment where aphids were confined to particular leaf side (abaxial or adaxial) and leaf orientation was manipulated.

Discussion

Our primary goal was to understand how UV radiation affects the soybean aphids as measured by the growth of small aphid populations. We focused on potential direct effects of UV as opposed to plant-mediated indirect effects by performing short term studies on plants that were unmanipulated before aphids were added and experimental treatments began. In the 1st experiment (UV addition in the lab) we found that the no UV control treatment had higher population growth compared to any of the three treatments that added artificial UV radiation, suggesting a deleterious effect of UV. However, in the field there was no difference in aphid growth rates between plants that were under a UV blocking film treatment (no UV control) and a UV transparent film treatment (ambient levels of UV radiation).

There are a tremendous number of logistical and biological reasons why we may have gotten different results in the 1st experiment (UV addition in the lab) and the 2nd experiment (UV blocking in the field). The most obvious difference between the experiments is the way we created our relatively low UV vs. relatively high UV treatments. Both artificial additions and

UV blocking plastics have been used to test for effects of UV radiation, but there are advantages and disadvantages to both methods (reviewed in Chapter 1), and it is not necessarily clear which methods offer the best insights. For example, in the laboratory experiment UV radiation is given in a constant amount for a set period of time, however, that method misses the day to day and hour to hour variation in UV radiation which naturally occurs (reviewed in Chapter 1). Additional logistical or methodological differences between the two experiments, e.g. physical and chemical differences in laboratory grown plants vs. field plants, exact amount and type of radiation, etc., could also play a role in the different results we received.

An additional biologically-based explanation for the different results appeared when we compared the aphid distribution in our laboratory and field experiments. Although aphid location was not different among the main treatments within each experiment, there was a dramatic difference in the distributions between the two experiments (Figure 8 vs. Figure 10); in the 1st experiment (UV addition in the lab), aphids were found throughout the plant, including on the adaxial leaf surface and the stem, whereas in the field, the aphids were found almost exclusively on the abaxial leaf surface.

There is a potential connection between this difference in aphid location and their risk of exposure to UV radiation. Our measurements showed that the amount of UV radiation was drastically lower on the undersurface of leaves compared to other areas. This means that the aphids on the abaxial surface could be less affected by UV radiation even if the plant was in a relatively high UV treatment. If this is the case, it could explain the difference in our experiments. In the laboratory, aphids were found throughout the plant including areas that should have received exposure to UV radiation. More exposure may have led to the lower growth rate for aphids in high UV treatments. In the field, aphids were almost exclusively found

in areas that receive little UV radiation. If aphids were receiving little exposure to UV in the high UV treatment, they may not have incurred the potential deleterious effects leading to similar growth rates across UV treatments.

We tested to see if this difference in being "under" a leaf or "on top" of a leaf could result in differences in soybean aphid population growth in the field. In the third experiment (leaf orientation) we found that when soybean aphids were confined to a particular leaf side we saw higher densities when the leaf was oriented downwards with the leaf between the aphids and the sun. We saw this effect regardless of whether the aphids were on the abaxial or adaxial surface of the leaf. This result is consistent with our idea that aphid location could mitigate some of the potential negative effects of UV radiation.

A similar idea about arthropod location and UV exposure was put forth by Onzo et al. (2010). They found that artificial addition of UV-B was extremely detrimental to three species of predatory mites, but that if the mites were inside the apex of the plant or on the underside of leaves, the effect of UV-B was lessened. Moreover, Kuhlman and Müller (2010) speculated that in their study there were few direct effects of UV on their aphids because the aphids were primarily on the underside of leaves.

In the third experiment, aphids confined to the adaxial leaf side had a much greater decrease in growth rate when faced up vs. faced down compared to aphids on the abaxial leaf surface. Leaf trichomes can influence the amount of UV-B radiation reaching the plant surface (Karabourniotis et al. 1992, Karabourniotis et al. 1995), and in some systems abaxial trichomes are more effective than those on the adaxial leaf surface (Karabourniotis and Bornman 1999).

There are a number of intriguing questions related to this study that could be pursued in the future. First, why did the aphids in the field change their distribution from the initial

placement on the top of the leaf to the underside of leaves, whereas in the lab aphids slowly spread throughout the plant without the same affinity for the abaxial side of the leaf? Perhaps there are cues related to natural sunlight, wind, or other abiotic factors that stimulate aphid movement in the field. Second, are there any indirect effects of UV on soybean aphids that are mitigated by changes to the plant? Many plants exhibit physical or chemical changes in response to UV (reviewed in Chapter 1). It is possible those changes also exist in soybeans and that those changes could subsequently affect soybean aphids. Further experiments would need to be done to be sure that any quickly acting plant-induced changes did not occur in this study as well. Despite trying to minimize such indirect effects by working on a small time scale, it is possible that some of the negative effects in the laboratory were due to changes in the plant. Moreover, some or all of the difference in the two experiments could have been influenced by the differences in how plants were raised in the laboratory versus the field or other artifacts of our methodology such as the exact intensity and duration of UV radiation in each experiment.

Our study demonstrates that UV radiation can potentially have negative effects on soybean aphids, but that this result was inconsistent across experiments, perhaps because of where aphids were located within the plant and the fact that some places on the plant seem to receive less UV exposure than others. These results, along with those of similar studies, suggest that aphids and other arthropods may be able to mitigate some of the potential negative effects of UV radiation. Thus, if we want to understand or predict the potential effects of changing abiotic variables, this studies furthers the argument that we need to account for not only the inherent tolerance of an organism, but also the potential behaviors that could alter how organisms are actually affected by that changing environment.

CONCLUSIONS

Soybean aphids are a potentially severe problem for soybean crops since their arrival in the United States, creating large yield losses if left untreated in years with high aphid densities (Ragsdale et al. 2011). Apterous aphids are relatively sessile with soft bodies, potentially limiting the adaptive means to deal with environmental stress which may make them more susceptible to environmental effects (Nguyen et al. 2009). On their secondary host, soybean, they are often found on the underside of leaves. There are some speculations as to why aphids are located below the leaf (Pettersson et al. 2007); these include potentially having easier access to phloem below the leaf, gravity and light direction preference, possibly to avoid predator detection, or its effort to avoid harmful environmental factors.

In a series of experiments, we examined how UV radiation impacted aphid population growth and aphid location. In the lab experiment (UV addition) the population growth of aphids was significantly greater in treatments absent of UV vs. treatments where UV was added. In the field experiment (UV blocking) the population growth did not significantly differ between treatments where UV was filtered and treatments where UV was allowed to penetrate. To further elucidate why we had a treatment difference in the lab experiment and not in the field experiment we examined aphid location.

Aphid location was not different between treatments within each experiment, however, aphid location was very different when comparing across experiments. This difference in aphid distribution between lab and field experiments may reveal a potential connection: when aphids are in a location, above the leaf, exposed to UV, population growth may be negatively affected, whereas when aphids are in a location shielded from UV, population growth may be less affected.

To test this hypothesis we conducted a third experiment where we confined aphids to a particular leaf side and orientated the leaf in positions that would either expose them to UV (aphid located between the sun and the leaf) or shield them from UV (leaf located between the sun and the aphid). We found that aphid population growth was significantly affected by the location of the aphid, independent of the leaf surface they were on (abaxial or adaxial).

Soybean aphid population growth appears to be affected by UV radiation, at least under certain conditions, but the size of the effect seems to depend on the location of the aphid. The mechanism by which the aphid population growth is affected is still uncertain. This study may be an example of the soybean aphid mitigating a potential negative environmental effect through behavior.

REFERENCES

Adams, R.M., C. Rosenzweig, J. Ritchie, R. Peart, J. Glyer, B. McCarl, B. Curry, and J. Jones. 1990. Global climate change and U.S. agriculture. Nature **345**:219–224.

Adamse, P., H. E. Reed, D.T. Krizek, S.J. Britz, R. M. and Mirecki. 1997. An inexpensive setup for assessing the impact of ambient solar ultraviolet radiation on seedlings. Journal of Natural Resource Life Science Education **26**:139-144.

Alberts, A.C. 1989. Ultraviolet visual sensitivity in desert iguanas: implications for pheromone detection. Animal Behavior **38**:129-137.

Allen Jr. L.H., and K.J. Boote. 2000. Crop ecosystem responses to climate changes: soybean. In: Reddy, K.R., Hoges, H.F. (Eds.). Climate Change and Global Crop Productivity. CABI Publishing, Oxon, UK. 133-160.

Allen, D.J., S. Nogués, and N.R. Baker. 1998. Ozone depletion and increased UV-B radiation: Is there a real threat to photosynthesis? Journal of Experimental Botany **49**:1775–88.

Ambasht, N. K., and M. Agrawal. 1998. Physiological and biochemical responses of Sorghum vulgare plants to supplemental ultraviolet-B radiation. Canadian Journal of Botany **76**:1290-1294.

Andersen, B. M., H. Bånrud, E. Bøe, O. Bjordal, and F. Drangsholt. 2006. Comparison of UV-C Light and Chemicals for Disinfection of Surfaces in Hospital Isolation Units. Infection Control and Hospital Epidemiology **27**:729-734.

Antignus, Y., M. Lapidot, N. Mor, R. Ben-Joseph, and S. Cohen. 1996. Ultra-violet absorbing plastic sheets protect crops from insect pests and virus diseases vectored by insects. Environmental Entomology **25**:919–924.

Antignus, Y., M. Lapidot, D. Hadar, Y. Messika, and S. Cohen. 1998. Ultraviolet-absorbing screens serve as optical barriers to protect crops from virus and insect pests. Journal of Economic Entomology **91**:1401–1405.

Antignus, Y. 2000. Manipulation of wavelength-dependent behavior of insects: an IPM tool to impede insects and restrict epidemics of insect-borne viruses. Virus Research **71**:213-220.

Antignus, Y. D., and Ben-Yakir. 2004. Ultraviolet-absorbing barriers, and efficient integrated pest management tool to protect greenhouses from insects and virus diseases. Insect Pest Management 319-335.

Ballare, C.L., A.L. Scopel, A.E. Stapleton, and M.J. Yanovsky. 1996. Solar ultraviolet-B radiation affects seedling emergence, DNA integrity, plant morphology, growth rate, and attractiveness to herbivore insects in Datura ferox. Plant Physiology **112**:161–70.

Ballare, C. L., M. C. Rousseaux, P. S. Searles, J. G. Zaller, C. V. Giordano, T. M. Robson, M. M. Caldwell, O. E. Sala and A. L. Scopel. 2001. Impacts of solar ultraviolet-B radiation on terrestrial ecosystems of Tierra del Fuego (southern Argentina)-an overview of recent progress. Journal of Photochemistry and Photobiology. B.**62**:67–77.

Bancroft, B. A., N.J. Baker, and A.R. Blaustein. 2007. Effects of UV-B radiation on marine and freshwater organisms: a synthesis through meta-analysis. Ecology Letters **10**:332-345.

Barcelo, J. A. 1981. Photoeffects of visible and ultraviolet radiation on the two-spotted spider mite, Tetranychus urticae. Photochem. Photobiol **33**:703-706.

Barnes, P. W., S.D. Flint, and M.M. Caldwell. 1990. Morphological Responses of Crop and Weed Species of Different Growth Forms to Ultraviolet-B Radiation. American Journal of Botany **77**:1354–1360.

Barton, B.T., and O.J. Schmitz. 2009. Experimental warming transforms multiple predator effects in a grassland food web. Ecology Letters **12**:1317–1325.

Bassman, J.H. 2004. Ecosystem Consequences of Enhanced Solar Ultraviolet Radiation: Secondary Plant Metabolites as Mediators of Multiple Prophic interactions in Terrestrial Plant Communities. Photochemistry and Photobiology **79**:382-398.

Berg, A.O. 2011. It's all in the fabric – coolibar suntect. The coolibar blog. http://blog.coolibar.com/coolibar-suntect/

Bidart-Bouzat, G.M, and A. Imeh-Nathaniel. 2008. Global change effects on plant chemical defenses against insect herbivores. Journal of Intergrated Plant Biology **50**:1339-1354.

Blaustein, A. R., Hoffman, P. D., Hokit, D. G., Kiesecker, J. M., Walls, S. C., and Hays, J. B. 1994. UV Repair and Resistance to Solar UV-B in Amphibian Eggs: A Link to Population Declines? Proceedings of the National Academy of Sciences **91**:1791–1795.

Bothwell, M.L., D.M.J. Sherbot, and C.M. Pollock. 1994. Ecosystem response to solar ultraviolet-B radiation: influence of trophic-level interactions. Science **265**:97–100.

Brandle, J. R., W.F. Campbell, W.B Sisson, and M.M. Caldwell. 1977. Net photosynthesis, electron transport capacity, and ultrastructure of Pisum sativum L. exposed to ultraviolet-B radiation. Plant Physiology **60**:165-169.

Briscoe, A.D. and L. Chittka. 2001. The evolution of colour vision in insects. Annual Review of Entomolgy **46**:471-510.

Brosche, M., and A. Strid. 2003. Molecular events following perception of ultraviolet-B radiation by plants. Physiologia Plantarum **117**:1-10.

Caldwell, M. M. 1977. The effects of solar UV-B radiation (280–315 nm) on higher plants: implications of stratospheric ozone reduction. In Research in photobiology pp. 597-607.

Caldwell, M.M., J.F. Bornman, C.L. Ballare, S.D. Flint, and G. Fulandaivelu. 2007. Terrestrial ecosystems, increased solar ultraviolet radiation, and interactions with other climate change factors. Photochemical and Photobiological Sciences **6**:252-266

Caldwell, M. M., L.O. Björn, J.F. Bornman, S.D. Flint, G. Kulandaivelu, A.H. Teramura, and M. Tevini. 1998a. Effects of increased solar ultraviolet radiation on terrestrial ecosystems. Journal of Photochemistry and Photobiology B: Biology **46**:40-52.

Caldwell, M.M., R. Robberechts, and S.D. Flint. 1983. Internal filters: prospects for UV-acclimation in higher plants. *Physiologia Plantarum* **58**: 445-450.

Caldwell, M. M., and S. D. Flint. 1994. Stratospheric ozone reduction, solar UV-B radiation and terrestrial ecosystems. Climate Change **28**:375–394.

Caldwell, M.M., P.S. Searles, S.D. Flint, and P.W. Barnes. 1998b. Terrestrial ecosystem responses to solar UV-B mediated by vegetation, microbes and abiotic photochemistry. In Physiological Plant Ecology, ed. MC Press, JD Scholes, MG Barker, 241–62.

Caputo, C.V., M. Rutitzky, C.L. Ballare. 2006. Solar UV-B radiation alters the attractiveness of Arabidopsis plants to diamondback moths (Plutella xylostella L.). Impacts on oviposition and involvement of the jasmonic acid pathway. Oecologia **149**:81–90.

Chyzik, R., S. Dobrinin, and Y. Antignus. 2003. Effect of a UV-deficient environment on the biology and flight activity of Myzus persicae and its hymenopterous parasite Aphidius matricariae. Phytoparasitica **31**:467-477

Cisneros, J. J., and J. A. Rosenheim. 1998. Changes in the foraging behavior, within-plant vertical distribution, and microhabitat selection of a generalist insect predator: an age analysis. Environmental Entomology **27**:949-957.

Clark, A.J., and K.L. Perry. 2002. Transmissibility of field isolates of soybean viruses by *Aphis glycines*. Plant Disease **86**:1219-1222.

Costa, H. S., K. L. Robb, and C.A. Wilen. 2002. Field Trials Measuring the Effects of Ultraviolet-Absorbing Greenhouse Plastic Films on Insect Populations. Journal of Economic Entomology **95**:113-120.

Crompton, D. 2007. Soybean aphid, Aphis glycines Matsumura (Homoptera: Aphididae): a new pest in the red river valley of north. Dissertation, North Dakota State University, Environmental and Biological Sciences. Fargo, ND.

Dai, Z. and J. Fan. 1991. Effects of aphid population dynamics and damage period on soybean yield. Journal of Shenyang Agricultural University **22**:135–139.

Davis, J. A., and E.B. Radcliffe. 2008. The importance of an invasive aphid species in vectoring a persistently transmitted potato virus: Aphis glycines is a vector of potato leafroll virus. Plant Disease, **92**:1515-1523.

Day, T.A. 2001. Multiple trophic levels in UV-B assessments: completing the ecosystem. New Phytologist **152**:183–86

Diaz, B.M., and A. Fereres. 2007. Ultraviolet-blocking materials as a physical barrier to control insect pests and plant pathogens in protected crops. Pest Technology **1**:85-95.

Dickson, J. G., and M.M. Caldwell. 1978. Leaf development of Rumex patientia L.(Polygonaceae) exposed to UV irradiation (280-320 nm). American Journal of Botany 857-863.

Dixon, A.F.G. 1998. Aphid ecology. Chapman & Hall London, England: 300pp.

Edwards, N.T. 1992. State-of-the-art exposure techniques do not qualitatively simulate expected global increases in UV-B irradiance. Bulletin of the Ecological Society of America **73**: 165.

Fehr, W.R., and C.E. Caviness. 1977. Stages of soybean development. Cooperative Extension Service, Agricultural and Home Economics Experiment Station, Iowa State University of Science and Technology, IA.

Feucht, W., D. Treutter, and E. Christ. 1996. Flavanols in grapevine: in vitro accumulation and defence reactions in shoots. Vitis-Geilweilerhof **35**:113-118.

Fleishman, L.J., E.R. Loew, and M. Leal. 1993. Ultraviolet vision in lizards. Nature **365**:39.

Freeman, P., J.S. Thomas, D.R. Buckner, C. Chang-Chi, and H.J. Thomas. 2001. Stylet penetration by Bemisia argentifolii (Homoptera: Aleyrodidae) into host leaf tissue. Annals of the Entomological Society of America **94**:761-768.

Fuhrer, J. 2003. Agroecosystem responses to combinations of elevated CO_2, ozone, and global climate changes. Agriculture, Ecosystems & Environment **97**:1-20.

Garcia, S., C. Garcia, H. Heinzen, and P. Moyna. 1997. Chemical basis of the resistance of barley seeds to pathogenic fungi. Phytochemistry **44**:415-418.

Gehrmann, W.H. 1994. Light requirements of captive amphibians and reptiles. Captive Management and Conservation of Reptiles and Amphibians. Society for the Study of Amphibians and Reptiles Contributions to Herpetology, Ithaca, NY **9**:53-59.

Gibson, L., and G. Benson. 2005. Origin, history, and uses of soybean (*Glycine max*). Iowa State University, Department of Agronomy.

Glassgen, W. E., A. Rose, J. Madlung, W. Koch, J. Gleitz, and H.U. Seitz. 1998. Regulation of enzymes involved in anthocyanin biosynthesis in carrot cell cultures in response to treatment with ultraviolet light and fungal elicitors. Planta, **204**:490-498.

Goggin, F.L. 2007. Plant–aphid interactions: molecular and ecological perspectives. Current Opinion in Plant Biology **10**:399–408.

Gold, W.G., and M.M. Caldwell. 1983. The effects of ultraviolet-B radiation on plant competition in terrestrial ecosystems. Physiologia Plantarum **31**:59-65.

Grammatikopoulos, G., Y. Petripoulou, and Y. Manetas. 1999. Site-dependent differences in transmittance and UV-B absorbing capacity of isolated leaf epidermis and mesophyll in *Urginea maritime* (L.) Journal of Experimental Botany **50**:517-521.

Grant-Petersson, J., J.A. Renwick. 1996. Effects of ultraviolet-B exposure of Arabidopsis thaliana on herbivory by two crucifer feeding insects (Lepidoptera). Environmental Entomology **25**:135-142.

Grau, C., B. Jensen, S. Myers, and J. Wedberg. 2002. Soybean Aphid. Fact Sheet, Team Grains Publication. University of Wisconsin, Madison, WI **1**:1.

Guay, J. F., S. Boudreault, D. Michaud, and C. Cloutier. 2009. Impact of environmental stress on aphid clonal resistance to parasitoids: Role of Hamiltonella defense bacterial symbiosis in association with a new facultative symbiont of the pea aphid. Journal of Insect Physiology **55**:919-926.

Gwynn-Jones, D., J. A. Lee and T. V. Callaghan. 1997. Effects of enhanced UV-B radiation and elevated carbon dioxide concentrations on a sub-arctic forest health ecosystem. Plant Ecology **128**:242–249.

Gwynn-Jones, Dylan. 1999. Enhanced UV-B radiation and herbivory. Ecological Bulletins 77-83.

Halbert, S.E., G. X. Zhang, and Z.Q. Pu. 1986. Comparison of sampling methods for alate aphids and observations on epidemiology of soybean mosaic virus in Nanjing, China. Annual Applied Biology **109**:479-483.

Han, B.A., L.B. Kats, R.C. Pommerening, R.P. Ferrer, M. Murry-Ewers, and A.R. Blaustein. 2007. Behavioral avoidance of ultraviolet-B radiation by two species of neotropical poison-dart frogs. Biotropica **39**:433–435.

Harris, N.R.P., G. Ancellet, L. Hofmann, D. J. Bishop, J. B. Kerr, R. D. McPeters, M. Prendez, W. J. Randel, J. Stahelin, B. H. Subbaraya, A. Volz-Thomas, J. Zawodny, and C. S. Zerefos. 1997. Trends in stratospheric and tropospheric ozone. Journal of Geophysical Research **102**:1570–1590.

Harvell, C.D., and R. Tollrian. 1999. Why inducible defenses? In the ecology and evolution of inducible defenses, eds. Princeton University Press 3-9.

Hastad, O., E. Ernstdotter, and A. Odeen. 2005. Ultraviolet vision and foraging in dip and plunge diving birds. Biology letters. **1**:306-309.

Hatcher, P.E., and N.D. Paul. 1994. The effect of elevated UV-B radiaiton on herbivory of pea by *Autographa gamma*. Entomologia Experimentalis et Applicata **71**:242-249.

Herman, J.R. 2010. Global increase in UV irradiance during the past 30 years (1979-2008) estimated from satellite data. Journal of Geophysical Research **115**.

Hill, J. H., R. Alleman, D. B. Hogg, and C. R. Grau. 2001. First report of transmission of Soybean mosaic virus and Alfalfa mosaic virus in the New World. Plant Disease **85**:561.

Houghton, R.A., and G.M. Woodwell. 1989. Global climatic change. Scientific American **260**:18-26.

Hunt, S., I.C. Cuthill, A.T. Bennett, S.C. Church, and J.C. Partridge. 2001. Is the ultraviolet waveband a special communication channel in avian mate choice? Journal of Experimental Biology **204**:2499-2507.

Hymowitz, T. and J. R. Harlan. 1983. Introduction of Soybean to North America by Samuel Bowen in 1765. Economic Botany. **37**:371-379

Ichihashi, M., M. Ueda, A. Budiyanto, T. Bito, M. Oka, M. Fukunaga, and T. Horikawa. 2003. UV-induced skin damage. Toxicology **189**: 21-39.

Iwaki, M. 1979. Virus and mycoplasma diseases of leguminous crops in Indonesia. Review of Plant Protection Research **12**:88–97.

Jansen, A.K., V. Gaba, and M. Greenberg. 1998. Higher plants and UV-B radiation: balancing damage, repair and acclimation. Trends in plant science **3**.

Kadur, G., B. Swapan, K. Sunita, Y. Sanjeev, T. Arjun, B. Sanjay,... & P. Mohanty. 2007. Growth enhancement of soybean (Glycine max) upon exclusion of UV-B and UV-B/A components of solar radiation: characterization of photosynthetic parameters in leaves. Photosynthesis Research **94**:299-306.

Kakani, V.G., K.R. Reddy, D. Zhao, and W. Gao. 2004. Senescence and hyperspectral reflectance of cotton leaves exposed to ultraviolet-B radiation. Journal of Plant Physiology **121**:250-257.

Karban, R., and I.T. Baldwin. 1997. Induced responses to herbivory. Chicago: *University of Chicago Press.*

Kearney, M., R. Shine, and W.P. Porter. 2009. The potential for behavioral thermoregulation to buffer ‘‘cold-blooded’’ animals against climate warming. Proceedings of the National Academy of Sciences of the United States of America **106**:3835–3840.

Kerr, R.A. 1988. Stratospheric ozone is decreasing. Science **239**:1489–1491

Kevan, P. G., L. Chittka, and A.G. Dyer. 2001. Limits to the salience of ultraviolet: lessons from colour vision in bees and birds. Journal of Experimental Biology **14**:2571-2580.

Kiesecker, J.M., and A.R. Blaustein. 1995. Synergism between UV-B radiaton and a pathogen magnifies aphibian embryo mortality in nature. Proceedings of the National Academy of Sciences of the United States of America **92**:11049-11052.

Kim J.H., B.W. Lee, F.C. Schroeder, and G. Jander. 2008. Identification of indole glucosinolate breakdown products with antifeedant effects on Myzus persicae (green peach aphid). Plant Journal **54**:1015–1026.

Kossuth, S.V., and R.H. Biggs. 1981. Ultraviolet-B radiation effects on early seedling growth of Pinaceae species. Canadian Journal of Forest Research 11:243–248.

Koti, S., K.R. Reddy, V. G. Kakani, D. Zhao, and W. Gao. 2007. Effects of carbon dioxide, temperature and ultraviolet-B radiation and their interactions on soybean (Glycine max L.) growth and development. Environmental and Experimental Botany **60**:1-10.

Krizek, D.T., S.J. Britz, and R.M. Mirecki. 1998. Inhibitory effects of ambient levels of solar UV-A and UV-B radiation on growth of New Red Fire lettuce. Plant Physiology **103**:1–7

Krizek, D.T., R.M. Mirecki, and S.J. Britz. 1997. Inhibitory effects of ambient levels of solar UV-A and UV-B radiation on growth of cucumber. Plant Physiology **100**:886–93

Kulandaivelu, G., N. Nedunchezhian, and K. Annamalainathan. 1991. Ultraviolet-B (280-320 nm) radiation induced changes in photochemical activities and polypeptide components of C3 and C4 chloroplasts. Photosynthetica **25**:333-340.

Kuhlmann, F., and C. Muller. 2009. Independent responses to ultraviolet radiation and herbivore attack in broccoli. Journal of Experimental Botany **60**:3467-3475.

Kuhlmann, F., and C. Muller. 2010. UV-B impact on aphid performance mediated by plant quality and plant changes induced by aphids. Plant Biology **12**:676-684.

Kumar, P., and H.M. Poehling. 2006. UV-blocking plastic films and nets influence vectors and virus transmission on greenhouse tomatoes in the humid tropics. Environmental entomology **35**:1069-1082.

Lake J.A., K.J. Field, M.P. Davey, D.J. Beerling, and B.H. Lomax. 2009. Metabolic and physiological responses reveal multi-phasic acclimation of Arabidopsis thaliana to chronic UV radiation. Plant, Cell & Environment **32**:1377-1389.

Lavola, A. 1998. Accumulation of flavonoids and related compounds in birch induced by UV-B irradiance. Tree Physiology **18**:53-58.

Lean, J. 1997. The sun's variable radiaiton and its relevance for the earth. Annual review of Astronomy and Astrophysics **35**:33-67.

Legarrea, S., B.M. Diaz, M. Plaza, L. Barrios, I. Morales, E. Viñuela, and A. Fereres. 2012. Diminished UV radiation reduces the spread and population density of Macrosiphum euphorbiae (Thomas)[Hemiptera:Aphididae] in lettuce crops. Horticultural Science-UZEI, **39**.

Liakoura, V., S. Stavrianakou, G. Liakopoulos, G. Karabourniotis, and Y. Manetas. 1999. Effects of UV-B radiation on Olea europaea: comparisons between a greenhouse and a field experiment. Tree physiology **19**:905-908.

Lin, C., L. Li, Y. Wang, Z. Xun, G. Zhang, and S. Li. 1993. Effects of aphid population on the major economic parameters of soybean. Soybean Science **12**:252–254.

Lindroth, R.L., R.W. Hofmann, B. D. Campbell, W. C. Mcnabb and D. Y. Hunt. 2000. Population differences in Trifolium repens L. response to ultraviolet-b radiation: foliar chemistry and consequences for two lepidopteran herbivores. Oecologia1 **22**:20–28.

Lindroth, R.L., K.K. Kinney, and C.L. Platz. 1993. Responses of deciduous trees to elevated atmospheric CO_2: productivity, phytochemistry, and insect performance. Ecology **74**:763-777.

Long, L.M., H.P. Patel, W.C. Cory, and A. E. Stapleton. 2003. The maize epicuticular wax layer provides UV protection. Functional Plant Biology **30**:75–81.

Macedo, T.B., C.S. Bastos, L.G. Higley, K.R. Ostlie, and S. Madhavan. 2003. Photosynthetic responses of soybean to soybean aphid (Homoptera: Aphididae) injury. Journal of Economic Entomology **96**:188–193.

Madronich, S., R.L. McKenzie, L.O. Bjorn, and M.M. Caldwell. 1998. Changes in biologically active ultraviolet radiation reaching the earth's surface. Journal of Photochemistry and Photobiology **46**:5-19.

Ma, C.S. 2000. Modelling and Simulation of the Population Dynamics of the Cereal Aphid Metopolophium dirhodum in Northern Germany. Verlag Franzbecker, Berlin, pp. 139–141.

Ma, G., C.S. Ma.2007a. Behavioral responses of bird cherry–oat aphid, Rhopalosiphum padi, to temperature gradients. Acta Phytophylacica Sinica **34**:624–630.

Ma, G., C.S. Ma. 2007b. Upper critical temperatures for behaviors of three species of cereal aphids in leaf temperature gradients. Acta Ecologica Sinica **27**:2449–2459.

Morandin, L. A., T.M. Laverty, P.G. Kevan, S. Khosla, and L. Shipp. 2001. Bumble bee (Hymenoptera: Apidae) activity and loss in commercial tomato greenhouses. The Canadian Entomologist **133**:883-893.

Massad, T., and L. Dyer. 2010. A meta-analysis of the effects of global environmental change on plant-herbivore interactions. Arthropod-Plant Interactions **4**:181-188.

Mazid, M., T.A. Khan, and F. Mohammad. 2011. Role of secondary metabolites in defense mechanisms of plants. Biology and Medicine **3**:232-249.

Mazza, C. A., D. Battista, A.M. Zima, M. SZWARCBERG-BRACCHITTA, C.V. Giordano, A. Acevedo, ... and C.L. Ballaré. 1999a. The effects of solar ultraviolet-B radiation on the growth and yield of barley are accompanied by increased DNA damage and antioxidant responses. Plant, Cell & Environment **22**:61-70.

Mazza, C.A., J. Zavala, A.L. Scopel, and C.L. Ballaré. 1999b. Perception of solar UV-B radiation by phytophagous insects: behavioral responses and ecosystem implications. Proceedings of the National Academy of Sciences, USA **96**:980–985.

Mazza, C.A., H. Boccalandro, C.V. Giordano, D. Battista, A.L. Scopel, and C.L. Ballare. 2000. Functional significance and induction by solar radiation of ultraviolet-absorbing sunscreens in field-grown crops. Plant Physiology **122**:117-125.

Mazza, C.A., M.M. Izaguirre, J. Curiale, and C.L. Ballaré. 2010. A look into the invisible: ultraviolet-B sensitivity in an insect (Caliothrips phaseoli) revealed through a behavioural action spectrum. Proceedings of the Royal Society B: Biological Sciences **277**:367-373.

McCloud, E. S. and M. R. Berenbaum. 1994. Stratospheric ozone depletion and plant-insect interactions: effects of UV-B radiation on foliage quality of Citrus jambhiri for Trichoplusia niJ. Chemical Ecology **20**:525–539.

McCloud, E. S. and M.R. Berenbaum. 1999. Effects of enhanced UV-B radiation on a weedy forb (Plantago lanceolata) and its interactions with a generalist and specialist herbivore. Entomologia Experimentalis et Applicata **93**:233–247.

McKenzie, R., B. Conner, and G. Bodeker. 1999. Increased summertime UV radiation in New Zealand in response to ozone loss. Science **285**:1709–1711.

McCornack, B. P., D.W. Ragsdale, and R.C. Venette. 2004. Demography of soybean aphid (Homoptera: Aphididae) at summer temperatures. Journal of Economic Entomology **97**:854-861.

McCornack, B. P., M. A. Carrillo, R. C. Venette, and D. W. Ragsdale. 2005. Physiological constraints on the overwintering potential of the soybean aphid (Homoptera: Aphididae). Environmental Entomology **34**:235–240.

Mead, M. Nathaniel. 2008. Benefits of sunlight: A bright spot for human health. Environmental Health Perspectives **116**:A160.

Mignault, M. P., M. Roy, and J. Brodeur. 2006. Soybean aphid predators in Quebec and the suitability of Aphis glycines as prey for three Coccinellidae. BioControl **51**:89-106.

Muller, C. 2008. Resistance at the plant cuticle. Induced Plant Resistance to Herbivory **4**:107-129.

Muller C., and M. Riederer. 2005. Plant surface properties in chemical ecology. Journal of Chemical Ecology **31**:2621–2651.

Nakagaki, S., K. Sekiguchi, and K. Onuma. 1982. The growth of vegetable crops and establishment of insect and mite pests in a plastic greenhouse treated to exclude near UV radiation. Bull Ibaraki Ken Horticultural Experiment Station **10**:39-47.

National Aeronautics and Space Administration (NASA). 1988. Executive Summary of the Ozone Trends Panel, NASA, Washington, D.C.

NDAWN. 2010 and 2011. Historical data archives. North Dakota Agricultural Weather Network. North Dakota State University.

Nguyen, T.T.A., D. Michaud, and C. Cloutier. 2009. A proteomic analysis of the aphid Macrosiphum euphorbiae under heat and radiation stress. Insect biochemistry and molecular biology **39**:20-30.

Niyogi, K.K. 1999. Photoprotection revisited: genetic and molecular approaches. Annual review of plant biology **50**:333-359.

Noorudeen, A.M., and G. Kulandaivelu. 1982. On the possible site of inhibition of photosynthetic electron transport by ultraviolet-B (UV-B) radiation. Physiologia Plantarum **55**:161-166.

Norton, R.A. 1999. Inhibition of aflatoxin B1 biosynthesis in Aspergillus flavus by anthocyanidins and related flavonoids. Journal of Agricultural and Food Chemistry **47**:1230-1235.

Onzo A, M.W. Sabelis, and R. Hanna. 2010. Effects of ultraviolet radiation on predatory mites and the role of refuges in plant structures. Environmental Entomology **39**:695–701.

Ostlie, K. 2005. Soybean Aphid in Minnesota. University of Minnesota, St. Paul, MN.

Paul, N.D., and D. Gwynn-Jones. 2003. Ecological roles of solar UV radiation: towards an integrated approach. Trends in Ecology & Evolution **18**:48-55.

Paul, N.D., S. Rasanayagam, S.A. Moody, P.E. Hatcher, and P.G. Ayres. 1997. The role of interactions between trophic levels in determining the effects of UV-B on terrestrial ecosystems. Plant Ecology **128**:196-308.

Parisi, A., and M.G. Kimlin. 1997. Ozone and ultraviolet radiation. Australasian Science **18**:44-46.

Pettersson, J., W.F. Tjallingii, and J. Hardie. 2007. Host-plant Selection and Feeding. Aphids as crop pests **4**:87-107.

Pfundel, E.E., G. Agati, and Z.G. Cerovic. 2006. Optical properties of plant surfaces. In: RiedererM., Muller. (Eds), Biology of the Plant Cuticle. Blackwell Publishing, London, UK: pp. 216–249.

Picman, A. K., E.F. Schneider, and J. Picman. 1995. Effect of flavonoids on mycelial growth of Verticillium albo-atrum. Biochemical Systematics and Ecology **23**:683-693.

Pincebourde, S., H. Sinoquet, D. Combes, and J. Casas. 2007. Regional climate modulates the canopy mosaic of favorable and risky microclimates for insects. Journal of Animal Ecology **76**:424–438.

Pinto, M., G.E. Edwards, A.A. Riquelme and M.S.B. Ku. 2002. Enhancement of nodulation in bean (Phaseolus vulgaris) by UV-B irradiation. Functional plant biology **29**:1189–1196.

Ragsdale, D.W., D.J. Voegtlin, and R.J.O'Neil. 2004. Soybean aphid biology in North America. Annals of the Entomological Society of America **97**:204–208.

Ragsdale, D.W., B.P. McCormack, R.C. Venette, B.D. Potter, I.V. MacCrae, E.W. Hodgson, M.E. O'neal, K. D. Johnson, R.J.O'Neil, C.D. Difonzo, T.E. Hunt, P.A. Glogaza, and

E.M. Cullen. 2007. Economic threshold for soybean aphid (Hemiptera: Aphididae). Journal of Economic Entomology **100**:1258-1267.

Ragsdale, D.W., D.A. Landis, J. Brodeur, G.E. Heimpel, and N. Desneux. 2011. Ecology and management of the soybean aphid in North America. Annual Review of Entomology **56**:375-399.

Rajendiran, K., & M.P. Ramanujam. 2006. Interactive effects of UV-B irradiation and triadimefon on nodulation and nitrogen metabolism in Vigna radiata plants. Biologia plantarum **50**:709-712.

Reifenrath, K., and C. Muller. 2007. Species-specific and leaf-age dependent effects of ultraviolet radiation on two Brassicaceae. Phytochemistry **68**:875-885.

Rosenthal, G.A. 1991. The biochemical basis for deleterious effects of L-canavanine. Phytochemistry **30**:1055-1058.

Rousseaux, M.C., C.L. Ballaré, A.L Scopel, P.S. Searles, and M.M. Caldwell. 1998. Solar ultraviolet-B radiation affects plant-insect interactions in a natural ecosystem of Tierra del Fuego (southern Argentina). Oecologia **116**:528-535.

Rousseaux, M.C., A. L. Scopel, P.S. Searles, M.M. Caldwell, O.E. Sala, and C.L. Ballaré. 2001. Responses to solar ultraviolet-B radiation in a shrub-dominated natural ecosystem of Tierra del Fuego (southern Argentina). Global Change Biology **7**:467–478.

Rozema, J., J. Van De Staaij, L. O. Björn and M. M. Caldwell. 1997. UV-B as an environmental factor in plant life: stress and regulation. TREE **12**:22.

Rozema, J. 1999. Stratospheric ozone depletion: the effects of enhanced UV-B radiation on terrestrial ecosystems. Backhuys Publishers.

Rice, M.E., M. O'Neal, and P. Pedersen. 2005. Soybean Aphids in Iowa-2005. Report No. SP 247, Iowa State University Extension, Iowa State University, Ames, IA.

Ryan, K.G., and W. Ireland. 1997. A small-scale outdoor plant growth chamber with modulated enhancement of solar UV-B radiation. Journal of Environmental Quality **26**:866-871.

Salt, D. T., S. A. Moody, J. B. Whitaker and N. D. Paul. 1998. Effects of enhanced UV-B on populations of the phloem feeding insect Strophingia ericaek (Homoptera: Psylloidea) on heathwer (Calluna vulgaris). Global Change Biology **4**:91–96.

SAS Institute Inc. 2010. JMP Statistics and Graphics Guide. SAS Institute Inc., Cary, NC.

Savenstrand, H., M. Brosche, and A. Strid. 2002. Regulation of gene expression by low levels of ultraviolet-B radiation in Pisum sativum: isolation of novel genes by suppression subtractive hybridisation. Plant and cell physiology **43**:402-410.

Saviranta, N.M.M., R. Julkunen-Tiitto, E. Oksanen and R.O. Karjalainen. 2010. Leaf phenolic compounds in red clover (Trifolium pratense L.) induced by exposure to moderately elevated ozone. Environmental Pollution **158**:440–446.

Searles, P.S., M.M. Caldwell, and K. Winter. 1995. The response of five tropical dicotyledon species to solar ultraviolet-B radiation. American Journal of Botany **82**:445–53.

Siemens, D.H., S.H. Garner, T. Mitchell-Olds, and R.M. Callaway. 2002. Cost of defense in the context of plant competition: *Brassica rapa* may grow and defend. Ecology **83**:505-517.

Simms, E.L. 1992. Costs of plant resistance to herbivory. In plant resistance to herbivores and pathogens. Ecology, evolution, and genetics. Fritz R.S. and Simms E.L., Chicago: University of Chicago Press 392-425.

Sisson, W. B., and M. M. Caldwell. 1976. Photosythesis, dark resperation, and growth of *Rumex patientia* L. exposed to ultraviolet irradiance (288 to 315 nanometers) simulating a reduced atmospheric ozone column. Plant Physiology **58**:563-568.

Strid, A., W.S. Chow, and J.M. Anderson. 1994. UV-B damage and protection at the molecular level in plants. Photosynthesis Research **39**:475-489.

Taalas, P., G. T. Amanatidis, and A. Heikkila. 2000. European conference on atmospheric UV radiation: overview. Journal of Geophysical Research: Atmospheres **105**:4777–4785.

Taalas, P., J. Kaurola, A. Kylling, D. Shindell, R. Sausen, M. Dameris, V. Grewe, J. Herman, J. Damski, and B. Steil. 2000. The impact of greenhouse gases and halogenated species on future solar UV radiation doses, Geophysical Research Letters **27**:1127-1130.

Teramura, A. H. 1980. Effects of ultraviolet-B irradiances on soybean. I. Importance of photosynthetically active radiation in evaluating ultraviolet-B irradiance effects on soybean and wheat growth. Physiologia Plantarum **48**:333-339.

Teramura, A.H., and M.M. Caldwell. 1981. Effect of UV-B irradiance on soybean. IV. Leaf morphology as a factor in evaluating UV-B irradiance effects on net photosynthesis. American Journal of Botany **68**:934-941.

Teramura, A.H, M. Tevini, and W. Iwanzik. 1983. Effects of ultraviolet-B irradiance on plants during mild water stress. I. Effects on diurnal stomatal resistance. Physiologia Plant **57**:175–180

Teramura, A.H., and Murali, N.S. 1986. Intreapecific differences in growth and yield of soybean exposed to ultraviolet-B radiation under greenhouse and field conditions. Environmental Experimental Botany **26**:89-95.

Teramura, A.H., and J.H. Sullivan. 1994. Effects of UV-B radiaiton on photosynthesis and growth of terrestrial plants. Photosynthesis Research **39**:463-473.

Tevini, M. 2004. Plant responses to ultraviolet radiation stress. In Chlorophyll a Fluorescence pp. 605-621.

Tevini, M., and A.H. Teramura. 1989. UV-B effects on terrestrial plants. Photochemistry Photobiology **50**:478-487.

USDA. 2011. U.S. Soybean Industry: Background Statistics and Information. http://www.ers.usda.gov/news/soybeancoverage.htm

Van de Mortel, T.F., and W.A. Buttemer. 1998. Avoidance of ultraviolet-B radiation in frogs and tadpoles of the species Litoria aurea, L. dentata, and L. peronii. Proceedings of the Linnean Society of New South Wales **119**:173-179.

Vanicek, K., T. Frei, Z. Litynska, and A. Schmalwieser. 2000. UV-Index for the Public. Publication of the European Communities, Brussels, Belgium.

Venette, R. C., and D. W. Ragsdale. 2004. Assessing the invasion by soybean aphid (Homoptera). Annals of the Entomological Society of America **97**.

Wang, C. L., N. J. Siang, G. S. Chang, and H. F. Chu. 1962. Studies on the soybean aphid, Aphis glycines Mastumura. ACTA entomologica SINICA **11**:31–44.

Wang, X., C. Fang, X. Zhang, and H. Wang. 1994. A study on the damage and economic threshold of the soybean aphid at the seedling stage. Journal of Plant Protection **20**:12-13.

Wang, S., X. Bao, Y. Sun, R. Chen, and B. Zhai. 1996. Study on the effect of population dynamics of soybean aphid (Aphis glycines) on both growth and yield of soybean. Soybean Science **15**:243–247.

Warren, J. M., J. H. Bassman, D. S. Mattinson, J. K. Fellman, G. E. Edwards and R. Robberecht. 2002. Alteration of foliar flavonoid composition induced by enhanced UV-B radiation in Pinus ponderosa, Quercus rubra, Pseudotsuga menziesii andPopulus trichocarpa. J. Photochem. Photobiol. B: Biol.**66**:125–133.

Whalen, R., and J.P. Harmon 2012. Rag1 Aphid Resistant Soybeans Alter the Movement and Distribution of Soybean Aphid (Hemiptera: Aphididae). Environmental entomology, **41**:1426-1434.

Wiktelius, S. 1987. Distribution of Rhopalosiphum padi (Homoptera: Aphididae) on spring barley plants. Annals of Applied Biology **110**:1–7.

Wink, M. 1999. Functions of plant secondary metabolites and their exploitation in biotechnology. Annual plant reviews. In-season prediction of corn yield using plant height under major corn production systems **3**.

Wu, Z., D. Schenk-Hamlin, W. Zhan, D. W. Ragsdale and G. E. Heimpel. 2004. The soybean aphid in China: a historical review. Annals of the Entomological Society of America **97**:209–218.

Xiong, F.S., and T.A. Day. 2001. Effect of solar ultraviolet-B radiation during springtime ozone depletion on photosynthesis and biomass production of Antarctic vascular plants. Plant Physiology **125**:738–51

Yin, X., J. N. Jaja, and M.A. McClure. 2011. Comparison of models in assessing relationship of corn yield with plant height measured during early to mid season. Journal of Agricultural Science **3.**

Zavala, J.A., A.L. Scopel, and C.L. Ballaré. 2001. Effects of ambient UV-B radiation on soybean crops: Impact on leaf herbivory by Anticarsi gemmatalis. Plant Ecology **156**:1–10.

Zerefos, C. S., A. F. Bais, C. Meleti, and I. C. Ziomas. 1995. A note on the recent increase of solar UV-B radiation over northern middle latitudes. Geophysical Research Letters **22**:1245–1247.

Ziska, L. H., A. H. Teramura, and J. H. Sullivan. 1992. Physiological Sensitivity of Plants Along an Elevation Gradient to UV-B Radiation. American Journal of Botany **79**:863-871.