

THE EFFECTS OF ABIOTIC CLIMATE CHANGE FACTORS ON APHID LIFE HISTORY
AND POPULATIONS

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Title

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North Dakota State University's regulations and meets the accepted standards
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ABSTRACT

While the effects of many abiotic factors associated with climate change have not been extensively studied, work on model organisms is beginning to help predict effects on similar species. Research on climate change has been dominated by temperature effects on organisms, but additional factors are being studied, including changes in atmospheric CO₂ concentrations, wind patterns, precipitation patterns, ultra-violet (UV) radiation, night-time temperatures, and daily high temperatures (heat shocks). We review these effects on aphids and present experimental findings on the effects of night-time warming on pea aphid population growth and birth rates. Aphid populations changed when warmed at different times of a day (overnight vs. during the day), but changes to individual aphid birth rate were not responsible for the population level pattern. Although the exact mechanism is still unclear, increasing nightly temperatures matters for pea aphid population growth and could influence interactions with other species.

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**CHAPTER 1. THE MANY FACES OF CLIMATE CHANGE: THE BIOLOGICAL
RESPONSE TO CHANGING ABIOTIC FACTORS APART FROM AVERAGE
TEMPERATURE**

Introduction

As we explore the topic of climate change, this review aims to highlight certain abiotic factors and their effects on a group of organisms. This first chapter is intended for publication in and written in the style of Nature Education Knowledge. Boxes are used to provide supplementary information to the main text because the journal is intended for a broad audience.

Background

Climate change is a broad topic, in terms of the numerous effects it can have on everything from individual organisms to large-scale ecological communities, as well as the many different abiotic factors that are changing (Walther et al. 2002, Bradshaw and Holzapfel 2006). Although the scientific community is actively researching the effects of many changing abiotic factors, certain factors have been given the majority of attention. This is especially evident when looking back at how climate change was portrayed in the public media around 10 years ago. At that time, the topic was widely talked about as “Global Warming”. This is not incorrect since global average temperatures have increased 0.6°C over the past century and much research has been performed to make the crucial link between changing temperature and its effects on organisms (Walther et al. 2002). However, increasing temperature is only one specific way that abiotic factors are changing in association with climate change. This article aims to highlight the complexity of the global climate system and how organisms have the potential to be affected by each of these many changing abiotic factors. Specifically, we address changes in atmospheric CO₂ concentrations, wind patterns, precipitation patterns, ultra-violet (UV) radiation, night-time

temperatures, and daily high temperatures (heat shocks). These factors are not only major players in climate, but we will discuss research demonstrating how changes to each factor can cause noticeable effects on organisms.

The effects of changing abiotic factors have been studied using a variety of organisms, but for this article, we will highlight the responses of a particular group of herbivorous insects called aphids (Box 1). The diversity of organisms and their ecology has already created a fascinating story of interactions with climate change that occur primarily because of the specific abiotic factors and the organism's natural history (Hofmann and Todgham 2010, Walther et al. 2009). However, by using a particular group of organisms as a model, we hope to focus more on the variety of abiotic factors and their wide spread potential effects on plants and animals. Aphids are a particularly useful group to discuss in climate change studies due to their life history characteristics, interactions with other species, and relative ease to study experimentally (Volkl et al. 1999, Harmon et al 2009, Hulle et al. 2010; Box 1). These hardy little insects may seem insignificant, but they can cause major damage to agricultural crops when populations are high enough. Their role in agricultural and other ecosystems has made them the subject of many scientific questions, including how they are affected by changing abiotic factors (Blackman and Eastop 2000). Moreover, because these aphids rely heavily on their host plants for food, shelter, and habitat, any abiotic factor that influences the plant has the potential to indirectly influence the aphid too (Box 2).

Box 1: Aphids

Aphids are a broad group of insects that contain almost 5,000 described species (International Aphid Genomics Consortium 2010). They are relatively small in size, with most being 1.5 to 3.5mm long with larger species up to 6mm long. Aphids are pear-shaped with soft

bodies that can be winged or wingless and they have needle-like mouth parts for tapping into plants and feeding on phloem contents (Blackman and Eastop 2000, International Aphid Genomics Consortium 2010).

Herbivorous in nature, aphids feed on a wide array of herbaceous and woody plants. Some aphid species specialize on one particular plant while other species are generalists and feed on a number of different plant species. Some generalist aphids are pests in agricultural systems as well as home gardens (Blackman and Eastop 2000). However, aphid species can be found in a variety of natural and anthropogenic environments around the world.

Due to unique reproductive characteristics, aphids are incredibly well suited for rapid population growth. Much of the time female aphids reproduce without having to mate with a male and they give birth to live young. Moreover, these offspring are genetically identical clones of their mother. One female can birth numerous offspring throughout her lifetime and each of those offspring will develop quickly then reproduce in the same way. As an area on a plant becomes crowded, winged aphids can be born and migrate to new plants to begin reproduction. These traits also make some aphids easy to keep in laboratory colonies and use in scientific experiments (Hulle et al. 2010).

Aphids also interact in a number of ways with many other insects. They compete for resources with other herbivorous insects, other aphid species, and even within the same aphid species (Blackman and Eastop 2000, Barton 2014). Many predatory insects, such as ladybugs, feed on aphids as a primary food source. In some cases, ant species develop mutualistic relationships with aphids where the ants feed on excess sugars aphids excrete, known as honeydew, and protect them from predation from other insects, such as ladybugs (Volkl et al. 1999). These interactions are crucial to these other species and to the fate of the aphids.

Box 2: Direct and Indirect Effects

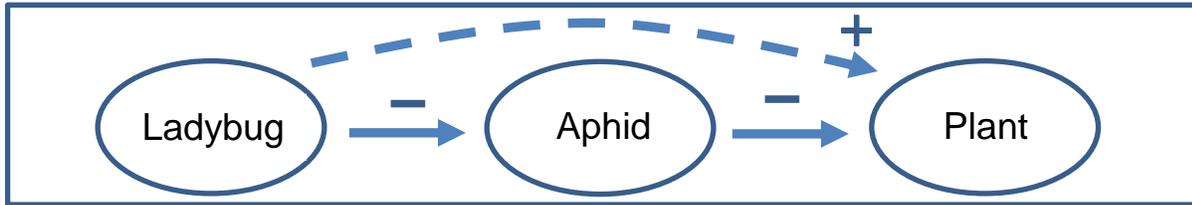


Figure 1. Direct and indirect effects illustrated by using an example among a predatory ladybug, herbivorous aphid, and plant. The solid arrows in Figure 1 represent direct effects while the dashed-line arrow represents an indirect effect. Aphids feed on a plant which has a direct negative effect on plant health. Since this interaction occurs just between the two organisms and no other organism is needed for it to occur, it is considered a direct effect. Similarly, ladybugs have a direct negative effect on aphid populations because they can eat aphids without needing another species present. In this case, the ladybug directly feeding on the aphids has an indirect positive effect on the plant. By directly eating the aphids that feed on the plant, there are fewer aphids, thereby reducing the aphid's negative effect on the plant.

Climate change effects on aphids can occur in two different ways: as direct effects or indirect effects. Direct effects occur when one organism affects a second without the need for any other organism to be present. These direct effects are what we normally think about when two organisms interact. Indirect effects differ from direct effects because they need an intermediate species present to happen. Although the example in Figure 1 uses three organisms, we can use the same concept to explain the effect of a changing abiotic factor by swapping one of the organisms for a changing abiotic factor (Harmon and Barton 2013). For example, we could have a particularly warm temperature in place of our ladybugs. This very warm temperature is directly detrimental to the aphids, which leads to an indirect benefit for the plant due to the reduced number of herbivores. It is important to remember that multiple direct and indirect effects can be occurring at the same time, especially when abiotic factors change. Moreover, indirect effects are not limited to just three interacting organisms (or abiotic factors). Thus strong ecological interactions like feeding and competition can result in even farther reaching effects of climate change throughout a tightly interacting system (e.g., Figure 2).

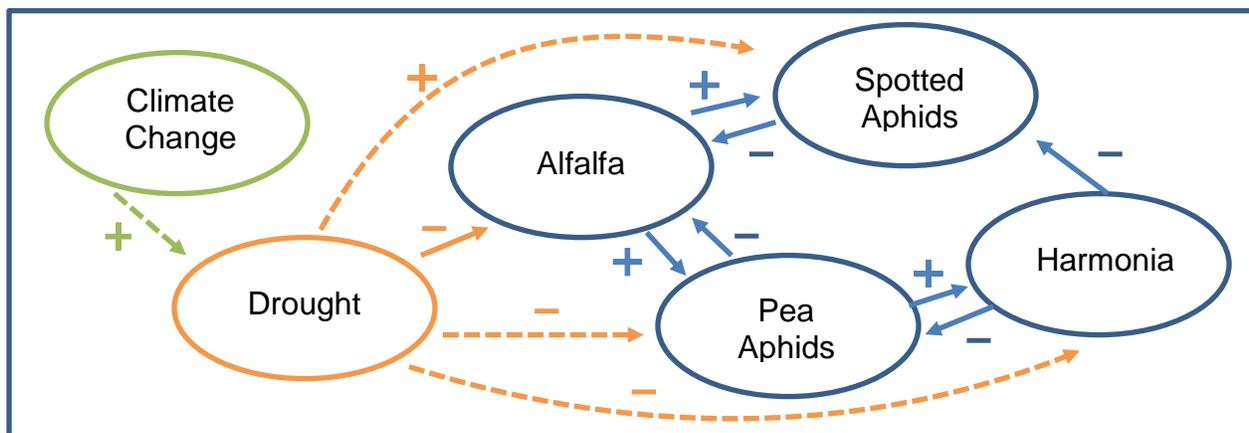


Figure 2. Direct and indirect effects from drought illustrated using an example in an alfalfa system. Solid-line arrows indicate direct effects while indirect effects are represented by dashed-line arrows. Positive and negative effects are also marked next to the effect arrow. This figure shows, in blue, how herbivores, a common predator, and a plant interact normally. Then orange arrows show direct and indirect effects on the different organisms in blue. Finally, climate change impacts on drought, shown in green, affect the subsequent interactions. A full description of the system and its origins is available in the *Precipitation* section.

Abiotic Factors

CO₂

Carbon dioxide (CO₂) is normally found in the atmosphere as part of the global carbon cycle, which moves various forms of carbon around the planet. Since the industrial revolution, CO₂ has increased approximately 40% and is predicted to increase up to twice the current level by 2100. CO₂ is often considered primarily as a greenhouse gas responsible for increases in global temperatures, but it can also have direct and indirect impacts on organisms (Oehme et al. 2013).

Changes in atmospheric CO₂ concentration do not typically affect animals directly in a major way, but instead are primarily known to have direct impacts on plants. Herbivores are indirectly affected by changes to the plants they feed on (Box 2). One study examined these indirect effects in two different systems by testing the effects of elevated CO₂ on aphids and their host plants (Oehme et al. 2013). They measured the performance of bird-cherry oat aphids (*Rhopalosiphum padi*) on spring wheat and the performance of green peach aphids (*Myzus*

persicae) on oilseed rape along with plant nutrients. Two nutrients in phloem sap, free amino acids and sugars, were measured in spring wheat and oilseed rape at varying growth stages while the relative growth rate of aphids on a specific plant was calculated. At elevated CO₂ concentrations, the bird-cherry oat aphid showed a significant increase in relative growth rate on spring wheat. For the green peach aphid, elevated CO₂ showed a significant decrease in the relative growth rate of aphids on oilseed rape. Because plants utilize CO₂ for metabolic processes, increasing CO₂ concentrations caused a chemical change in plant phloem contents which led to different responses from these two aphid species.

Wind

Over the last 30 years, wind intensity has decreased 5 – 15% worldwide and is predicted to continue decreasing over the 21st century. This change in wind is due to two different proposed mechanisms. First, the global thermal gradient between the poles and equator has been decreasing, which leads to a decrease in wind energy. Secondly, an increase in the number of physical structures on the landscape, such as manmade structures and reforested areas, obstruct wind (Barton 2014). Wind may seem like a very large culmination of environmental factors, but wind could be an important abiotic factor that has direct and indirect effects on even small organisms.

One study in a soybean agroecosystem showed how a major crop pest, the soybean aphid (*Aphis glycines*), indirectly benefits from wind because its predator, the multicolored Asian lady beetle (*Harmonia axyridis*), is directly impeded by wind (Barton 2014). In greenhouse and field experiments, aphids were not directly affected by wind when they were alone feeding on their plant. This is most likely due to their small body size and sessile lifestyle. However, the larger predatory lady beetle was directly affected by wind. When simulating only the plant movement

caused by wind, predators took more time to find aphids and begin eating. Additionally, once predators began consuming aphids, they ate fewer aphids when on moving plants compared to those on control plants. In the field, this translated to plants that were open to the wind having fewer predators compared to plants that were blocked from the wind. Likely because there were fewer predators on these plants, there were more aphids on plants with more wind. While the aphid was not directly affected by wind in this study, wind became important when considering the other species that aphids interact with.

Precipitation

Precipitation, like wind, is a large scale culmination of other climate events that influence landscapes and organisms alike. Fluctuating wind, air pressure, temperature, and moisture in the atmosphere determine local and large scale precipitation events (Trenberth 2011). In the global climate system, average global precipitation has increased 2% over the past century, but this increase is not consistent across locations or through time (Dore 2005). In fact, areas of high precipitation are expected to receive more precipitation while areas with little precipitation are expected to receive less (Dore 2005, Trenberth 2011). However, in both types of areas, individual precipitation events have become less frequent over time but have increased in magnitude (Trenberth 2011, Barton and Ives 2014). This trend is expected to continue and have significant effects across ecosystems (Dore 2005, Trenberth 2011, Barton and Ives 2014). Therefore, to understand how climate change may affect organisms through changes in precipitation there are a number of scenarios to potentially study, including both higher and lower than normal precipitation.

On the agricultural landscape, precipitation is incredibly important to many plants and therefore it can be important to the herbivores that feed on them. One study in alfalfa (*Medicago*

sativa) fields, found that the aphid community dominated by pea aphids (*Acyrtosiphon pisum*) and spotted aphids (*Therioaphis maculata*) changes with precipitation (Barton and Ives 2014). In Figure 2, the direct competitive interactions between these two aphid species are fairly weak, but drought conditions in alfalfa indirectly affect the populations of both aphids. Drought-stressed alfalfa is detrimental to pea aphids, but spotted aphids are not directly affected by lower moisture content in alfalfa. A shared predator, however, the multicolored Asian lady beetle (*Harmonia axyridis*), adds an additional indirect complication due to drought. These predators typically prefer areas with high pea aphid density, but decreasing alfalfa moisture leads to fewer pea aphids and therefore fewer lady beetles. This results in a negative indirect effect from drought to the lady beetles. Fewer lady beetles mean less predation on spotted aphids, thereby giving a positive indirect effect, from drought to spotted aphids, which is mediated by the other species in the system. So, in this instance, a local decrease in precipitation directly and indirectly affects pea aphid populations and predatory lady beetles, which eventually lead to an indirect increase in spotted aphids.

UV Radiation

Out of all the abiotic factors discussed in this article, a change in UV radiation is something people may not readily associate with climate change. UV radiation comes from the sun and reaches the earth's surface after traveling through the atmosphere (Burdick 2013). Climate change research focuses predominately on how UV radiation changes in relation to changes in the ozone layer, cloud cover, and air quality (McKenzie et al 2011). Although UV radiation had been increasing for some time as atmospheric ozone decreased, the Montreal Protocol enacted in 1989 has been effective in preventing further degradation of atmospheric ozone (McKenzie et al 2011, Williamson et al. 2014). Now, changes in UV radiation are

strongly linked to cloud cover, air pollution, and aerosols which are all influenced by other climate change factors (McKenzie et al. 2011).

Before predicting the overall effects of different UV radiation levels, one study set out to determine the direct effects of UV radiation on the soybean aphid (*A.glycines*) (Burdick 2013). In a field setting, the presence or absence of UV radiation was tested on aphid population growth but no effect was found; however, this may have been due to the distribution of aphids on the plant. Even though all the aphids started the experiment on the top of leaves, by the end of the experiment almost all aphids were located on the underside of leaves where there was a significantly lower level of UV radiation than above the leaf. Moving to areas of lower UV radiation may have allowed aphids to avoid any harmful effects. A follow up experiment supported this idea by modifying how much UV exposure an aphid had while controlling for which side of the leaf (abaxial vs. adaxial) the aphid was on. Aphids confined to always “face down” were exposed to less UV and showed higher growth than if they were “face up” and exposed to more UV. Although changes in UV radiation can have effects on aphid traits, their movement and distribution on a plant could help minimize exposure to harmful UV effects.

Temperature beyond the average

Global average temperatures are predicted to increase anywhere from 1.5°C to 6°C by the end of the century (Hulle et al. 2010, Clarke and Zani 2012), but long term averages are only one way to look at temperature changes, and they are definitely not the only way that temperature is important to organisms. For example, while daily average temperatures have increased, this increase is not symmetrical in terms of when warming occurs. Specifically, nightly minimum temperatures have increased at almost twice the rate of daily maximum temperatures (Alward et al. 1999, Peng et al. 2004, Clarke and Zani 2012). For animals that are only active at night, it is

clear that night time temperatures and warming at night are more important than what is happening on average over the entire day. However, species that are active throughout the entire day may also be influenced by whether warming occurs during the day or at night.

Another temperature trend in climate change that can affect organisms is the increase in daily maximum temperatures, sometimes referred to as heat shock events. These events are characterized as especially high temperatures for a fairly short time periods, like a particularly warm afternoon. This contrasts with heat waves, which would be over a longer time period and likely have cumulative effects. Heat shocks can exceed the thermal tolerance of an organism, which typically leads to negative effects on physiological and behavioral processes (Harmon et al. 2009, Hofmann and Todgham 2010).

Warming at night and heat shocks can directly and indirectly affect pea aphids (*A. pisum*). In one study (Chapter 2), warming at night was compared to warming during the day to determine if an equal increase in temperature differentially affected aphids by only changing when warming occurred. Pea aphid population growth increased with warming at either time, but warming at night caused greater population growth than warming during the day.

In a different study, heat shock effects were observed on pea aphids in combination with seven spotted lady beetles (*Coccinella septempunctata*) and multicolored Asian lady beetles (*Harmonia axyridis*) (Harmon et al. 2009). Testing aphid populations in the field showed a significant decrease in aphid populations with heat shock, but the addition of a predator species did not always cause an additive negative effect. With seven spotted lady beetles, heat shock and predator effects caused a greater decrease in aphid populations than each effect separately. When multicolored Asian lady beetles were present, the combined negative effect with heat shock was actually reduced. These two studies add to the understanding that temperature is

important to aphids, but do so with different types of temperature changes and through direct and indirect effects.

Closing

With the examples shown above, it is clear that aphids can be affected by a number of factors associated with climate change and that these factors affect aphids in a variety of ways. Each of these factors can show effects on its own, but it is important to remember that multiple factors likely occur simultaneously in a natural environment. Some research has taken this into account and studied the effect of multiple factors, such as elevated temperature and CO₂. For example, corn leaf aphids (*Rhopalosiphum maidis*) under increased temperature and CO₂ levels produced more offspring and had a higher proportion of winged aphids (Xie et al. 2014). This increase can cause faster population growth and a greater dispersion of aphids from this population. Understanding how multiple factors affect an organism is important when predicting the effects of climate change. However, it is not clear how many predictions can be made for multiple factors when only considering how they affect the organisms alone.

The natural history of a given organism will also be crucial to take into account. In cases like night warming, the effects may depend on when the organism is active throughout a 24-hour period. In cases like UV radiation, behaviors that compensate for changes in abiotic conditions may mediate the effects an organism experiences.

Looking at the many faces of climate change rather than “global warming” alone reveals the complexity of this issue and its potential effects. As more research is done, we will continue to gain a better understanding of the ways climate change and its multiple factors affect species and their interactions.

CHAPTER 2. TIMING IS KEY: HOW WARMING AT DIFFERENT TIMES OF DAY AFFECTS PEA APHID POPULATIONS

Introduction

Temperature effects due to climate change can potentially impact on organisms and ecosystems across the planet (McCarty 2001, Walther et al. 2002, Walther et al. 2009, Sunday et al. 2011, Kingsolver et al. 2013, Sharma and Prabhakar 2014). Changes in temperature have been shown to impact the physiological processes and behaviors of many plant and animal species across different environments (Bradshaw and Holzapfel 2006, Deutsch 2008, Hofmann and Todgham 2010). Over the past century, global averages have increased approximately 0.6°C and are predicted to increase anywhere from 1°C to 6°C during the next century (Rustad et al. 2001, Holopainen and Kainulainen 2004, Hulle et al. 2010, Paaijmans et al. 2013). While many studies address how changes in average temperature impact organisms, this may not be the best representation of the predicted annual change, as there is currently an unequal warming trend between daily maximum and nightly minimum temperatures. Minimum temperatures have increased at a greater rate than maximum temperatures, which indicates greater warming at night than during the day (Alward et al. 1999, Walther et al. 2002, Xia et al. 2010, Clarke and Zani 2012, Bai et al. 2012, Peng et al. 2013). This leads us to ask: does the particular time of day that warming takes place matter?

Research comparing the effects of warming at different times of a day is relatively scarce in the literature, but the available studies show that increasing temperatures just at night can have important impacts on plant and animal species. For plants, increased temperatures at night decreased net primary production and yield for grasses and rice (Alward et al. 1999, Peng et al. 2004). Other taxa, such as insects and reptiles, have shown some beneficial and detrimental

effects from warming at night. The side-blotched lizard (*Uta stansburiana* Baird and Girard) showed several beneficial effects from night warming, including decreased duration of the reproductive cycle, increased hatchling size, decreased incubation period, and increased rate of offspring development. The temperature threshold of these activities is such that warming at night increases the reproductive capabilities of the population (Clarke and Zani 2012). In contrast, reproductive activity and nymph survival rate of the English grain aphid (*Sitobion avenae*) decreased, but optimal temperature for development increased (Zhao et al. 2014). The imported cabbage worm (*Pieris rapae* L.) showed variation in response to night warming, but on average, increased nightly temperatures caused faster development rates (Whitney-Johnson et al. 2005). These studies show that warming does not need to occur over an entire day nor during the normal hours of light to be important.

As global temperature trends are continuously investigated, it is interesting to ask ecological questions comparing the effects of warming during the day and at night on organisms. Effects from warming at these specific times may be similar or different depending on the daily activity of the organism in question. Warming at night may have significant effects on a nocturnal species and one active throughout an entire day, but it is also possible that the species active all day would not respond in the same way as a nocturnal species. The goal of our research was to examine the effects of warming at different times on pea aphids (*Acyrtosiphon pisum*) and determine if the response to warming at night differs from warming during the day.

The experiments we performed focused on changes observed in population growth and reproduction rates of individual aphids while manipulating environmental temperatures. These experiments follow a set of questions we posed in order to explore the role time of day plays in warming effects on this species. (1) Do pea aphids express a certain behavior that is present and

susceptible to the effects of warming, such as reproduction, during the day and at night? (2)
Does the time of day a population of aphids experience warming affect population growth? (3)
Does a mechanism of population growth, individual birth rate, change in a similar way to
population growth when warmed at different times of a day? (4) And finally, how does the birth
rate of aphids change with increasing constant temperatures? Observing birth rate at increasing
temperatures helps to distinguish the effects of absolute temperature from time of warming on
individual aphid birth rates.

Methods

Aphids

Pea aphids (*Acyrtosiphon pisum*) are small, phloem feeding insects with needle-like mouth parts. During summer months, they feed on a broad range of host plants and populations can grow rapidly through asexual reproduction (Blackman and Eastop 2000, International Aphid Genomics Consortium 2010). As winter approaches, aphids lay overwintering eggs through sexual reproduction (Le Trionnaire et al. 2012). These life history traits, along with other factors, make aphids a good model system to observe the effects of temperature (Morgan et al. 2001, Lu and Kuo 2008). Additionally, pea aphid genetics (International Aphid Genomics Consortium 2010) and interactions (Charles and Ishikawa 1999, Snyder and Ives 2003, Harmon et al. 2009) with other organisms are well documented.

Pea aphid colonies began from individuals collected from alfalfa fields in Fargo, North Dakota, USA and maintained in North Dakota State University greenhouses. Colonies were reared on fava, *Vicia faba*, under ambient temperature and humidity with a 16:8 L:D (Light:Dark) cycle.

Measuring the Reproductive Activity of the Pea Aphid, *Acyrtosiphon pisum*

We first examined the daily reproductive activity of pea aphids to assess their suitability as subjects in this study. For some animals, their activity and behaviors can be more clearly separated in time to be either nocturnal or diurnal (Kelber et al. 2002, Warrant et al. 2004). Thus, examining the effects of night vs day warming on pea aphids would be difficult if their behaviors showed a clear separation in time. Before we began manipulating warming times, we first set out to establish the biological activity pattern of pea aphids. We wanted to compare night vs day warming with a behavior that is present at all times of the day rather than only night or day.

To understand the daily reproductive cycle of pea aphids, we observed the number of nymphs each adult laid throughout the day and night for 48 h. Reproductive output was measured with individual adults placed on a fava leaf removed from a grown plant. Leaves were placed in 5cm Petri dishes then secured in a thin layer of agar gel to prevent water loss. Adult pea aphids were selected at random from laboratory colonies, then each was placed on an individual fava leaf. The number of nymphs laid every 8 hours was recorded and removed from the leaves to prevent any potential crowding effects and the experiment was repeated twice.

Petri dishes were placed in walk-in growth chambers (Kysor Sherer model no. CER 55, 1.35 m x 1.5 m x 2.35 m) located in the NDSU Entomology department and monitored every 8 hours. Walk-in chambers ran at 28°C on average during the day and 27°C during the night with a 16:8 L:D cycle. These 8-hour time periods represented morning (6:00am to 2:00pm), afternoon (2:00pm to 10:00pm), and night (10:00pm to 6:00am) which corresponded to the time when the lights were off.

Of the aphids used in this experiment, not all were used in the data analysis. Aphids that died or laid zero nymphs over the course of the experiment were excluded from analyses. We also removed any replicate where the aphid molted during the course of the experiment, as this indicates we started with an older juvenile and not an adult. We did not include the period after the molt, as preliminary results indicate that a recently-molted individual has a lower birth rate over the rest of the experiment compared to the rate of reproduction for other adult aphids. The remaining 39 aphids were analyzed with a repeated measures ANOVA to determine how the number of nymphs each adult laid changed across time periods (morning, afternoon, or night) (JMP 9.0, SAS Institute Inc. 2010). We then combined the morning and afternoon time periods and ran a repeated measures ANOVA to compare the number of nymphs laid during the day and at night.

Aphid Population Growth with Differential Warming Time

In this experiment, we tested how population growth of groups of aphids changed with warming at different times. To do this, climate controlled growth chambers simulated three different warming treatments: a control, day warming, and night warming (Figure 3). We used three climate controlled growth chambers (2 Vemco BOD Low Temp Incubators, model no. 2015 with Watlow EZ-Zone controller and one Conviron model no 125L) with treatments randomly assigned to chambers each replicate block of the experiment. Chambers were set to run repeating 8 hour temperature cycles each day with the first two cycles done in the light and the last one done in the dark for a total 16:8 hour Light:Dark(LD) cycle. Temperature cycles ran at the same morning, afternoon, and night time periods as previously described. The day warming treatment was set to increase 3°C for the 8-hour afternoon period, while the night warming treatment increased 3°C during the night and a control treatment had no increase.

Warming during the day occurred for 8 hours in the afternoon instead of all day to give an equal amount of time warmed between treatments.

Temperatures used were based on data from the North Dakota Agricultural Weather Network (NDAWN) and a metabolically active range for the pea aphid (Mackay et al. 1993, Ma and Ma 2012, Moraiti et al. 2012, NDAWN 2012). Average monthly highs and lows from May to September in Fargo, ND were used to determine the minimum and maximum range of experimental temperatures. The 3°C temperature increase was chosen based on methods from previous research and because it avoids any potential effect of heat shock on aphids (Asin and Pons 2001, Newman 2004, Ma and Ma 2012).

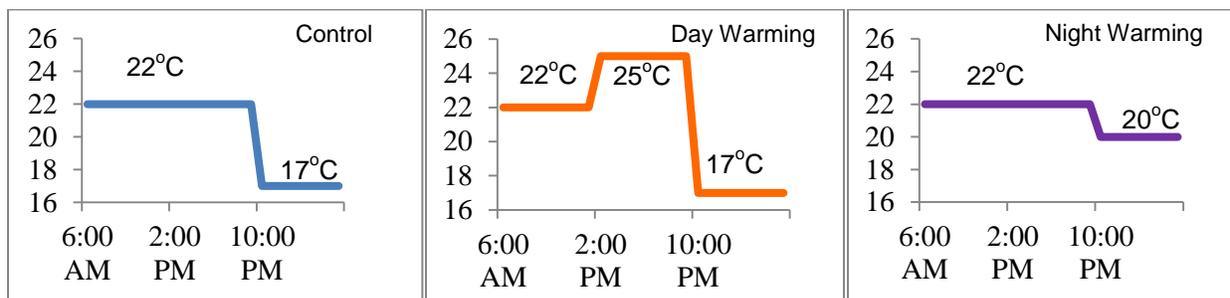


Figure 3. Simulated temperature treatments: Control, Day Warming, and Night Warming. Warming treatments increased 3°C for 8 hours during the afternoon in day warming or overnight in night warming treatments.

A small population of pea aphids were used to test warming effects at different times; fifteen adults and fifteen mixed-age nymphs were placed on a fava plant after the first leaves unfurled and a tube cage housed the plant. Plants were each randomly infested with one population of aphids and assigned to each of the temperature treatments. After 9 days in the chambers, all plants were removed and aphids counted on each plant. Growth chambers were reprogrammed to a different temperature treatment at the beginning of each trial to avoid confounding effects of temperature treatment with any differences in each individual chamber.

Thirty-six populations of aphids were used in the experiment, but one group was left out of analysis due to a ladybug larvae present in the tube cage. The population growth of aphids was compared across warming treatments with ANOVA using the temperature treatment as the independent variable, a blocking factor for the two temporal blocks of the experiment, and the number of aphids on each plant as the dependent variable (JMP 9.0, SAS Institute Inc. 2010).

Reproductive Output of Individuals under Differential Warming Treatments

In this experiment, the birth rate of individual aphids was observed under the same control, day warming, and night warming treatments as the previous experiment (Figure 3). After observing the effect of the three warming treatments on the population growth of aphids, we attempted to determine if the population pattern could be explained by differences in individual birth rates under the same treatments. Birth rate is often a major factor in population growth and may prove to be the reason behind our population growth differences (Newman 2004, Hulle et al. 2010, Jeffs and Leather 2014).

Pea aphid adults were randomly assigned to one of the three treatments and each adult was placed on an excised fava leaf in a 5 cm Petri dish. Whether a leaf is attached to the plant or removed can be important to aphid performance (e.g., Soffan and Aldawood 2014), but a preliminary test comparing individual pea aphid reproduction on attached leaves versus detached leaves under three warming treatments showed no significant differences in the number of nymphs laid. In this experiment, detached fava leaves were kept hydrated in petri dishes with a damp cotton wick, then placed in one of the three treatments (control, day warming, and night warming). Adults reproduced for 2 days while nymphs were counted and removed every 24 hours to prevent any crowding effects.

This experiment was repeated 7 times with a total of 136 aphids after excluding individuals from analysis that molted, laid no nymphs, or died. Aphids then were compared using ANOVA with temperature treatment as the independent variable, a random temporal block, and the number of nymphs each adult laid as the dependent variable (JMP 9.0, SAS Institute Inc. 2010).

Reproductive Output of Individuals under Constant Temperatures

Our day-warming and night-warming treatments were different in several ways. Besides changing when warming occurred, we also changed the absolute temperatures that the aphids experienced. Even though we changed the temperature by the same amount (3°C), warming during the day meant the aphids went from about 22°C to 25°C and warming during the night meant the aphids went from 17°C to 20°C . If the aphids react differently when going from 17°C to 20°C compared to going from 22°C to 25°C , it could explain our population level results.

To further explore the effects of warming on aphid population growth we measured the birth rate of individual aphids under constant temperatures (initially 17°C , 20°C , 22°C and 25°C). Observing how individual birth rates changed at different constant temperatures could give insight to the effects seen at the population level. Specifically, if the observed effects were caused by the actual temperatures used in the treatments (Figure 3) rather than the time warming occurred. Thus, for the day warming we are interested in the difference in reproduction at 22°C (afternoon temperature in the control) vs. 25°C (afternoon temperature in day warmed treatment), and we want to compare that to 17°C (night temperature in the control) vs. 20°C (night temperature in night warmed treatment).

Individual adult aphids experienced constant temperature treatments on a fava leaf in a 5cm petri plate. Leaves were kept hydrated with a damp cotton wick and placed in a climate-

controlled growth chamber. Growth chambers were run at one of four temperature treatments: 17°C, 20°C, 22°C, 25°C. Between trials, each chamber was programmed to a different constant temperature to reduce confounding a temperature treatment with any chamber-specific effects. Nymphs were counted and removed every 24 hours over 2 days to prevent crowding effects. This experiment was repeated 7 times with 374 aphids total after those that molted, died, or laid 0 nymphs were excluded from analysis.

Despite attempting to measure birth rate at each of those 4 temperatures (17, 20, 22, and 25°C), the actual temperatures experienced by the aphids were somewhat variable. The chambers we used were all set to run under the same conditions, but the actual temperatures they ran varied. Across the seven trials of this experiment, we were close to the target temperature for the low temperature (range 16.8-17.3°C, average 17.1°C) and the high temperature (range 24.7-26.4°C, average 25.4°C). However, the medium temperatures were sometimes difficult to distinguish (target 20°C: range 19.5-20.5°C, average 20.0°C; target 22°C range: 20-22.6°C, average 21.0°C). Therefore, we analyzed the results two ways: once by taking the average of the results from the 20°C and 22°C treatments to make a combined “medium” treatment to go along with the low (17°C) and high (25°C) and a second time using the exact temperature experienced in a chamber but still differentiating the two middle treatments. The second analysis gave almost the exact same results as the first, so we focus on just the first analysis for the sake of brevity.

We took a conservative approach to analyzing the results of this experiment by focusing on the average results from each of the seven separate trials of this experiment rather than the individual results from each of the aphids tested within each treatment every time it was run. This approach allowed us to focus on the main point of this experiment, determining how birth rate changed when aphid temperature was changed from low to medium compared to medium to

high, without assuming any particular relationships (linear or otherwise) between temperature and birth rate. We started with an ANOVA using the temperature class as the independent variable and the average nymphs in a temperature class for each of the seven trials as the dependent variable. We then used those same averages to find the difference in birth rate that came from each temperature change associated with a warming treatment (medium to high for day warming vs. low to medium for night warming) and then used a paired t-test to compare the magnitude of these differences.

Finally, because the exact temperatures aphids experienced varied somewhat between trials, we found the average temperature experienced by aphids in each temperature class (low, medium, high) for each trial and then determined the change in birth rate per degree change. This allowed us to account for the actual temperature experienced in each run of the experiment. For example, in trial 1, going from medium to high showed a 4 aphid increase with a 6.1°C increase in temperature, equating to a change of 0.6 aphids per degree. We performed another paired t-test to compare the change in aphids laid per degree increase from high to medium vs. medium to low.

Results

Measuring the Reproductive Activity of the Pea Aphid, *Acyrtosiphon pisum*

The goal of this experiment was to determine the daily activity of pea aphid adults by observing reproductive activity through a 24-hour period. Reproductive activity changed between the morning, afternoon, and night time periods (Figure 4, $F_{2,52} = 5.5$, $p = 0.0066$). The number of nymphs laid by each adult was highest in the morning, decreased through the afternoon, and was lowest overnight. Combining the morning and afternoon time periods showed that reproductive activity was different between “day” and “night” periods. (Figure 5,

$F_{1,53}=10.5, p=0.0021$). Although fewer aphids were laid overnight, reproduction still occurred, therefore making reproductive behavior a potentially useful tool for comparing warming during the day vs. at night.

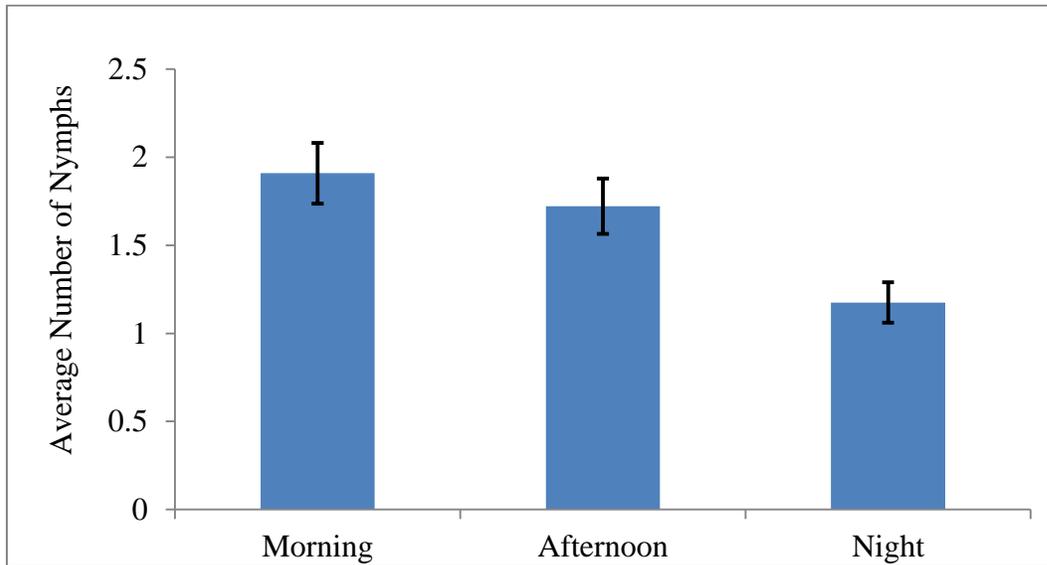


Figure 4. Average number of nymphs laid per adult over three daily time periods (mean ± SE).

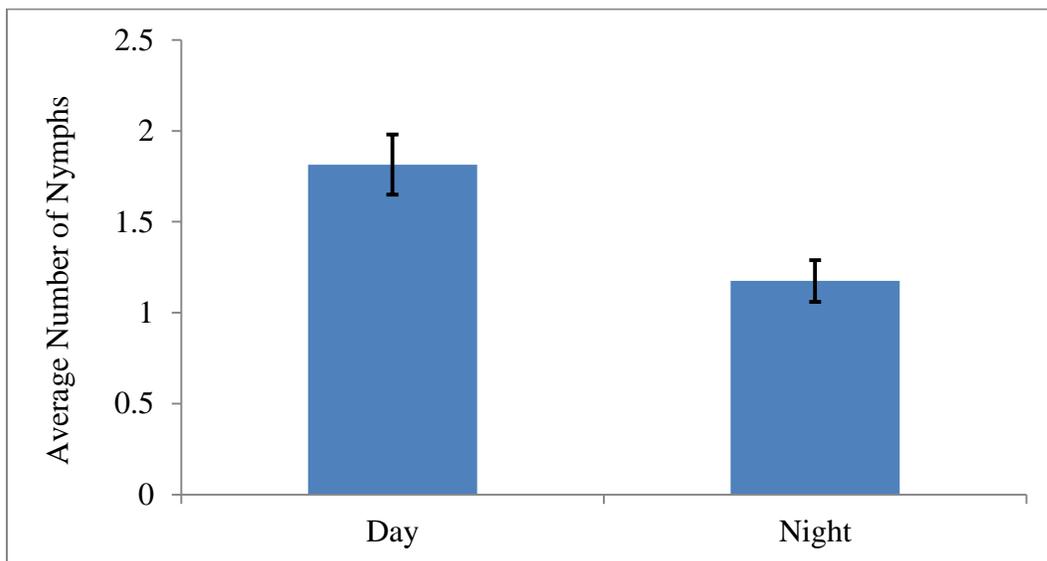


Figure 5. Average number of nymphs laid per adult during the day vs. night time periods (mean ± SE).

Aphid Population Growth with Warming at Different Times

To compare the effects of night warming and day warming, aphids were subjected to three different temperature treatments and population growth was measured. Populations of aphids over 9 days were different between warming treatments (Figure 6, ANOVA $F_{2,31} = 15.6$, $p < 0.001$). Contrasts showed aphid populations in both night warming and day warming treatments were significantly higher than in the control, which was expected because warming in general has been shown to increase aphid populations (Asin and Pons 2001, Lu and Kuo 2008, Moraiti et al. 2012). However, contrasts also show aphids in the night warming treatment experienced significantly greater population growth, resulting in greater populations than those in the day warming treatment ($p < 0.05$).

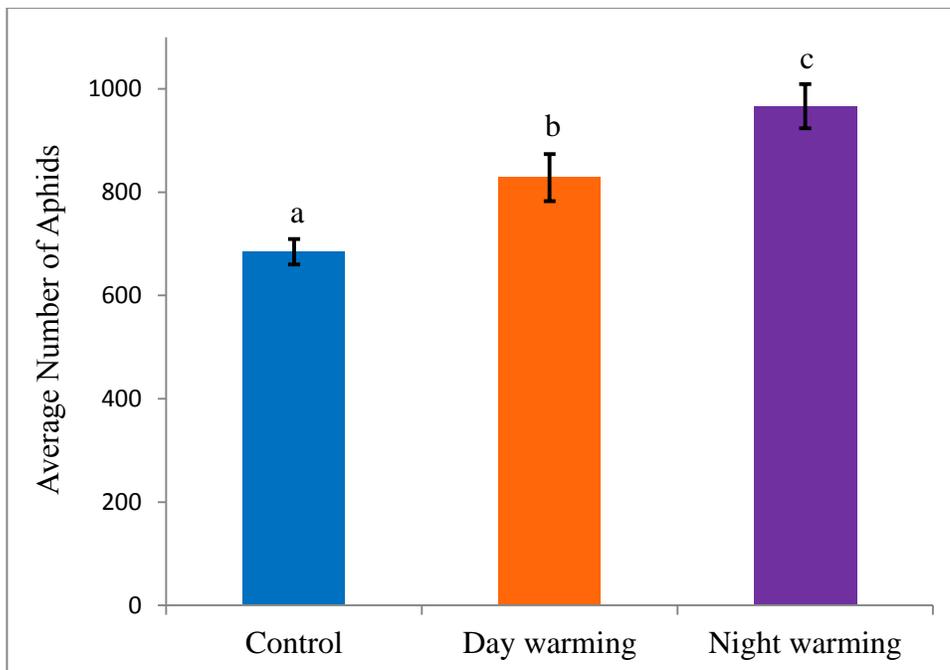


Figure 6. Average number of aphids per plant after experiencing warming at different times (mean \pm SE). Letters show significant difference between treatments. Different letters indicate significantly different means with contrasts at $p < 0.05$

Reproductive Output of Individuals under Differential Warming Treatments

To test the effects of when warming happens on individual reproduction rate, adult aphids were subject to three warming treatments and the number of nymphs laid was measured. Aphid reproduction was different between treatments (Figure 7, $F_{2,130} = 3.45$ $p = 0.0347$). Contrasts between warming treatments showed the day warming and night warming were each significantly different from the control ($p < 0.05$). However, day warming and night warming treatments were not significantly different from one another ($p > 0.05$).

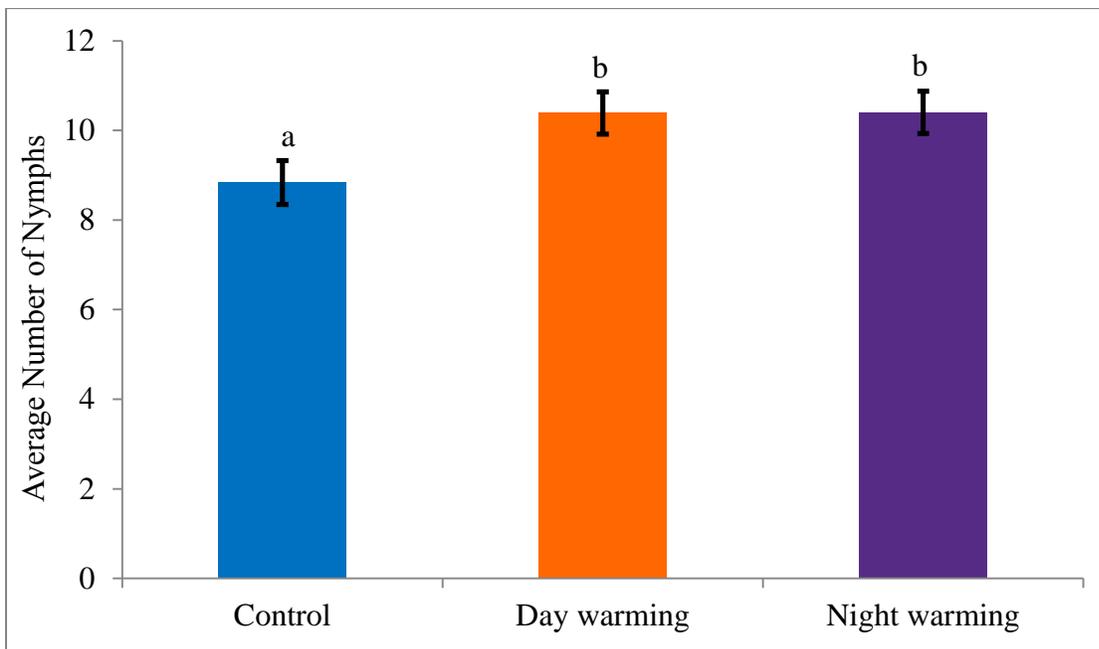


Figure 7. Average number of nymphs laid per adult after experiencing warming at different times (mean \pm SE). Different letters indicate significantly different means with contrasts at $p < 0.05$

Reproductive Output of Individuals under Constant Temperatures

As expected, temperature affected aphid birth rate (Table 1, $F_{2,18} = 4.9$ $p = 0.020$) with the average nymphs per trial increasing with temperature (Lu and Kuo 2008, Stacey et al. 2003, Morgan et al. 2001). When looking at the differences between temperature classes within each trial, there were always more aphids produced in the high temperature compared to the medium

temperature and almost always (6/7 trials) more aphids produced in the medium temperature compared to the lower temperature treatments. Yet, the size of these differences were not the same (paired t-test $t_6=3.5$ $p=0.012$). On average, the difference between high and medium was almost 2.5 times the difference between medium and low. That means that the boost in aphid production was bigger going from medium to high than it was going from low to medium, the opposite of what was needed to explain the population pattern. Even when accounting for the exact temperatures the aphids experienced, the fecundity difference per degree change was twice as big going from medium to high compared with going from low to medium (paired t-test $t_6=3.4$ $p=0.014$).

Table 1. Aphid birthrate parameters in different temperature classes. (a) Average number of nymphs laid in each temperature class per trial and per treatment. (b) Difference in birth rate between temperature classes per trial and on average. (c) Change in birth rate per degree per trial and on average.

(a) Number of Nymphs Laid								
Trial	1	2	3	4	5	6	7	Average
High	13.7	13.1	7.7	10.9	5.6	11.1	12.7	10.7
Medium	9.7	9.9	7.0	8.8	4.5	7.5	8.4	8.0
Low	9.4	9.4	7.4	6.7	4.0	5.8	5.2	6.8
(b) Birth Rate Differences								
High minus medium	4.0	3.2	0.7	2.2	1.1	3.6	4.3	2.7
Medium minus low	0.4	0.5	-0.4	2.1	0.5	1.7	3.2	1.1
(c) Change in Births Rate per Degree								
High minus medium	0.6	0.5	0.1	0.6	0.3	0.8	0.9	0.6
Medium minus low	0.1	0.2	-0.1	0.5	0.1	0.5	1.0	0.3

Discussion

Despite the potential importance of warming at night, studies comparing warming during the day to at night are lacking. Therefore, we set out to examine how warming at different times affects the population growth of an insect. For this comparison to be as relevant and likely as

possible, we needed to make sure the pea aphid would demonstrate a potential behavior or demographic activity during the day and night. After observing the number of offspring laid over a 24-hour period, we concluded that reproduction occurred through the night and pea aphids were continuously active, even if the magnitude of activity varied.

Testing the effects of warming at night compared to day showed that pea aphid population growth was higher due to night warming compared to warming during the day, and both warming treatments were higher than the control. Higher average temperatures often cause higher population growth rates in pea aphids (Asin and Pons 2001, Walther et al. 2002, Holopainen and Kainulainen 2004); however, our results show that the timing of warming can also play a significant role in determining the population level response. These results are somewhat surprising based on the daily activity of pea aphids. In this instance, warming at night, when biological activity is lower, caused a greater increase in population growth over warming at a time with more reproductive activity.

To better understand the difference in night warming vs. day warming, we used our same treatments to test one particular potential demographic mechanism, birth rate. Warming, no matter the time, increased the number of nymphs laid, but we saw no difference between day and night warming. This experiment seems to suggest that while higher temperatures may play a role in greater population growth over the control, birth rate doesn't seem to explain the difference between night and day warming. This is inconsistent with the observed population level effects of night warming, but those results could be explained by other demographic mechanisms of population growth such as development or death rates. Alternatively, it is possible that the differences among treatments at the population level are not observable at the individual level. For example, interactions with other aphids involving competition for food or space would not

be seen with individuals in our experimental setup (Barton and Ives 2014). Effects from competition or crowding could have been a factor in our population level experiment, but would not have affected aphids at the individual level.

Differences in plant quality and response may also be overlooked with individuals on removed leaves as compared to populations on whole plants. Although preliminary testing showed no difference between fecundity on whole plants when compared to excised leaves, there could have been plant effects in the longer population experiment. Pea aphid activity was still present at night, but plant processes change between night and day (Kramer and Decker 1944). Warming at different times could affect the processes at day differently than those at night, which could lead to a difference in treatment effects. Because photosynthesis occurs with light and respiration occurs in darkness, warming during the day and night would affect these processes differently. Generally, respiration is more sensitive to temperature changes than photosynthesis (Gifford 1995), thus warming at night would have a greater effect on respiration than warming during the day would on photosynthesis. This change in respiration may alter plant chemistry in a way that benefits aphids and could potentially explain the greater population growth with our night warming treatments.

The most straightforward explanation for our population level pattern is that certain demographic rates of the aphid change when warming is happening during the day vs. when at night; however an alternative explanation for our overall pattern is that pea aphids responded to the absolute temperature they experienced. We chose temperatures that seem to be in the normal range for pea aphids (Mackay et al. 1993, Lu and Kuo 2008, Hulle et al. 2010), but it is possible that experiencing a maximum of 25°C in the day warming treatment (22°C day temperature + 3°C increase) elicited a different response than experiencing a maximum of 20°C in the night

warming treatment (17°C night temperature +3°C increase). To test the response of our aphids, we looked at the difference in fecundity when aphids were kept at one of those constant temperatures. Generally speaking, offspring numbers increased with temperature, which is the standard result for these types of thermal performance curves, at least until temperatures get too warm (Lamb et al. 1987, Stacey et al. 2003, Lu and Kuo 2008, Ma and Ma 2012). The question is what the shape of that increase was. If fecundity was increasing but starting to hit an asymptote at the higher temperatures, the overall shape would start to be concave and we would expect that warming from 22 to 25°C would give less of a benefit than warming from 17 to 20°C. However, this was not the case. We actually saw that on an individual level, there was a larger benefit of going from 22 to 25°C. If this pattern would have held at the population level, we would have seen higher populations for the day warming treatment than the night warming because of the effect of the absolute temperatures. Instead we saw the opposite effect which could be because of the variable temperatures used and its effects on population growth. We cannot completely rule out that absolute temperature is important for producing the population level response (Figure 6) as our follow up experiment seemed to indicate that measuring fecundity alone as the response variable (Figure 7) may not elicit the same response as testing full populations (Figure 6). Potentially, increasing nightly temperatures could have raised the reproductive output of the aphids at night, extended the time when aphids are more active, or increased the reproductive activity during the day. In addition, the development rate of aphids is expected to be faster at higher temperatures than lower temperatures (Morgan et al. 2001, Stacey et al. 2003, Lu and Kuo 2008, Moraiti et al. 2012) but night warming populations were greater despite a lower maximum temperature experienced.

Our research shows how night warming effects on one insect species; however, there are implications for other species and interspecies interactions. We know populations are constantly changing through interactions with other species, and night warming has the potential to influence these interactions (Harmon et al. 2009, Barton et al. 2009). For example, certain behaviors are regulated by temperature; therefore, night warming can increase or decrease such behaviors. A species that is less active or inactive at night may increase its activity or now become active with warmer night temperatures. One example of a species that would benefit from warmer nights is the pea aphid parasitoid *Aphidius ervi*. Walking speed of *A.ervi* is correlated to temperature and an increase from 17°C to 20°C would actually double movement speed which could allow for greater parasitism rates (Gilchrist 1996). Conversely, a species active at night due to cooler temperatures may decrease its activity with higher temperatures if the upper thermal limit is reached. This means that dynamics between diurnal and nocturnal species could overlap with time and become more complex.

Increasing temperature related to climate change has been shown to have significant effects on insects (Walther et al. 2002, Bradshaw and Holzapfel 2006, Barton 2010, Jeffs and Leather 2014), but our results expand this effect to demonstrate that when the increase happens can also be important. Studies have shown significant effects from warming at night (Whitney-Johnson et al. 2005, Battisti et al. 2006, Scriber and Sonke 2011, Wu et al. 2012 Zhao et al. 2014), but we set out to directly compare the effects of night warming and day warming. Our results support the importance of night warming, but also show how its effects can vary depending on the life history trait in question or scale of focus in a pea aphid system. Thus, it is beneficial to understand the many ways that night warming may affect different organisms and ecosystems as climate change research continues and integrates other abiotic factors.

REFERENCES

- Alward, R. D., J. K. Detling, and D. G. Milchunas. 1999. Grassland vegetation changes and nocturnal global warming. *Science* **283**:229-231.
- Asin, L., and X. Pons. 2001. Effect of high temperature on the growth and reproduction of corn aphids (Homoptera : Aphididae) and implications for their population dynamics on the northeastern Iberian peninsula. *Environmental Entomology* **30**:1127-1134.
- Bai, W. M., J. Y. Xia, S. Q. Wan, W. H. Zhang, and L. H. Li. 2012. Day and night warming have different effect on root lifespan. *Biogeosciences* **9**:375-384.
- Barton, B. T. 2010. Climate warming and predation risk during herbivore ontogeny. *Ecology* **91**:2811-2818.
- Barton, B. T. 2014. Reduced wind strengthens top-down control of an insect herbivore. *Ecology* **95**:2375-2381.
- Barton, B. T., A. P. Beckerman, and O. J. Schmitz. 2009. Climate warming strengthens indirect interactions in an old-field food web. *Ecology* **90**:2346-2351.
- Barton, B. T., and A. R. Ives. 2014. Species interactions and a chain of indirect effects driven by reduced precipitation. *Ecology* **95**:486-494.
- Battisti, A., M. Stastny, E. Buffo, and S. Larsson. 2006. A rapid altitudinal range expansion in the pine processionary moth produced by the 2003 climatic anomaly. *Global Change Biology* **12**:662-671.
- Blackman, R. L., and V. F. Eastop. 2000. Aphids on the world's crops: an identification and information guide. Second edition. Aphids on the world's crops: an identification and information guide. Second edition.:i-x, 1-466.

- Bradshaw, W. E., and C. M. Holzapfel. 2006. Climate change - Evolutionary response to rapid climate change. *Science* **312**:1477-1478.
- Burdick, S.C. 2013. Interactions between ultraviolet light and soybean aphid, *Aphis glycines* Matsumura (Hemiptera: Aphididae). Thesis, North Dakota State University, Fargo, North Dakota, USA.
- Charles, H., and H. Ishikawa. 1999. Physical and genetic map of the genome of Buchnera, the primary endosymbiont of the pea aphid *Acyrtosiphon pisum*. *Journal of Molecular Evolution* **48**:142-150.
- Clarke, D. N., and P. A. Zani. 2012. Effects of night-time warming on temperate ectotherm reproduction: potential fitness benefits of climate change for side-blotched lizards. *Journal of Experimental Biology* **215**:1117-1127.
- Deutsch, C. A., J. J. Tewksbury, R. B. Huey, K. S. Sheldon, C. K. Ghalambor, D. C. Haak, and P. R. Martin. 2008. Impacts of climate warming on terrestrial ectotherms across latitude. *Proceedings of the National Academy of Sciences of the United States of America* **105**:6668-6672.
- Dore, M. H. I. 2005. Climate change and changes in global precipitation patterns: What do we know? *Environment International* **31**:1167-1181.
- Gifford, R.M. 1995. Whole plant respiration and photosynthesis of wheat under increased CO₂ concentration and temperature: long-term vs. short-term distinctions for modeling. *Global Change Biology* **1**: 385-396.
- Gilchrist, G. W. 1996. A quantitative genetic analysis of thermal sensitivity in the locomotor performance curve of *Aphidius ervi*. *Evolution* **50**:1560-1572.

- Harmon, J. P., and B. T. Barton. 2013. On their best behavior: how animal behavior can help determine the combined effects of species interactions and climate change. *Climate Change and Species Interactions: Ways Forward* **1297**:139-147.
- Harmon, J. P., N. A. Moran, and A. R. Ives. 2009. Species Response to Environmental Change: Impacts of Food Web Interactions and Evolution. *Science* **323**:1347-1350.
- Hofmann, G. E., and A. E. Todgham. 2010. Living in the Now: Physiological Mechanisms to Tolerate a Rapidly Changing Environment. Pages 127-145 *Annual Review of Physiology*. Annual Reviews, Palo Alto.
- Holopainen, J. K., and P. Kainulainen. 2004. Reproductive capacity of the grey pine aphid and allocation response of Scots pine seedlings across temperature gradients: a test of hypotheses predicting outcomes of global warming. *Canadian Journal of Forest Research-Revue Canadienne De Recherche Forestiere* **34**:94-102.
- Hulle, M., A. Coeur d'Acier, S. Bankhead-Dronnet, and R. Harrington. 2010. Aphids in the face of global changes. *Comptes Rendus Biologies* **333**:497-503.
- International Aphid Genomics Consortium. 2010. Genome Sequence of the Pea Aphid *Acyrtosiphon pisum*. *Plos Biology* **8**.
- Jeffs, C. T., and S. R. Leather. 2014. Effects of extreme, fluctuating temperature events on life history traits of the grain aphid, *Sitobion avenae*. *Entomologia Experimentalis Et Applicata* **150**:240-249.
- Kelber, A., A. Balkenius, and E. J. Warrant. 2002. Scotopic colour vision in nocturnal hawkmoths. *Nature* **419**:922-925.

- Kingsolver, J. G., S. E. Diamond, and L. B. Buckley. 2013. Heat stress and the fitness consequences of climate change for terrestrial ectotherms. *Functional Ecology* **27**:1415-1423.
- Kramer, P.J., Decker, J.P. 1944. Relation between light intensity and rate of photosynthesis of loblolly pine and certain hardwoods. *Plant Physiology* **19**:350-358.
- Lamb, R. J., P. A. Mackay, and G. H. Gerber. 1987. Are Development and growth of pea aphids, *Acyrtosiphon pisum*, in North-America adapted to local temperatures. *Oecologia* **72**:170-177.
- Le Trionnaire, G., S. Jaubert-Possamai, J. Bonhomme, J. P. Gauthier, G. Guernec, A. Le Cam, F. Legeai, J. Monfort, and D. Tagu. 2012. Transcriptomic profiling of the reproductive mode switch in the pea aphid in response to natural autumnal photoperiod. *Journal of Insect Physiology* **58**:1517-1524.
- Lu, W.-N., and M.-H. Kuo. 2008. Life table and heat tolerance of *Acyrtosiphon pisum* (Hemiptera : Aphididae) in subtropical Taiwan. *Entomological Science* **11**:273-279.
- Ma, G., and C. S. Ma. 2012. Climate warming may increase aphids' dropping probabilities in response to high temperatures. *Journal of Insect Physiology* **58**:1456-1462.
- Mackay, P. A., R. J. Lamb, and M. A. H. Smith. 1993. Variability in life-history traits of the aphid, *Acyrtosiphon pisum* (Harris), from sexual and asexual populations. *Oecologia* **94**:330-338.
- McCarty, J. P. 2001. Ecological consequences of recent climate change. *Conservation Biology* **15**:320-331.

- McKenzie, R. L., P. J. Aucamp, A. F. Bais, L. O. Bjoern, M. Ilyas, and S. Madronich. 2011. Ozone depletion and climate change: impacts on UV radiation. *Photochemical & Photobiological Sciences* **10**:182-198.
- Moraiti, C. A., J. T. Margaritopoulos, K. D. Zarpas, and J. A. Tsitsipis. 2012. The grapevine aphid, *Aphis illinoisensis*: thermal requirements for development and its performance on six grapevine cultivars. *Bulletin of Insectology* **65**:29-35.
- Morgan, D., K. F. A. Walters, and J. N. Aegerter. 2001. Effect of temperature and cultivar on pea aphid, *Acyrtosiphon pisum* (Hemiptera : Aphididae) life history. *Bulletin of Entomological Research* **91**:47-52.
- NDAWN. 2012. Historical data archives. North Dakota Agricultural Weather Network. North Dakota State University.
- Newman, J. A. 2004. Climate change and cereal aphids: the relative effects of increasing CO₂ and temperature on aphid population dynamics. *Global Change Biology* **10**:5-15.
- Oehme, V., P. Hoegy, C. P. W. Zebitz, and A. Fangmeier. 2013. Effects of elevated atmospheric CO₂ concentrations on phloem sap composition of spring crops and aphid performance. *Journal of Plant Interactions* **8**:74-84.
- Paaijmans, K. P., R. L. Heinig, R. A. Seliga, J. I. Blanford, S. Blanford, C. C. Murdock, and M. B. Thomas. 2013. Temperature variation makes ectotherms more sensitive to climate change. *Global Change Biology* **19**:2373-2380.
- Parmesan, C. 2006. Ecological and evolutionary responses to recent climate change. *Annual Review of Ecology Evolution and Systematics* **37**:637-669.
- Parmesan, C., and G. Yohe. 2003. A globally coherent fingerprint of climate change impacts across natural systems. *Nature* **421**:37-42.

- Peng, S. B., J. L. Huang, J. E. Sheehy, R. C. Laza, R. M. Visperas, X. H. Zhong, G. S. Centeno, G. S. Khush, and K. G. Cassman. 2004. Rice yields decline with higher night temperature from global warming. *Proceedings of the National Academy of Sciences of the United States of America* **101**:9971-9975.
- Peng, S. S., S. L. Piao, P. Ciais, R. B. Myneni, A. P. Chen, F. Chevallier, A. J. Dolman, I. A. Janssens, J. Penuelas, G. X. Zhang, S. Vicca, S. Q. Wan, S. P. Wang, and H. Zeng. 2013. Asymmetric effects of daytime and night-time warming on Northern Hemisphere vegetation. *Nature* **501**:88-+.
- Rustad, L. E., J. L. Campbell, G. M. Marion, R. J. Norby, M. J. Mitchell, A. E. Hartley, J. H. C. Cornelissen, J. Gurevitch, and N. Gcte. 2001. A meta-analysis of the response of soil respiration, net nitrogen mineralization, and aboveground plant growth to experimental ecosystem warming. *Oecologia* **126**:543-562.
- Scriber, J. M., and B. Sonke. 2011. Effects of diurnal temperature range on adult size and emergence times from diapausing pupae in *Papilio glaucus* and *P. canadensis* (Papilionidae). *Insect Science* **18**:435-442.
- Sharma, H. C., and C. S. Prabhakar. 2014. Impact of Climate Change on Pest Management and Food Security. *Integrated Pest Management: Current Concepts and Ecological Perspective*:23-36.
- Snyder, W. E., and A. R. Ives. 2003. Interactions between specialist and generalist natural enemies: Parasitoids, predators, and pea aphid biocontrol. *Ecology* **84**:91-107.
- Soffan, A., and A. S. Aldawood. 2014. Biology and demographic growth parameters of cowpea aphid (*Aphis craccivora*) on faba bean (*Vicia faba*) cultivars. *Journal of Insect Science* **14**.

- Stacey, D. A., M. B. Thomas, S. Blanford, J. K. Pell, C. Pugh, and M. D. E. Fellowes. 2003. Genotype and temperature influence pea aphid resistance to a fungal entomopathogen. *Physiological Entomology* **28**:75-81.
- Sunday, J. M., A. E. Bates, and N. K. Dulvy. 2011. Global analysis of thermal tolerance and latitude in ectotherms. *Proceedings of the Royal Society B-Biological Sciences* **278**:1823-1830.
- Trenberth, K. E. 2011. Changes in precipitation with climate change. *Climate Research* **47**:123-138.
- Volkl, W., J. Woodring, M. Fischer, M. W. Lorenz, and K. H. Hoffmann. 1999. Ant-aphid mutualisms: the impact of honeydew production and honeydew sugar composition on ant preferences. *Oecologia* **118**:483-491.
- Walther, G.-R., A. Roques, P. E. Hulme, M. T. Sykes, P. Pysek, I. Kuehn, M. Zobel, S. Bacher, Z. Botta-Dukat, H. Bugmann, B. Czucz, J. Dauber, T. Hickler, V. Jarosik, M. Kenis, S. Klotz, D. Minchin, M. Moora, W. Nentwig, J. Ott, V. E. Panov, B. Reineking, C. Robinet, V. Semchenko, W. Solarz, W. Thuiller, M. Vila, K. Vohland, and J. Settele. 2009. Alien species in a warmer world: risks and opportunities. *Trends in Ecology & Evolution* **24**:686-693.
- Walther, G. R., E. Post, P. Convey, A. Menzel, C. Parmesan, T. J. C. Beebee, J. M. Fromentin, O. Hoegh-Guldberg, and F. Bairlein. 2002. Ecological responses to recent climate change. *Nature* **416**:389-395.
- Warrant, E. J., A. Kelber, A. Gislén, B. Greiner, W. Ribi, and W. T. Weislo. 2004. Nocturnal vision and landmark orientation in a tropical halictid bee. *Current Biology* **14**:1309-1318.

- Whitney-Johnson, A., M. Thompson, and E. Hon. 2005. Responses to predicted global warming in *Pieris rapae* L. (Lepidoptera): Consequences of nocturnal versus diurnal temperature change on fitness components. *Environmental Entomology* **34**:535-540.
- Williamson, C. E., R. G. Zepp, R. M. Lucas, S. Madronich, A. T. Austin, C. L. Ballare, M. Norval, B. Sulzberger, A. F. Bais, R. L. McKenzie, S. A. Robinson, D.-P. Haeder, N. D. Paul, and J. F. Bornman. 2014. Solar ultraviolet radiation in a changing climate. *Nature Climate Change* **4**:434-441.
- Wu, T., S. Hao, O. J. Sun, and L. Kang. 2012. Specificity Responses of Grasshoppers in Temperate Grasslands to Diel Asymmetric Warming. *Plos One* **7**.
- Xia, J. Y., S. P. Chen, and S. G. Wan. 2010. Impacts of day versus night warming on soil microclimate: Results from a semiarid temperate steppe. *Science of the Total Environment* **408**:2807-2816.
- Xie, H., L. Zhao, W. Wang, Z. Wang, X. Ni, W. Cai, and K. He. 2014. Changes in Life History Parameters of *Rhopalosiphum maidis* (Homoptera: Aphididae) Under Four Different Elevated Temperature and CO₂ Combinations. *Journal of Economic Entomology* **107**:1411-1418.
- Zhao, F., W. Zhang, A. A. Hoffmann, and C. S. Ma. 2014. Night warming on hot days produces novel impacts on development, survival and reproduction in a small arthropod. *Journal of Animal Ecology* **83**:769-778.