EFFECTS OF HEAT WAVE TIMING ON PLANT, HERBIVORE, DISEASE INTERACTIONS

By

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ABSTRACT

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Climate change research has primarily focused on the consequences of increasing average global temperature. However, extreme weather events are another consequence of climate change that has been harder to examine because of their rarity and unpredictability. Heat waves are brief events of extreme heat that pose an immediate threat to food security and agricultural sustainability and are predicted to increase exponentially in frequency over the next fifty years. The effect of heat stress on single organisms are well known, but we have a poor understanding of how the timing of heat wave events influence plant-insect-disease interactions in agroecosystems. In this thesis, I address two primary research questions: 1) How does timing of a heat wave impact crop-pest interactions? 2) How are multi-species interactions influenced by heat waves? I used environmental chambers in the lab and open-top chambers fitted with infrared heaters in the field to simulate heat wave conditions at early and late development stages of potato (*Solanum tuberosum*) inoculated with an associated herbivore the Colorado potato beetle (CPB, *Leptinotarsa decemlineata, (Say)* (Coleoptera: Chrysomelidae)) and foliar disease early blight caused by (*Alternaria solani)*. I found that heat waves increased CPB larval mass by 26-44% and sped up development time, suggesting there could be an additional generation of herbivores per season. Heat waves combined with herbivore and disease stress caused 20-40% lower yield on potatoes in early reproductive stages. Actions should be taken to protect crops in early reproductive stages from stress through diligent irrigation practices and more frequent scouting to inform pest management strategies prior to and during heat waves.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1: Effects of heat wave timing on plant, herbivore, disease interactions

INTRODUCTION

While a gradual increase in global mean temperatures is a widely appreciated aspect of climate change, concomitant increases in climatic variability and extreme weather events pose a much more immediate threat to humans and the resources we depend on (Bisbis et al., 2018; Meehl and Tebaldi 2004; Rahmstorf and Coumou 2011; Fischer and Knutti 2015; Perkins-Kirkpatrick and Gibson 2017; Price 2020). Drought and heat waves are predicted to impact 11% and 10% of global crop and grassland with Europe, Africa and the Middle East, and Asia being areas hardest hit (Myers et al., 2017). Technological advancements in agriculture like large scale irrigation systems have reduced the damage of heat waves on agriculture but growing enough crops to sustain an increasing human population is a major challenge of the modern world, with food scarcity being a widespread problem (IPCC, 2014). New farming practices and resilient crop varieties are constantly being developed to maintain optimal productivity of the landscape. However, most food crops are produced in specialized monocultures making them especially susceptible to disease and herbivore pest outbreaks, as well as extreme events like flooding, drought, and heat waves. Millions of dollars are spent controlling agricultural pests every year yet much of how extreme events like heat waves affect pest management is largely unknown (Alyokhin, 2009).

Growth chamber studies exposing crops, insects, and pathogens to a temperature gradient identifies their thermal minima, optima, and maxima, but predictive models derived from such studies typically fail to incorporate fluctuating temperatures and interspecific interactions (Ferro et al. 1985; Chaerani & Voorrips, 2006; Logan et al., 1985; Levy & Veilleux, 2007). Daily and seasonal fluctuations create an ever-changing environment in which organisms must live and adapt, with some organisms exhibiting behavioral movements to avoid stressful temperatures and intense sunlight, making it difficult to predict the outcome of an extreme event on mobile organisms. Plants also have mechanisms to avoid the negative effects of heat stress by lowering leaf temperatures through transpiration, but periods of low precipitation preceding and accompanying heat waves reduce available soil moisture limiting this protective process (Robinson 2001). The combination of high heat and low precipitation have caused massive yield losses leading to development of highly sophisticated irrigation systems to combat heat and drought conditions (Moriondo & Bindi, 2007). This technology has greatly reduced yield loss of crops like potatoes but this is not a sustainable solution.

The timing of an extreme event could drastically alter the impacts of the event on phytopathogens, insect herbivores, and their host plants, depending on when the event occurs during the ontogeny of each organism and the temporal sequence of their interactions (Yang and Rudolf 2010). A deeper understanding of the timing of heat waves on the interaction between plants and their pests is vital for maintaining sustainable agriculture and resilient natural systems. This thesis addressed three primary research questions: 1) what are the direct and indirect effects of heat waves on plant–insect interactions?; 2) how does timing of a heat wave alter its impacts on plant–pest interactions?; and 3) how do heat waves alter inset pest, disease pest, host plant interactions?

To address the first question, I conducted a growth chamber experiment to identify the direct and indirect effects of heat waves on plant-insect interactions. With clear direct and indirect effects of heat waves on CPB survival and potato yield identified, next I developed a field experiment that exposed four different age classes of potato to both heat wave and herbivore pressure to identify the role of heat wave timing on plant insect interactions. I used the specialist herbivore CPB in our experiments. I chose the larval life stages to use in my experiments because they can be found on plants soon after emergence through till plant senescence, while adult beetle abundance is more variable. Larvae also lack the hardened exoskeleton of adults making them more susceptible to desiccation from heat. Lastly, in the summer of 2019, I incorporated potato early blight, which is a common foliar disease, into a fully crossed field experiment that identified the impact of heat waves on potato (*Solanum tuberosum*), insect herbivore (CPB), and disease interaction. *Alternaria solani* is a fungal pathogen that affects solanaceous plants like potato, tomato, pepper, and eggplant as well as other wild night shade species (Thomma, 2003). It persists in dead plant tissue from previous year's crop and also in solanaceous weeds around field margins. Its spores are transferred by wind and rain droplet splash to new host plants. Young solanaceous plants however are considered resistant until near flowering when plant resources begin to be used for reproduction (Tsedaley, 2014). I focused the experiment around early reproductive life stages of potato tuber initiation and early tuber bulking when the disease begins to infect the lower leaves (Douglas, 1972). These experiments combined identify key life stages of potato that are especially susceptible to heat waves and herbivores, and gain insight into complex interactions with plant disease that will aide in the preservation of sustainable agriculture in the face of a changing climate.

CHAPTER 2: The effect of heat wave timing on plant-herbivore interactions

ABSTRACT

Brief periods of intense heat and decreased precipitation (heat waves) are becoming more common globally. The immediate negative effects of heat stress on plants and insects are well understood, but we have a poor understanding of the potentially lasting ecological effects of heat waves on plant-insect interactions. An understanding of how heat waves alter the dynamics of plant-herbivore interactions in agroecosystems would improve our ability to manage pests in an increasingly variable climate. I examined how heat waves influence the growth and development of potatoes and the associated herbivore CPB. I experimentally imposed heat wave conditions at multiple times during potato ontogeny in the lab in environmental chambers and in the field using open-top chambers and ceramic heating elements. I found that heat waves decreased CPB larval survival on young plants. Moreover, survival was also reduced for larvae that fed on plants that had previously experienced a heat wave, even if the larvae themselves did not experience the heat wave. Despite the negative consequences of heat waves for CPB performance, potato plants in early reproductive life stages exposed to both CPB herbivory and heat waves had significantly reduced yield. Our results indicate that even though heat waves may reduce the performance of insect pests, they may also act interactively with the stress of herbivore damage to worsen the effects of pests on plant growth. This suggests that best management practices should prioritize reducing CPB populations prior to forecasted heat wave events especially for potatoes in early reproductive stages.

INTRODUCTION

While climate change biology has mainly focused on gradual changes in mean temperatures (Bale 2002; Dyer and Letourneau 2013; Leisner et al. 2018; Brown et al. 2004; Harrington et al. 1999; Parmesan and Yohe 2003), there has been less attention on the impacts of extreme weather events, including heat waves, which have been increasing in frequency, duration and intensity in recent years (Meehl and Tebaldi 2004; Rahmstorf and Coumou 2011; Fischer and Knutti 2015; Perkins-Kirkpatrick and Gibson 2017). While there is no widely accepted single definition of a heat wave, for purposes of this work we describe them as extreme and infrequent events with ≥ 2 d of stressfully high temperatures. Heat waves have been estimated to have occurred less than 4 times per decade per region of the U.S. in 1951–1990 but they are predicted to become more common in many parts of the world (Robinson 2001).

Heat waves are important not only for their impacts on urban environments and natural ecosystems but also because they have potential impacts on food security, especially since major food crops in the world are produced in specialized monocultures. These agroecosystems are fine-tuned to produce maximal yields and often lack resilience to rebound after extreme weather events. Because they result in extreme conditions that may exceed some physiological tolerances, they can disrupt ecological processes (Smith 2011) in ways that may alter pest control. Indeed, extreme weather has already had severe negative impacts on agriculture (Bisbis et al. 2018), including the reduction of national cereal production by 9.1% by heat waves during 1964–2007 (Lesk et al. 2016). These reductions can be the result of direct and indirect effects on interacting plants and their herbivores in crop fields. Predicting the net effect of an extreme event on pest control requires examination of how the individual effects of an event combine and interact across trophic levels. To do this we need studies that explore both the direct, physiological effects of events on individual organisms and the ecological effects that occur when organisms interact in field settings.

A major challenge to understanding how heat waves affect crop-pest interactions is that the sensitivity of organisms likely changes through different life stages. Heat waves occurring at different life stages could have different consequences for the plant and herbivores feeding on it (Yang and Rudolf 2010), and potentially change what crop or pest management practices should be followed in the wake of a heat wave. For example, soybeans and corn responded differently to heat stress in certain life stages than others (Siebers et al. 2015, 2017). Heat stress also had varying effects on different life stages of aphids, with younger nymphs more susceptible than older instars and adults (Chiu et al. 2015; Zhang et al. 2015). However, previous studies often did not incorporate the daily variation in temperature between night and day, which is key to linking research results to practical predictions (Dowd et al. 2015).

I examined the impacts of heat waves and their timing on a crop–insect pest interaction using both laboratory and field experiments that exposed multiple crop life stages to heat wave conditions in the presence or absence of herbivores. I did this using the specialist insect herbivore CPB and its host plant, the potato. I used growth chambers to create heat waves in the laboratory and open top chambers fitted with ceramic heaters to simulate heat waves over field-grown potatoes. I aimed to identify the potato life stages that are most susceptible to heat stress and how beetle survival and weight are impacted by feeding on heat-stressed plants.

METHODS

Experimental organisms

For both lab and field experiments, we grew potato plants from certified seed potatoes (*Solanum tuberosum* cv. 'Atlantic'). Prior to planting, seed potatoes were cut and allowed to suberize overnight for the growth chamber experiment and for 7 d for the field experiment (~40 g per seed piece for lab experiments and ~64 g for field experiment). For all experiments, we obtained CPB from a lab colony reared on potatoes (cv. 'Atlantic'). The colony was started with individuals collected from a potato field in Stanton, Michigan in 2017 and kept in continuous culture in the laboratory (16L:8D, 23ºC).

Growth chamber experiment

In a growth chamber experiment (01/2018 – 06/2018) I investigated the effects of heat stress and its timing on potatoes, larvae, and their interaction. This experiment exposed 156 potato plants to either constant control conditions, an early heat wave, or a late heat wave (52 per heat wave treatment), allowing me to examine the effects of heat wave timing. I added three 1st instar beetle larvae to half of the plants in each treatment, making six total treatments with 26 plants per treatment. The larvae were added all at the same time, after the early heat wave but before the late heat wave, so that some larvae fed on plants that already experienced a heat wave, while another group of larvae fed on plants during a heat wave. This allowed the determination of direct and indirect effects of heat stress on the larvae and their interaction with the plants.

I started the experiment by planting 200 potato seed pieces into 450 ml round plastic pots filled with soil (Suremix perlite, Galesburg, MI) and placing them into a growth chamber (BioChambers Inc., Model FxC19; Winnipeg, Canada). This chamber—the constant conditions control chamber—was set to 16L: 8D photocycle, 26ºC during the day, and 14ºC at night, which are the average June temperatures for Southern Michigan (Coldwater, MI weather station; MSU Enviroweather). Light levels were measured at bench level and set to 170 µmol $m^{-2}s^{-1}$ using a light meter (Apogee Instruments, Inc., Logan, UT). I used a 2 h linear ramp between day and night temperatures to prevent sudden heat shocks, resulting in 12 h of day and 8 h of night temperatures. Humidity was set at 40% and fan speed was at 75%. When plants were 2 weeks old, we selected 156 fully emerged plants of similar size to be used in the growth chamber experiment. Trays of plants were rotated 180 degrees and to different benches within the chamber on watering days to avoid uneven conditions. All plants were watered three times per week (25 ml of water/plant). I fertilized once per week with 25 ml of 750 ppm 20-20-20 N-P-K solution (Jack's Professional, J. R. Peters Inc., Allentown, PA).

When plants were 45 d old in the vegetative growth phase, I applied the early heat wave treatment on 51 plants by moving them from the control chamber to a second chamber set to heat wave conditions. This chamber was set to simulate heat waves with a 40ºC day maximum and a 26ºC night minimum. I chose these conditions because an analysis of 1897–2016 temperature data from Michigan (Coldwater, MI weather station) indicated such an event was in the 99th percentile of past events, and these rare events are predicted to become 8–20 times more likely after 3°C of mean warming (Fischer and Knutti 2015). The heat wave lasted for 4 d, after which plants were moved back to the control chamber. Neither control nor treatment plants received fertilizer during heat waves to minimize negative effects of the fertilizer during a heat stress event (Slafer & Savin 2018).

When plants were 56 d old, 7 d after the end of the early heat wave treatment, I placed three 1st instars on half of all the plants in control, early heat wave, and late heat wave treatments. This delayed placement allowed us to detect the lasting impact of the early heat wave on plant nutritional quality while not exposing those larvae to the direct heat stress. Conversely, the larvae in the late heat wave treatment did experience the extreme heat conditions. Larvae were contained within bags (California Containers, 19 L, 200-micron white Nylon mesh material) placed over each plant. A 38-cm bamboo stake was placed in the soil to support the bag and the plant; the bag was twist-tied closed around the base of the plant and bottom of the stake. Bags and stakes were also placed over all control plants which did not receive any larvae. Only egg clutches large enough to contribute 3 larvae to each treatment were used. The clutch number that the larvae came from was randomly assigned to the plants.

I applied the late heat wave treatment when plants were 59 d old and in the tuber initiation stage by moving another 51 plants from the control chamber to the heat wave chamber for 4 d. This was 3 d after beetle larvae were added to plants, which means that larvae and plants experienced heat wave conditions together. After the late heat wave, plants and their larvae were moved back to the control chamber. Larvae were left on plants for another 3 d before being removed. All larvae were on plants for a total of 10 d which is the minimum time from egg to pupation at constant 28˚C (Ferro et al., 1985). On d 66, larvae were removed and larval survival, mass, and instar were recorded. Plants were destructively sampled to obtain tuber mass 70 d after planting.

Field experiment

I examined the effects of heat wave timing on potatoes, CPB larvae, and their interaction using a field experiment at the Michigan State University Kellogg Biological Station (Hickory Corners, MI). I generated heat wave conditions in field plots using ceramic heaters and open-top chambers at two different times in the growing season for two different age classes of plants.

In the field, I set up 96 1.5 x 1.5 m plots. Each plot had 10 potato plants total, 5 in each of two rows, which were planted 14 d apart (05/25/2018 and 06/08/2018) to obtain two age classes of plants. The field had 0.76 m spacing between rows and 0.25 m between seed pieces within a row. I randomly assigned two plants from each row, and or age class, within each plot to a beetle treatment or a no-beetle control. Finally, I randomly assigned each plot to a July heat wave (n= 24), or an August heat wave (n= 24). This design resulted in 12 treatment combinations of heat wave timing, beetle presence, and plant age (Table 2.1).

Three d before the start of each heat wave, plants assigned a beetle treatment were covered with a 19 L mesh bag with five 1st instars per bag. Plants in each paired no-beetle control were simultaneously bagged without larvae. We began simulating heat wave conditions on plots assigned to heat wave treatments 3 d after larvae were placed on plants, (07/17/2018, 08/13/2018) for each timing treatment and continued treatment for 96 h.

At the conclusion of each heat wave, we removed the chambers and left the larvae on the plants for another 3 d. After a total of 10 d on the plants the larvae were removed and counted. The survival, mass, and instar of each larva were recorded and the amount of insect defoliation was visually estimated.

On (09/22/2018), the four experimental plants in every plot were dug up by hand and placed in paper bags. These potatoes were transported to the laboratory and stored at 2.8°C. They were assigned a scab rating from 0-5 (0 = no scab and 5 = > 50% of tuber covered with pitted lesions) and sorted into standard potato size classes (USDA, 1997). Total tuber mass was recorded for each plant, then potatoes with deformities were removed and healthy potatoes were counted and weighed to assess the marketable yield of each size class.

Heat wave methods

The heat waves were created by using open-top chambers and ceramic heaters (300-watt, Tempco, Inc., IL). The chambers were constructed of wood and anti-condensate greenhouse plastic (6 mil, 91% light transmittance, Poly-Ag Corp., CA). The chambers were 1.5 by 1.5 m, 1.2 m tall, with a 0.6 m^2 square opening at the top. These chambers were fitted with ceramic heaters that were turned on at 2100h and off at 0900h to maintain elevated plant canopy temperatures at night. Leaf temperatures were recorded for a subset of plants (digital infrared thermometer, Fluke Corporation, Everett, WA) on the 3rd d of the experiment between 1200h and 1300h and the 4th d from 0400h to 0600h. The soil surface temperature and soil temperature 10 cm below the surface were recorded at the same time. Ambient air temperatures were recorded every 15 min (HOBO Pendant data loggers in radiation shields, Onset Computer Corporation, Bourne, MA) in four heat-treated plots and in four control plots for the duration of each heatwave. Two of the data loggers recorded humidity (HOBO Pro v2® data loggers in radiation shields, Onset Computer Corporation, Bourne, MA). In order to calculate the average temperatures for the two treatments, we selected two 3-hr time periods (0300-0600h, 1300-1600h) to represent night and day temperatures, respectively.

The heat wave treatment elevated air temperatures in the July experiment by 8.7°C to 43.0°C (+/- 0.1°C) during the day and by 3.5°C to 21.4°C (+/- 0.4°C) at night, relative to the control (day and night temperatures averaged over 3-h block in middle of day and night). Potato leaf temperatures increased by 3.6°C during the day and 8.4°C at night, relative to the control (Table 2.2). In the August experiment, air temperatures were elevated by 4.0°C to 38.3°C (+/- 0.5 °C) during the day and by 4.7°C to 24.7°C (+/- 0.3°C) at night (see Table 2.2 & 2.3 for details). Leaf temperatures were elevated by 1.4°C during the day and 6.8°C at night, relative to the control (Table 2.3). The lower increase in temperature during the August experiment was caused by slightly different weather conditions during the August experiment.

Statistical analyses

I analyzed the effects of heat waves on beetle survival and rate of development to 4th instar using generalized linear mixed-effects models with a binomial distribution. I analyzed tuber mass and beetle mass using a linear mixed effects model. In the growth chamber experiment, I included larval clutch as a random effect to account for potential correlations among larvae from the same clutch. In the field experiment, I included plant age during the heat wave treatment (the ontogenetic heat wave timing) as a predictor in all models. For potato yield, I also included the beetle treatment and its interaction with ontogenetic heat wave timing as predictors. Our models accounted for correlations among responses from plants within the same plot using a plot-level random effect. I repeated analyses for both the July and August field experiments. I conducted all analyses in the lme4 and glmmTMB packages in R (Bates et al., 2015, R Core Team 2019). I used likelihood ratio tests to compare models with and without each of our fixed-effect predictors (Bolker, 2009). When treatment effects were significant ($\alpha = 0.05$), Tukey's HSD was performed to determine differences among treatments using the 'emmeans' package.

RESULTS

Growth chamber experiment

Beetle performance

The environmental chamber heat wave treatments significantly reduced CPB larval survival (χ^2=23.1, df=2, P<0.01; Fig. 2.1.A). Larvae that experienced the late heat wave had 48% lower survival than larvae on control plants (z =4.32, P<0.01). Larvae in the early heat wave treatment, in which larvae were placed on plants 7 d following the conclusion of heat wave temperatures, had 20% lower survival relative to controls (z =2.13, P=0.03), indicating that the heat wave treatment indirectly reduced beetle survival by altering plant quality. In contrast, neither heat wave timing treatment significantly influenced beetle larval mass (χ^2=0.67, df=2, P=0.71) or larval instar development rate (χ^2=0.32, df=2, P=0.85), suggesting that larvae that survived a heat wave grew normally (Fig. 2.1.B).

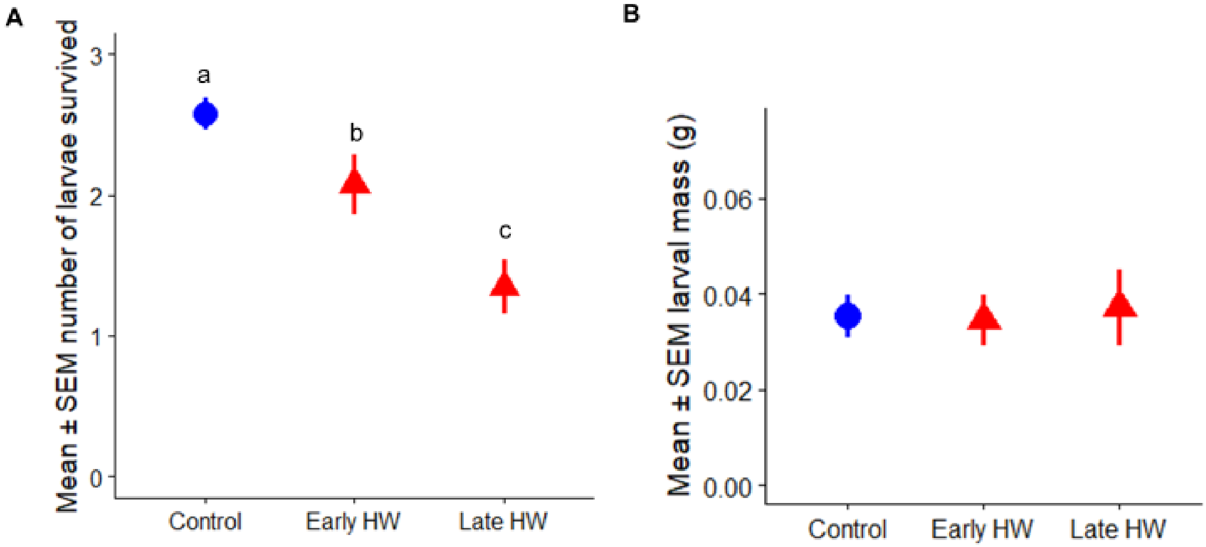

Figure 2.1. Survival and mass of Colorado potato beetle larvae feeding on plants exposed to an early heat wave and late heat wave treatments. Blue circles denote larvae feeding on control plants and red triangles represent larvae feeding on plants exposed to a heat wave treatment. (A) Larval survival was lowest in the late heat wave treatment, in which larvae were present on plants during the heat wave. Larval survival was slightly reduced by the early heat wave treatment in which the heat wave occurred on plants one week prior to larval addition. (B) Larval mass did not differ between control and heat wave treatments. Points and error bars show means +/- one standard error. Means with different letters are significantly different from each other (Statistical test p<0.05)

Potato yield

The environmental chamber heat waves significantly reduced potato tuber mass ($F_{2, 154}$=17.94, P<0.01; Fig. 2), and these effects depended on heat wave timing. The early heatwave (45 d after planting) reduced tuber mass by 87% relative to the controls (t_{154}= 5.95, P < 0.01). The late heat wave treatment (59 d after planting) led to a 35% reduction in tuber mass (t_{154}= 5.95, P = 0.02). There was no overall effect of larval presence on tuber mass in this experiment ($F_{1, 155}$= 0.23, P = 0.63), nor was there a significant interaction between the heat wave treatments and larval presence ($F_{1,151}$= 0.22, P = 0.8). However, there was a trend for CPB presence to have more negative effect on yield when combined with heat waves, especially the late heat wave (Fig. 2.2.). In the late heat wave, plants with CPB produced 43% lower yield than control plants without larvae (t_{151}=2.02, P=0.075), a substantially larger reduction than the only 26% decline in yield as result of the heat wave alone compared to control plants (t_{151}=1.20, P = 0.26).

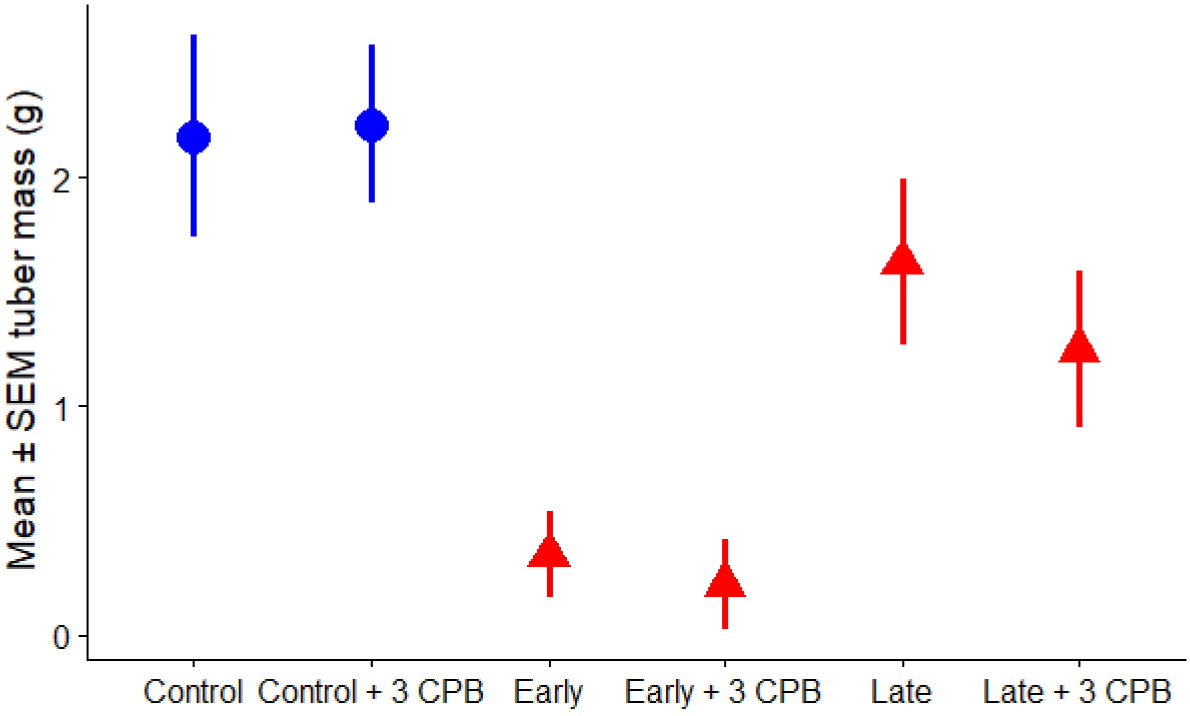

Figure 2.2. Average tuber mass produced per plant for all heat wave treatment combinations after 70 d. Blue circles denote control plants and red triangles represent plants exposed to a heat wave treatment. The early heat wave had the greatest negative effect on tuber mass. The addition of 3 beetle larvae had no effect on yield compared to plants without larvae and of the same heat wave treatment. The addition of 3 beetle larvae to the late heat wave treatment did decrease tuber production compared to control groups. Points and error bars show means +/- one standard error.

Field experiment

Beetle performance

In the July block, the heat wave treatment decreased larval survival by 25% regardless of plant age (χ^2=8.94, Df=1, P=0.004). There was a 19% reduction in larval survival for those experiencing a heat wave and feeding on 6-week-old plants (Z=2.8, P=0.02) and a 31% reduction for larva experiencing a heat wave and feeding on 8-week-old plants (Z=2.8, P=0.02; Fig. 2.3.A) compared to survival of larva feeding on non-heat wave 6 and 8-week-old control plants respectively. Age of plants alone only accounted for a 6% decrease of larval survival for those feeding on 8-week-old plants compared to 6-week-old plants (χ^2=0.60, Df=1, P=0.44).

In contrast, in the August block, heat wave treatment was not a significant predictor, only causing a 5% reduction in larval survival (χ^2 = 0.27, Df= 1, P= 0.61). However, plant age at time of larval placement was an important factor for larval survival with 12-week-old plants having a 27% lower survival compared to larvae on 10-week-old plants (χ^2 = 9.01, Df = 1, P = 0.003; Fig. 2.3.B). Larvae feeding on 12-week-old control plants had a 17% decrease in larval survival compared to those feeding on 10-week-old control plants (Z = 3.04, P = 0.007). The impact of the heat wave in August on larval survival varied drastically depending on the age of plants they were feeding on. Larvae feeding on 10-week-old plants and experiencing a heat wave saw a 6% increase of larval survival (Z =-0.57, P = 0.57). In contrast, larval feeding on 12-week-old plants and experiencing a heat wave had a 19% reduction in larval survival (Z = 1.25, P = 0.26). Larvae feeding on 12-week-old plants during the August heat wave treatment had 37% lower survival than larvae feeding on 10-week-old heat wave plants (Z = 3.04, P=0.01). This suggests that older potatoes interact with the heatwave in a way that decreases palatability or nutrition to cause lower larval survival rates.

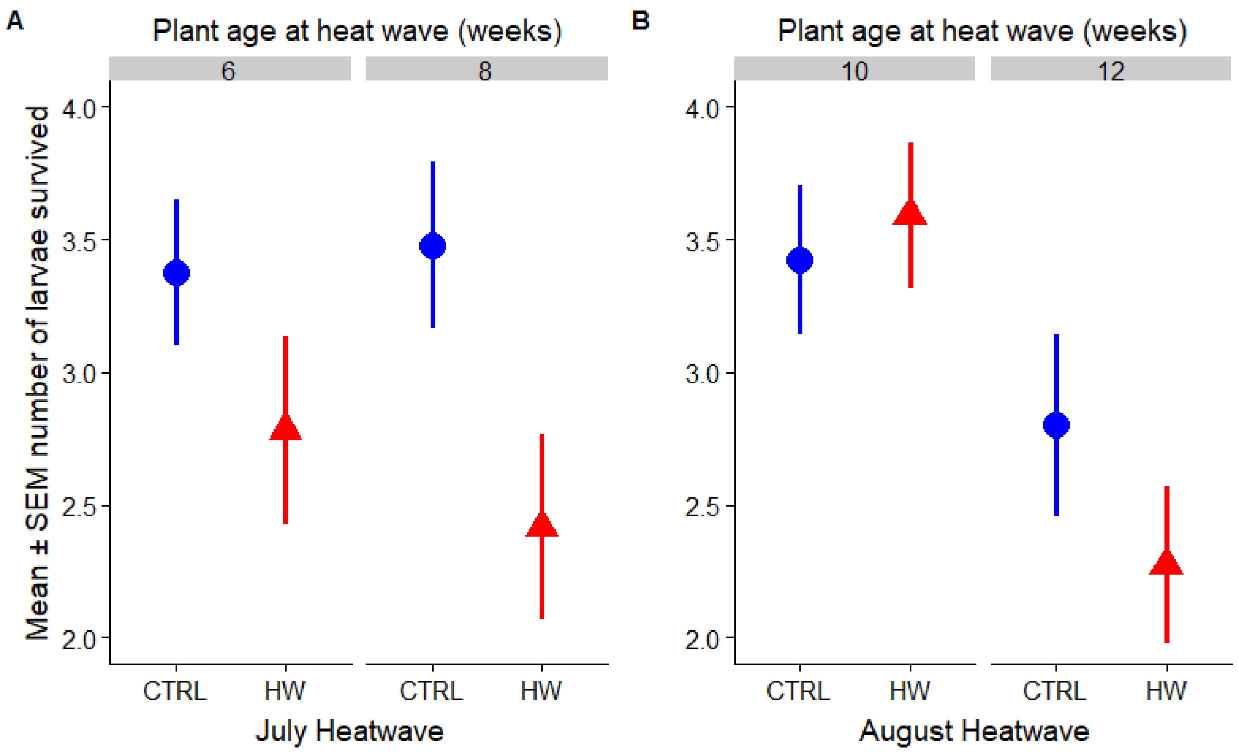

Figure 2.3. Average survival of Colorado potato beetle larvae feeding on different age plants for (A) July and (B) August heat wave treatments in a field at the Kellogg Biological Station. Blue circles denote control plants and red triangles represent plants exposed to a heat wave treatment. Larval survival was lower for those that experienced a July heatwave regardless of plant age. There was no difference in survival of larvae between control and heatwave treatments for the August heat wave, but the larvae that were feeding on younger 10-week-old plants and experienced a heat wave had a much higher survival rate than those that were feeding on the 12-week-old plants and experienced a heat wave.

In the July block, regardless of plant age, larval mass increased by 13% in heat wave treatments compared to larvae feeding on no-heat wave control plants (χ^2= 3.89, df = 1, P = 0.049; Fig. 2.4.A). Average larval mass increased by 5% for larvae feeding on 6-week-old plants exposed to heat wave treatments (t=-0.509, df=80, P=0.61) and by 21% for larvae feeding on 8-week-old plants in the heat wave treatment (t=-2.304, df=80, P=0.14) compared to larvae feeding on control plants of the same age. Plant age alone did not have an impact on larval mass in the July block (χ^2= 0.07, df = 1, P = 0.79). The greatest impact on larval mass occurred in larvae that fed on 8-week-old potato plants and experienced a July heat wave, with 21% higher mass than larvae that fed on 8-week-old non-heat wave control plants. In the August block, larval mass was not impacted by the heat wave treatment (χ^2= 1.87, df = 1, P = 0.17; Fig. 2.4.B), or plant age (χ^2 = 2.68, df = 2, P = 0.26).

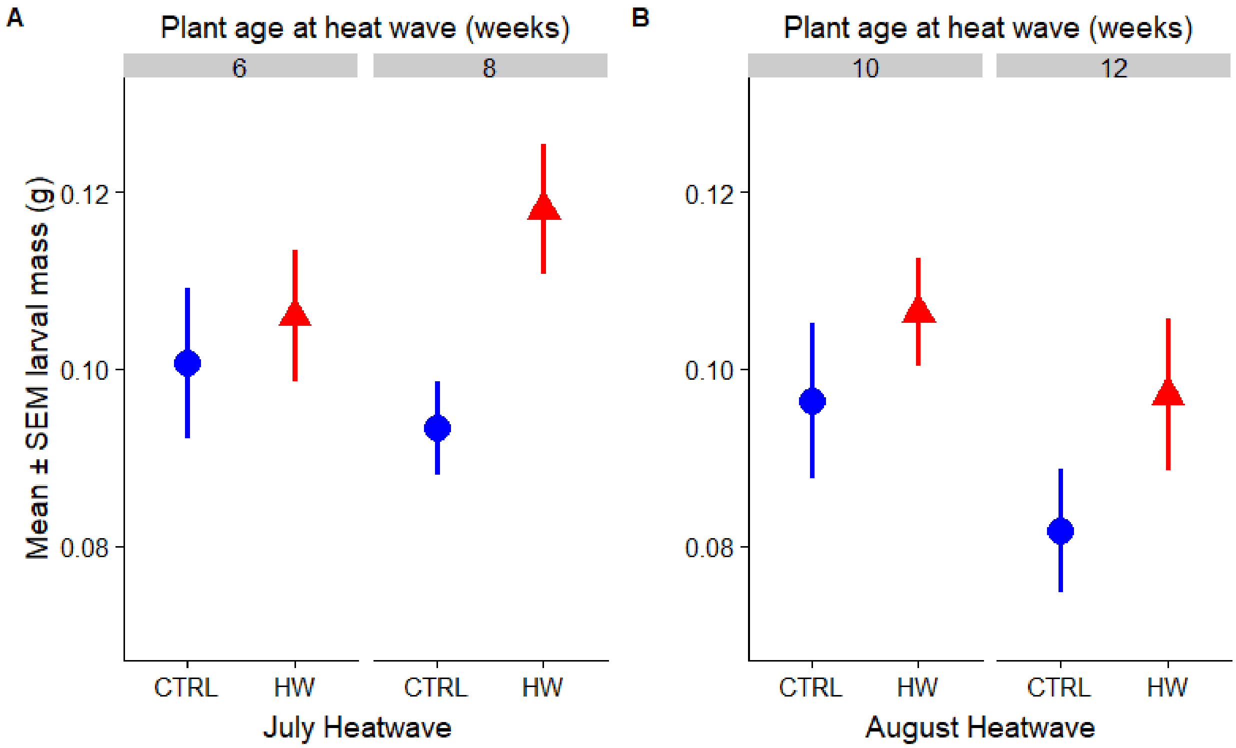

Figure 2.4. Mean mass of Colorado potato beetle larvae feeding on different age plants exposed to (A) July and (B) August heat wave in a field at the Kellogg Biological Station. Blue circles denote control plants and red triangles represent plants exposed to a heat wave treatment. Larval mass was 30% higher for larvae that fed on 8-week-old potatoes and were exposed to a July heat wave ($P<0.05$) compared to non-heat wave controls. Heat waves significantly influenced larval mass when modeled collectively (χ^2 =6.0094, df=6, P= 0.01423).

Potato yield

Older plants (those planted in May vs June) had 27% higher total yield (χ^2= 50.038, df = 1, P< 0.01) and 46% higher marketable yield then younger plants regardless of heatwave or beetle treatments (χ^2= 53.34, df = 1, P< 0.01). Because marketable tuber mass is most important to production that is what we focus on here.

In the July experiment, plant age was the most important predictor of marketable tuber mass with older 8-week-old plants having a 47% higher marketable yield than 6-week-old plants (χ^2=23.56, df=1, P<0.001). The beetle treatment and heatwave treatment had no effect on marketable tuber yield as single factors (χ^2=0.25, df=1, P=0.62; χ^2=2.57, df=1, P=0.11 respectively). However, a model with an interaction between plant age and heat wave was found to be significantly better than the model with plant age alone (χ^2=6.7, df=1, P=0.035). We also found 8-week-old plants that experience a heat wave had a 28% decrease in marketable yield (t=1.97, df=182, P=0.12), and plants that experienced both heat wave and beetle treatments have 41% lower marketable yield than plants in the control treatment (t=-2.8, df=182, P=0.02 ; Fig. 2.5.A).

In the August experiment, plant age was the only significant predictor of marketable tuber mass with older 12-week-old plants having a 45% higher marketable yield than 10-week-old plants (χ^2=27.1, df=1, P<0.001; Fig. 2.5.B). The beetle treatment and heatwave treatment had no significant effect on marketable tuber yield as single factors (χ^2=0.4, df=1, P=0.52; χ^2=0.2, df=1, P=0.65 respectively).

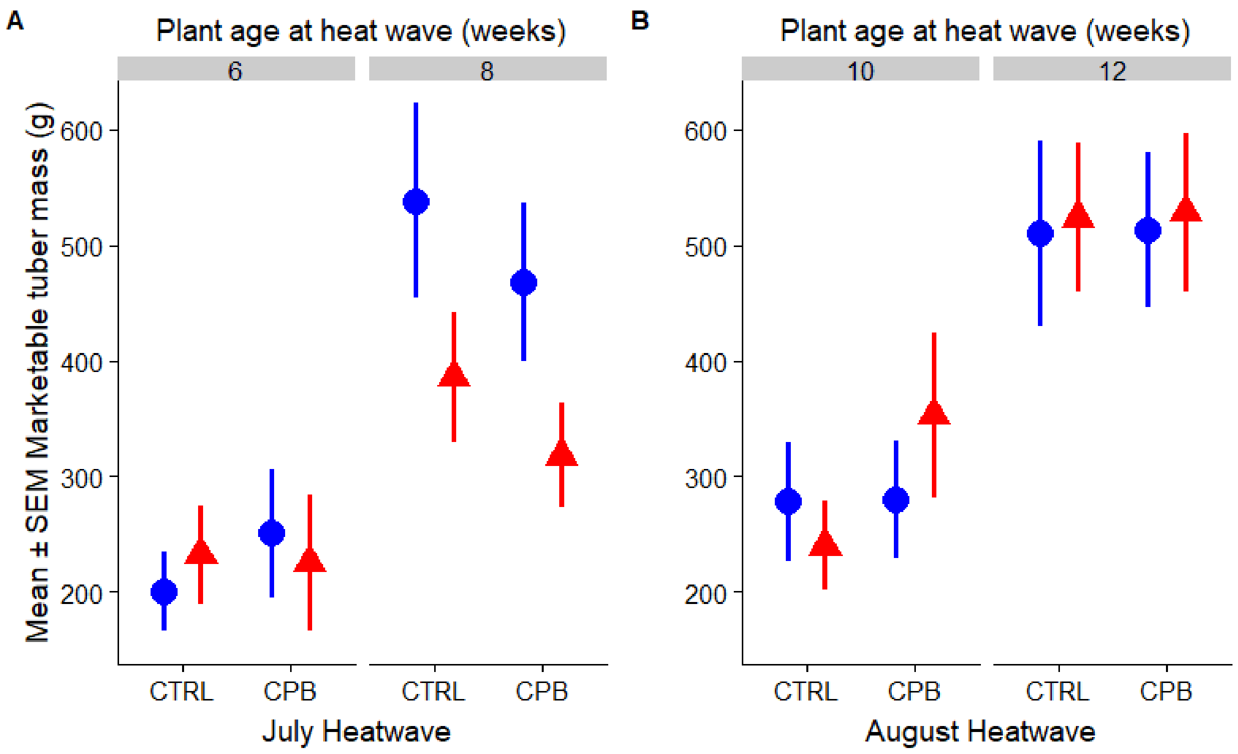

Figure 2.5. Blue circles denote control plants and red triangles represent plants exposed to a heat wave treatment. (A) Heat wave treatment significantly reduces marketable tuber mass in 8-week-old plants that experienced a July heat wave. (B) Marketable tuber mass in the August heat wave was significantly influenced by plant age but heat wave and beetle treatments had no effect. Blue circles denote non heat wave controls and red triangles denote heatwave treatments.

DISCUSSION

I examined the impact of heat waves and heat wave timing on insect-plant interactions on potato using an environmental chamber experiment and a field experiment. The chamber experiment revealed that the timing of both heat wave and herbivory is important: heat waves significantly reduced herbivore survival regardless of plant age and whether the larvae experienced the heat waves directly, although the greatest reduction in survival occurred when herbivores were directly exposed to the heat wave. This indicates that the direct effect of heat waves is not necessary for herbivores to experience negative effects and that these events can have both direct and indirect effects, possibly leading to an emergent effect on herbivore survival. It is interesting to note that larval mass and instar was similar across control and heat wave treatments; this could be due to behavioral changes whereby surviving larvae were able to locate microclimates on the plant that allowed them to temper the effects of the extreme heat as observed in spiders and other beetles (Barton & Schmitz, 2009;Ward & Seely, 1996). This similarity in mass and development is perhaps best explained by overlaying average temperatures experienced by larvae in control (21˚C) and heat wave (34˚C) treatments. These two groups of larvae both experienced similar average temperatures at the optimal CPB developmental temperature of 28˚C (Ferro et al., 1985). Due to the experimental set up, we cannot conclusively distinguish between the effect of plant age and direct/indirect larval heat wave exposure on larval survival, but our results indicate that the effects of heat stress on potato persist in the leaf tissue for at least a week after a heat wave. Direct heat wave effects on undamaged plants were more pronounced when the plants were younger, indicating that younger potatoes are more sensitive to stress than older plants.

In the field, heat wave effects on larval survival depended on heat wave timing, with more consistent negative impacts earlier in the season on younger plants and variable effects on older plants later in the season. It is likely that heat waves still reduced palatability and nutrient value in older plants but effects on survival are not as drastic. Larval mass however, was consistently higher on heat waved plants regardless of their age. This over compensatory response is potentially caused by larvae having higher metabolic rates and consuming more plant tissue during heat waves, although we did not measure leaf area consumed (Neven, 2000). The difference in patterns of larval weight between the laboratory and field experiments could be due to the fact that larvae experiencing 40°C for 14 h in the environmental chamber was near their thermal maximum in but in the field experiment heat waves had similar high temperatures with a shorter duration of high heat making average temperatures closer to developmental optimum. Heat waves also had negative impacts on potato yield but only for plants in the early reproductive life stages of tuber initiation and early tuber bulking (8-wk-old). Potatoes are most sensitive to temperature extremes during the tuber initiation phase, and are less responsive once the tubers are formed, during tuber bulking (Kim and Lee 2019), therefore it is possible that the lack of consistent negative yield response was because some plants were still in the vegetative stage (6-wk-old) or had already started bulking (10 & 12-wk-old). With heat waves in the Midwest occurring most commonly from early June to mid-August the risk of these sensitive life stages of potato being exposed to stressful temperatures is high, especially for later crop plantings (Anderson et al., 2011).

Extreme heat affects numerous aspects of plant-herbivore relationships, but a decrease in herbivore survival is a frequent outcome (Sentis et al., 2003, Ferro et al., 1985, Chen et al., 201, Logan et al., 1985). While heat waves are relatively brief, the stress or heat-shock they cause can persist in plants and affect herbivores indirectly after the event as demonstrated with the lab experiment (Al-Whaibi, 2011). Our study highlighted that heat waves on potatoes at different developmental stages could differ in how the heat wave impacts them and require different management responses. Studying extreme events therefore requires the adoption of a temporally-explicit framework that links seasonal variation, organismal ontogeny, and event timing to ecological outcomes. In addition, heat waves may speed up herbivore development with significant negative impacts on crop production such as an extra herbivore generation is possible earlier than predicted by the gradual warming models. In this system, similar to other crops, the most sensitive life stage of potato to heat waves and herbivore stress are the early reproductive stages suggesting management practices such as irrigation or pesticide applications should be diligently used to mitigate stressful conditions during this sensitive growth stage (Kim and Lee 2019; Siebers et al. 2015). Research that manipulates these various stressors and the timing at which they occur is key to informing an adaptive management strategy and should continue to be investigated in the future.

APPENDIX

Table 2.1 The number of replicates by month of planting, heat wave timing and presence or absence of Colorado potato beetle larvae in a field experiment at the Michigan State University Kellogg Biological Station in 2018. We generated heat wave conditions in field plots using ceramic heaters and open-top chambers each with 10 potato plants (5/month).

	May		June	
	0 larvae	5 larvae	0 larvae	5 larvae
Control	44*	44	44	44
July Heat Wave	22	22	22	22
August Heat Wave	22	22	22	22

*Controls were paired within blocks with heat treatments, resulting in twice as many control plants as heat treatment plants.

Table 2.2 Mean ± SEM temperatures (°C) for 3-hour increments during heat of the day and cool of night during field heat wave experiments in Michigan in an experimental potato field in 2018. Heat waves were created using open top chambers and ceramic heaters.

	July				August			
	Control		Heat wave		Control		Heat wave	
	Day	Night	Day	Night	Day	Night	Day	Night
Air temperature	34.3±0.8	17.9±0.1	43.0±0.1	21.4±0.4	34.3±0.5	20.0±0.03	38.3±0.5	24.7±0.3
Leaf surface	28.8±0.3	17.3±0.1	32.4±0.3	25.7±0.5	24.9±0.1	18.9±0.1	26.3±0.2	25.7±0.2
Soil surface	33.3±0.3	19.3±0.2	35.8±0.4	25.7±0.3	27.0±0.2	20.8±0.3	28.7±0.3	25.8±0.3
Soil sub-surface	27.3±0.2	24.0±0.1	29.4±0.2	26.1±0.2	25.9±0.2	23.4±0.1	27.1±0.2	25.4±0.2

Table 2.3 Temperature (°C) differences between heat wave and control treatments in open top chambers for heat waves created in an experimental potato field in Michigan in 2018.

	July		August	
	Day	Night	Day	Night
Air temperature	8.7	3.5	4	4.7
Leaf temp	3.6	8.4	1.4	6.8
Soil surface	2.5	6.4	1.7	5
Soil sub-surface	2.1	2.1	1.2	2

CHAPTER 3: Consequences of heat waves on plant, herbivore, disease interactions

ABSTRACT

Heat waves, brief periods of intense heat and decreased precipitation, are becoming more common globally. The immediate negative effects of heat stress on organisms are well understood, but we have a poor understanding of the potentially lasting ecological effects of heat waves on biotic interactions among plants, insect herbivores, and pathogens. An understanding of how heat waves alter the dynamics of ecological interactions in agricultural systems would improve our ability to manage crop pests in an increasingly variable climate. I examined how heat waves influence the growth and development of potatoes and two potato pests: CPB and potato early blight (A. *solani*). I inoculated field grown potato plants with early blight and CPB 3 d prior to simulating heat wave conditions in the field using open-top chambers and ceramic heating elements. Heat waves and early blight treatments had no effect on CPB survival, but larval mass was 16% and 44% higher for larvae feeding on blighted plants and plants exposed to heat waves respectively. Larvae developed faster in heat wave treatments suggesting an increased likelihood of an additional generation of the pest. Potato yield was not affected by any single stressor, but when heat waves were added to plants with early blight and CPB total yield was reduced by 14% and 17% respectively. These results suggest that multi-species interactions are heavily influenced by short duration, high intensity heat waves and the projected increase in frequency of these extreme events justifies future research on their ecological effect to improve agricultural sustainability and food security

INTRODUCTION

Most climate change research has focused on the gradual increase in mean temperature predicted to occur in the next 30-100 years, neglecting the inherent variability of extreme weather such as heat waves (Lloret et al. 2012; Parmesan and Yohe 2003). The current and predicted increase in the frequency and intensity of extreme weather events has become a major focus of climate change research (Meehl and Tebaldi 2004; Rahmstorf and Coumou 2011; Fischer and Knutti 2015; Perkins-Kirkpatrick and Gibson 2017). Extreme events, such as heat waves, cold snaps, droughts, and floods, are localized abiotic disturbances that have direct negative effects on organisms but also disrupt trophic interactions (Barlow et al. 2015). These stressors have directly reduced crop yields and caused phenological mismatches of plants and insects altering community composition for years afterwards which is of ecological significance (Bisbis et al. 2018; Lesk et al. 2016; Smith 2011; Thompson et al. 2013).

Temperature influences the development and life cycle of all organisms differently as each ectothermic organism has its own thermal optimum, minimum and maximum for growth and reproduction. While heat waves have direct effects on insects, diseases, and their host plants, the impact on the interaction among them is much less studied (Szczepaniec and Finke 2019). Increased temperatures can increase development rates for insects, diseases, and plants which can disrupt the synchronicity of tritrophic interactions (Szczepaniec and Finke 2019; Dyer et al. 2013). Due to the difficulty of replicating heat waves under field conditions, most research on heat waves to date has been done in growth chambers or greenhouses limiting their ability to let ecological interactions play out (Brown et al. 2004; Harrington et al. 1999; Parmesan and Yohe

2003). Moreover, many of these studies are conducted at constant temperatures that do not capture key daily variation that occurs during a heat wave (Lloret et al. 2012).

The goal of this research was to improve our understanding of how heat waves alter multi-species interactions in a crop system, thereby enhancing the sustainability of agriculture in the face of an increasingly variable climate. I addressed this by using open-top chambers and ceramic heaters to generate heat wave conditions on crop plants, an insect herbivore, and a common fungal disease in the field. I used a fully-crossed experimental design to address how extreme heat affects the fitness of potato, the survival and growth of CPB, and the severity and incidence of a common fungal disease, potato early blight (A. *solani*). I addressed the following questions: (1) How do heat waves and early blight influence CPB growth and survival? (2) How do heat waves and CPB impact early blight infection rate and severity? (3) How do heat waves, CPB, and early blight interactions influence potato yield?

METHODS

Experimental organisms

Insect

I used CPB and early blight because they are both common pests of potato plants, occur at the same time during the season in potato fields, and are hypothesized to have synergistic effects on one another (Tsedaley, 2014). We obtained CPB from a lab colony reared on potatoes (cv. ‘Reba’). The colony was started with individuals collected from a potato field in Stanton, Michigan in July 2019 and kept in the laboratory at a light period of 16 h light :8 h dark and constant temperature at 23ºC. Clutches of eggs were collected over 2 d to obtain enough insects to place 15 larvae per plot, for a total of 1,200 larvae for the experiment. All larvae were randomly mixed together in a container before selecting 15 individuals to be added to each plot.

Disease

An isolate of Alternaria solani was obtained from a Michigan State University collection (isolate A00219; collected Sanilac Co., MI in March 2019). The isolate was originally collected from a symptomatic potato tuber, variety Onaway. An approximately 0.5 cm3 piece of tissue was excised and surface disinfested by submerging for 1 min in 0.6% sodium hypochlorite followed by rinsing in sterile deionized water and blotting on sterile filter paper. The disinfested tissue was plated on 1.5% water agar and incubated at 21-24 °C for 3-5 days. Resulting mycelial growth and conidial sporulation was morphologically characterized as A. solani. For molecular identification, fragments of the internal transcribed spacer (ITS) were amplified using the ITS1 and ITS4 primers (White et al. 1990) and sequenced; resulting sequences were matched with known sequences in GenBank using the BLAST algorithm. Characterized growth was hyphal tipped onto clarified V8 agar (Rosenzweig et al. 2008) – 16 g L-1 agar, 3 g L-1 calcium carbonate, and 0.05 g L-1 benomyl – amended with 30 µg ml-1 streptomycin. The isolate was stored on sterile filter paper at -20 °C to preserve viability.

Active cultures of the isolate were maintained on clarified V8 agar amended with 30 µg ml-1 streptomycin, as described previously. After 10-14 days of colony growth at 21-24 °C under a 17 h light: 7 h dark regime, conidia were harvested by gently scraping the surface of the sporulating colony with a microspatula. Collected spores were diluted with deionized water to achieve a conidial spore suspension at a concentration of ~$1x10^4$ spores/ml with 10% Tween 20 (v/v) added to act as a surfactant. Plants were infected with this solution using a hand sprayer by spraying 25 ml of inoculum, which was until it dripped off the leaves. Non-blight treatments received the same treatment with distilled water and the surfactant but without the spores. Plants were sprayed in the last two hours of daylight on (08/01/2019).

Plant

For this field experiment, I grew potato plants from certified seed potatoes (*Solanum tuberosum* cv. 'Reba'). Prior to planting, seed potatoes were cut into pieces approximately 57-71g and allowed to suberize for 5 d at 13 ℃. Potato pieces were transported to the Kellogg Biological Station organic fields and planted on (06/12/2019).

Experimental design

I investigated the effect of heat stress in the form of a 5-d heat wave on the interaction among *A. solani*, CPB larvae and potato using a full-factorial field experiment at the Michigan State University's Kellogg Biological Station (Hickory Corners, MI). I generated heat wave conditions in field plots using ceramic heaters and open-top chambers.

In the field, I set up 130 1.5 x 1.5 m plots on June 12 2019. Each plot had 4 potato plants with 2m spacing between rows and 0.25 m between seed pieces within a row. I randomly assigned the two center plants in each plot to a beetle treatment or a no-beetle control. Finally, I randomly assigned each plot to a control treatment (n= 20), early blight treatment (n= 20), a heat wave treatment (n= 20), or an early blight + heat wave treatment (n= 20). This design resulted in 8 treatment combinations of early blight, heat wave, and beetle.

The morning after plants were inoculated with early blight, 3 d prior to start of the heat wave, plants assigned to a beetle treatment received 15 1st instar larvae and were covered with a 19 L mesh bag and secured with a twist tie around the base of the stem. The no-beetle control plant in each plot was bagged in the same manner at the same time without larvae. I began simulating heat wave conditions on plots assigned to heat wave treatments 3 d after larvae were placed on plants (08/05/2019) and continued for 120 h.

At the conclusion of the heat wave, open-top chambers were removed on (08/10/2019) and the larvae were left bagged on the plants for another 2 d. After a total of 10 d on the plants the larvae were removed and counted on (08/12/2019). The survival, mass, and instar of each larva were recorded and the percent of insect defoliation was visually estimated. Plant height, % dead tissue, and % defoliation was recorded weekly up until harvest (omitting the first week of September).

On (09/14/2019), the potatoes produced by the two experimental plants in every plot were harvested by digging them up by hand and placing them in paper bags. These potatoes were transported to the laboratory and stored at 2.8°C for approximately three months. They were then removed and assigned a scab rating from 0-5 (0 = no scab and 5 = > 50% of tuber covered with pitted lesions) and sorted into standard potato size classes (USDA, 1997). Total tuber mass was recorded for each plant, then potatoes with deformities and rot were removed and healthy potatoes were counted and weighed to assess the marketable yield of each size class. Results were similar for total tuber yield and marketable yield, so I focus on total yield below.

Heat wave methods

The heat waves were created by using open top chambers and ceramic heaters (300-watt, Tempco, Inc., IL). The chambers were constructed of wood and anti-condensate greenhouse plastic (6 mil, 91% light transmittance, Poly-Ag Corp., CA). The chambers were 1.5 by 1.5 m at the base, 1.2 m tall, with a 0.6 m^2 square opening at the top of the plastic sides. These chambers were fitted with ceramic heaters 90 cm above the soil surface that were turned on at 2100h and off at 0900h to maintain elevated plant canopy temperatures at night. Heaters were turned on outside of those hours when there was greater than 50% cloud cover for a minimum of 30 minutes, as the chambers increased solar radiation during the day to elevate the ambient air temperature. Leaf temperatures were recorded for a subset of plants (digital infrared thermometer, Fluke Corporation, Everett, WA) on the 4th d of the heat wave between 0400h and 0600h and the 4th d from 1200h to 1400h. The soil surface temperature and soil temperature 10 cm below the surface were recorded at the same time. Ambient air temperatures were recorded every 15 min (HOBO Pendant® data loggers in radiation shields, Onset Computer Corporation, Bourne, MA) in four heat treated plots and in four control plots for the duration of each heatwave. Two of the data loggers recorded humidity (HOBO Pro v2® data loggers in radiation shields, Onset Computer Corporation). In order to calculate the average temperatures for the two treatments, I selected two 3-hr time periods (0300-0600h, 1300-1600h) to represent night and day temperatures, respectively. The heat wave treatment elevated ambient air temperatures by 6°C to 38.1°C (+/- 0.6 SE) during the day and by 3.9°C to 21.1°C (+/- 0.2 SE) at night, relative to the control. Potato leaf temperatures increased by 5.1°C during the day and 9.6°C at night, relative to the control (Table 3.1 and 3.2).

Statistical analyses

I conducted all analyses in the lme4 package in R (Bates et al., 2015, R Core Team 2019). I used likelihood ratio tests to compare models with and without each of our fixed-effect predictors (Bolker 2009). When treatment effects were significant ($\alpha = 0.05$), Tukey's HSD was performed to determine differences among treatments using the 'emmeans' package. I analyzed tuber mass and beetle mass using a linear mixed-effect models with a normal distribution, and survival and development to 4th instar using generalized linear mixed-effects models with a binomial distribution. The models accounted for correlations among responses from plants within the same plot using a plot-level random effect, and correlations among CPB on the same plant with plant and plot level random effects. For potato yield, we included the beetle treatment and its interaction with early blight as predictors.

RESULTS

Beetle performance

Larval survival

Early blight did not affect larval survival (χ^2=1.13, df=1, P=0.29), nor did the heat wave treatment (χ^2=0.2, df=1, P=0.66). The combined effect of early blight and heat wave on larval survival was also not significant (χ^2=1.34, df=1, P=0.51; Fig. 3.1.).

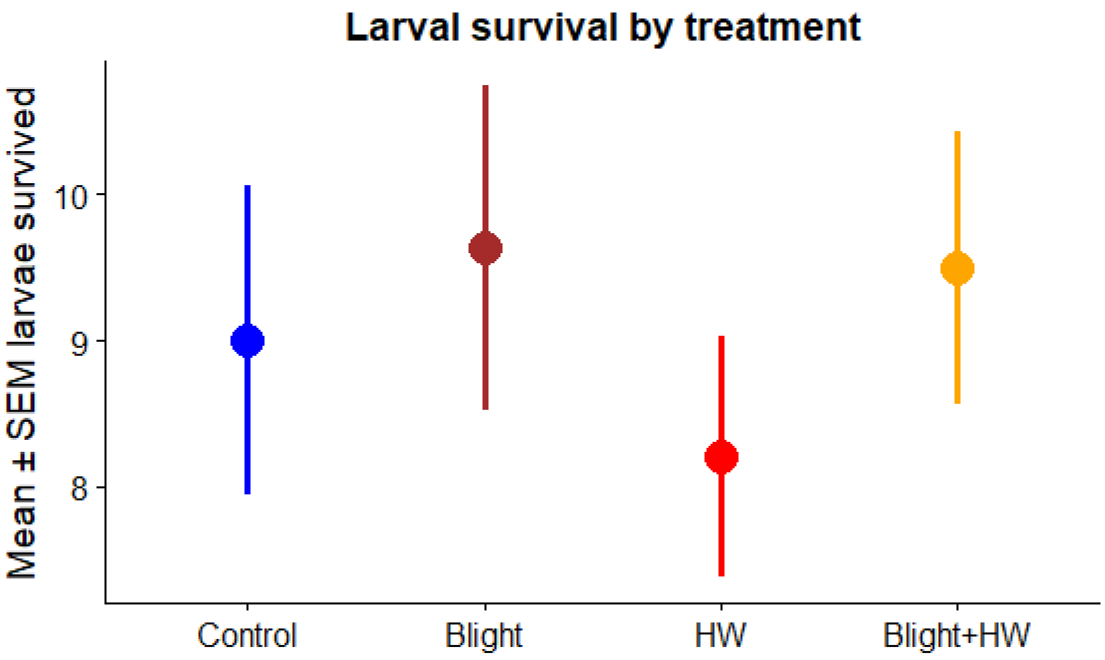

Figure 3.1. Mean (+/- SEM) survival of Colorado potato beetle larvae feeding on control plants (blue dot), early blight inoculated plants (brown dot), heat wave (HW) plants (red dot), and plants exposed to both early blight and heat wave treatments (yellow dot).

Larval mass

Larvae exposed to heat wave treatments had a 44% higher larval mass than those feeding on control plants (t=-4.5, df= 80.3, P<0.01; Fig. 3.2.). Larvae feeding on blighted plants and exposed to heat wave treatments also had a 43% increase in mass compared to control (t=-4.67, df= 77.6, P<0.01) and 25% increase compared to those feeding on blighted plants (t=-2.95, df= 74.4, P=0.02). Larvae feeding on early blight inoculated plants had a 15% increase in mean mass compared to those feeding on control plants (t=-1.6, df=74.5, P=0.37). Any positive effect of early blight on larval mass appears to be canceled out by effects of the heat wave, demonstrated by a comparison between larvae in the heat wave treatment compared to those in the early blight and heat wave treatment (t=-0.12, df=80.3, P=0.99).

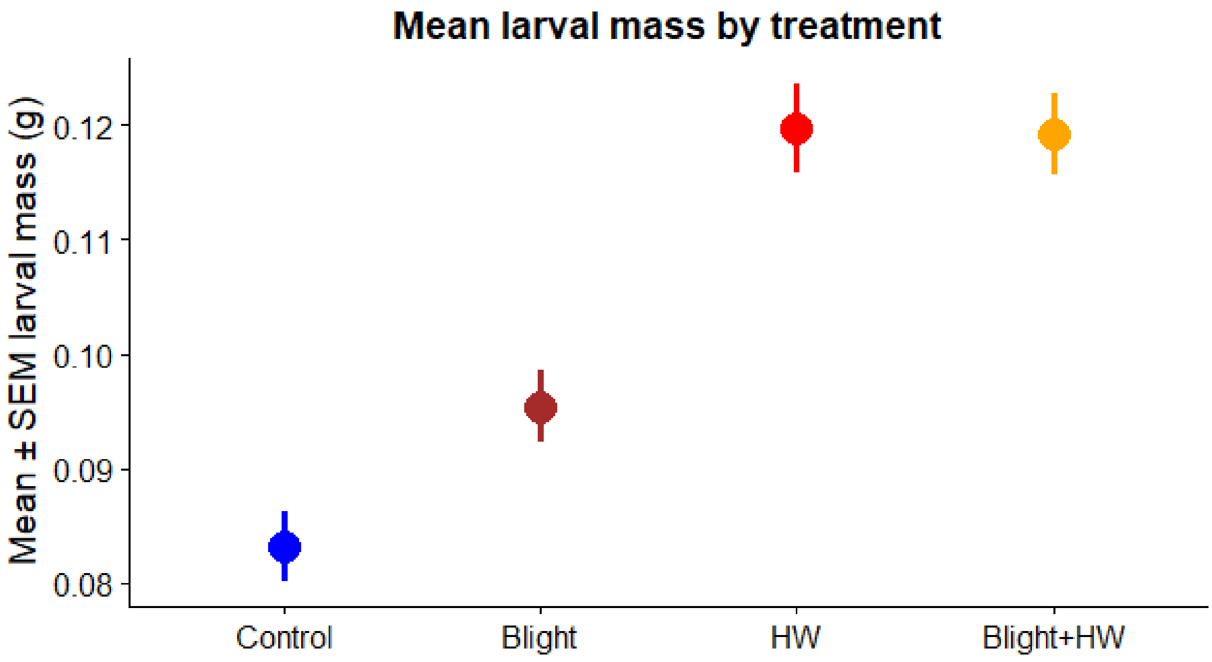

Figure 3.2. Mean mass of Colorado potato beetle larvae feeding on control plants (blue dot), early blight inoculated plants (brown dot), heat wave (HW) treated plants (red dot), and plants exposed to both early blight and heat wave treatments (yellow dot) in a field at the Kellogg Biological Station.

Larval development

The average larval instar reached was most influenced by whether the larvae experienced a heat wave or not, with 5% more larvae achieving the final 4th instar in the heat wave treatment (z=-3.1, df=inf, P=0.01) compared to control. The larvae experiencing early blight and the heat wave treatment had a similar proportion of 4th instars as control treatments (z=-2.1, df=inf, P=0.13), but 4.4% more than those feeding on just blighted plants (z=-3.1, df=inf, P=0.01; Fig. 3.3.).

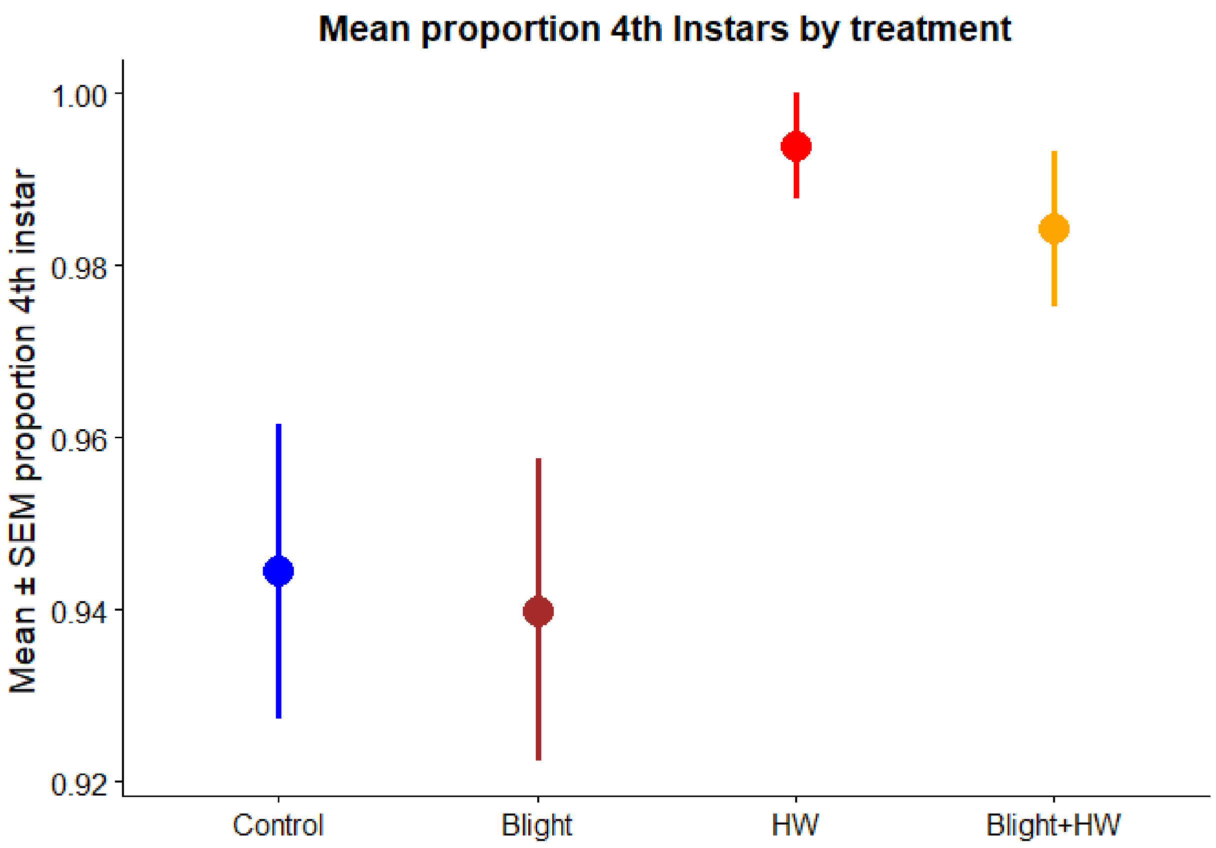

Figure 3.3. Proportion of surviving larvae that achieved 4th instar feeding on control plants (blue dot), early blight inoculated plants (brown dot), heat wave (HW) treated plants (red dot), and plants exposed to both early blight and heat wave treatments (yellow dot). Larvae were placed on plants as first instars and removed after 10 d, with the heat wave treatment occurring from day 3 to day 8.

Disease development

Disease prevalence, calculated as the cumulative sum of percent leaf with disease, was significantly higher on plants inoculated with the *A. solani* solution (χ^2=79.6, df=1, P<0.01), indicating that our early blight treatment was successful (Fig. 3.4.). The best model of disease prevalence including an additive effect of the early blight treatment and the heat wave treatment (χ^2=8.9, df=1, P=0.003). The interactive model between early blight and heat wave was not better than the additive model (χ^2=0.9, df=1, P=0.33), suggesting that heat waves have similar effects on disease prevalence on control and early blight-inoculated plants. The heat wave treatment in non-inoculated plants reduced natural occurring early blight by a non-significant 12% when compared to control treatments (t=-3.016, df= 83.1, P=0.06). For plants that were inoculated with early blight, adding a heat wave treatment reduced early blight by 13% (t=-3.016, df=83.1, P=0.06) compared to control plants. The beetle treatment had no effect on early blight (χ^2=0.07, df=1, P=0.79).

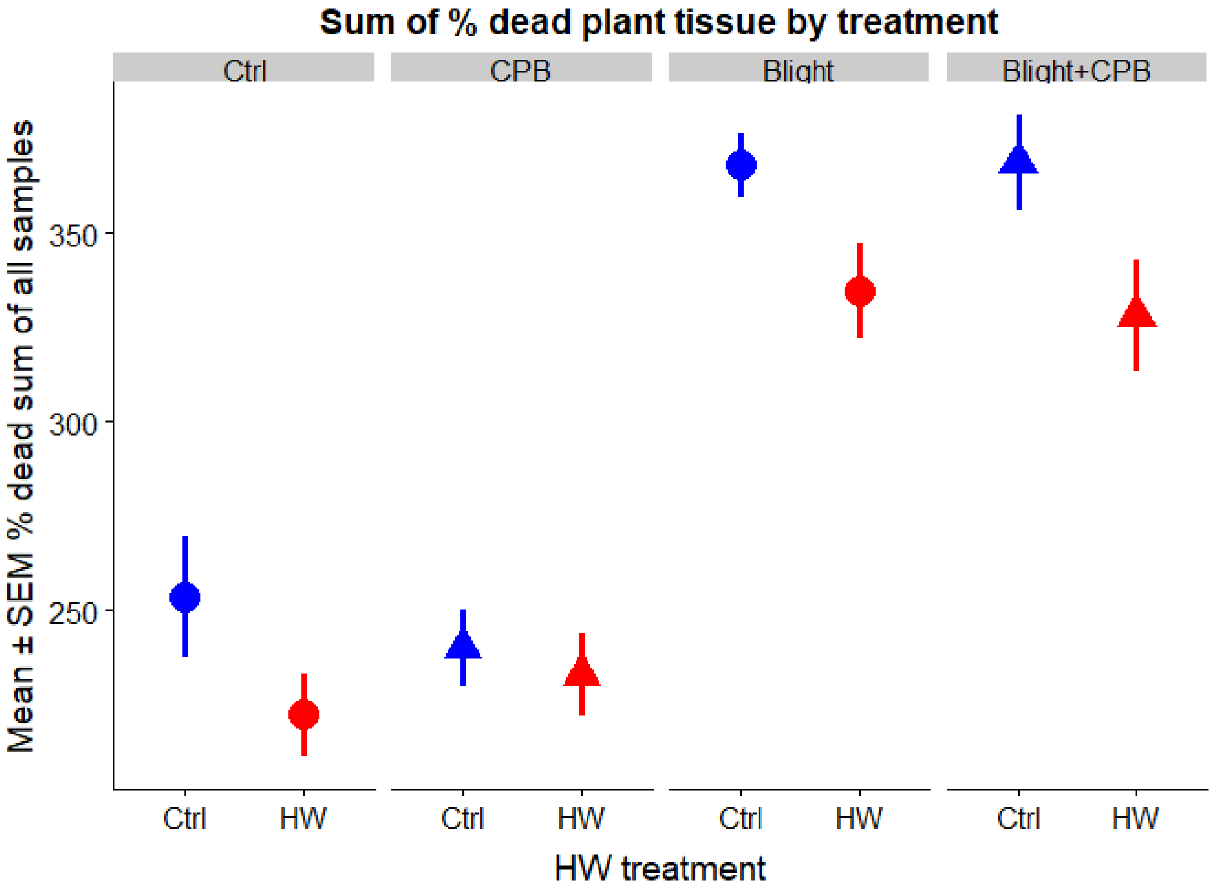

Figure 3.4. Sum of percent dead potato foliage for all seven sampling dates combined. The circles represent plants with no CPB treatment and the triangles represent those that received 15 first instar Colorado potato beetles (blue shapes = control and red = heat wave (HW) treatment).

Potato yield

Plants exposed to early blight treatments had lower yield (χ^2= 8.11, df = 1, P=0.004) regardless of heat wave or beetle treatments. The beetle treatments also had negative impacts on total tuber mass (χ^2= 5.15, df = 1, P=0.02). Heat wave treatments alone had no effect on total tuber mass (χ^2= 1.28, df = 1, P=0.26; Fig. 3.5.). We found a 20% reduction in total yield for plants that were exposed to early blight and beetles (t=3.53, df=156, P=0.01), 17% reduction for heat wave and beetles (t=3.53, df=156, P=0.01), and 14% for early blight and heat wave (t=2.0, df=83.1, P=0.47). The exposure to all three stressors reduced yield by 21% (t=3.1, df=129.3, P=0.046) but did not have more detrimental effects than any of the dual stressor treatment combinations (t=0.56, df=83.1, P=0.99; t=2.3, df=83.1, P=0.30; t=2.7, df=81, P=0.11). We were interested in the effect heat waves would have on plants when combined with beetle damage so we used a subset of the data to only include those treatments. We found that adding beetles to the heat wave treatment decreased total tuber yield by 24% (χ^2= 5.3, df = 1, P=0.02) compared to plants just experiencing a heat wave.

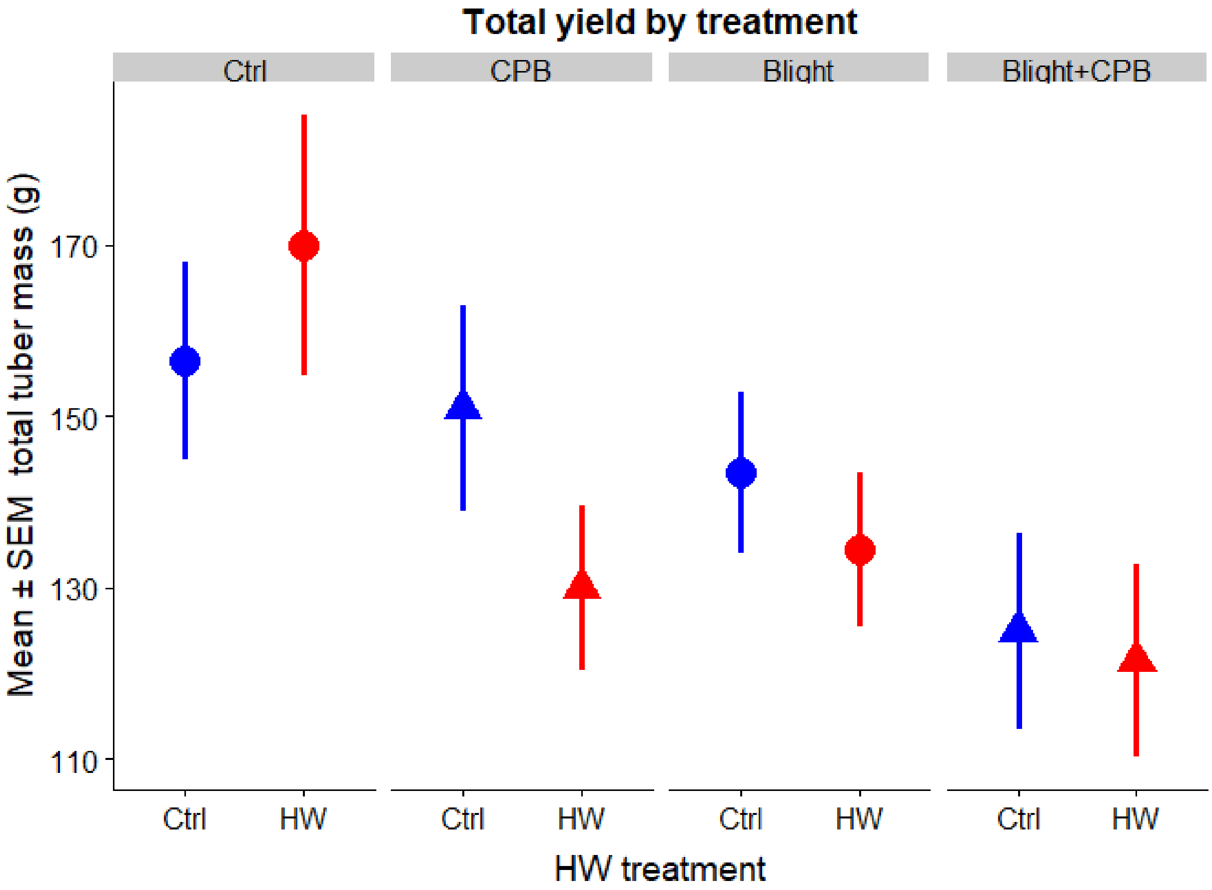

Figure 3.5. Average total tuber mass produced per plant for control (blue points) and heat wave (HW) plants (red points) exposed to beetle (CPB), early blight inoculation treatments (Blight), and both beetle and early blight exposure (Blight+CPB). Circles denote plants without CPB treatments and triangles represent plants exposed 15 first instar Colorado potato beetles. Points and error bars show means +- one standard error from 20 replicates per treatment.

DISCUSSION

In this study, I investigated the consequences of heat waves on the interaction between three stressors of potato. First, I found that beetle larvae feeding on diseased plants, plants exposed to heat waves and plants experiencing both stressors simultaneously had higher larval mass indicating faster development rates. Second, heat waves slowed the progression of early blight infecting and spreading among the leaves of potato plants. Finally, the effect of the heat wave, herbivory, and disease had no impact on potato tuber yield except when two stressors occurred simultaneously which caused up to 20% potato yield loss. Overall, heat waves influenced interactions between plants, herbivores, and diseases by decreasing development rate of CPB and changing environmental conditions to be less favorable for early blight and more favorable for CPB. The results suggest that if there is a higher frequency of heat waves, then an additional CPB generation is likely to occur in the latitudes where these experiments were performed.

The spread of early blight on infected plants was slowed by the extreme heat conditions in our simulated heat wave experiment. In contrast to a pilot experiment that suggested more rapid disease spread in the presence of herbivores via movement of spores and wounding of leaves, I found the amount of disease was not greater for those plants that were inoculated with early blight and received herbivore damage. This experiment was conducted in the last month of the growing season and would likely have shown CPB to facilitate the spread of disease if the herbivory and disease treatment additions were timed so that beetle damage occurred a week prior to disease inoculation as done in the pilot study. The larvae were only on the plant for 10 d, while herbivore pressure starts as soon as the plant emerges and continues for the duration of the plant's life cycle (approximately 90-100 d) which would likely increase the impact of herbivory on disease spread (Tsedaley, 2014).

Insect survival rate was not impacted by any of the treatments, one possible explanation of this is that the parent population was collected from the newly emerged summer generation of CPB (offspring of the overwintering population) which had been exposed to daily variation and the high heat in mid-late June and July, possibly allowing individuals to acclimate physiologically to high heat and potentially passing that acclimation on to their offspring epigenetically. Our previous field experiments found significant reductions in survival of CPB larvae sourced from a parent colony kept in the lab at a constant 22˚C. Research suggests that offspring often inherit resistance to stressors accumulated by their parents which might be the case here (Onstad 2014). This lack of mortality from heat treatments could also be due to behavioral changes whereby larvae were able to locate microclimates on the plant that allowed them to temper the effects of the extreme heat as observed in spiders and Tenebrionid beetles (Barton & Schmitz, 2009; Ward & Seely, 1996). The final instar achieved after 10 d of feeding on the plant was not affected by early blight but 5% more larvae that experienced the 5-d heat wave treatment achieved the final 4th instar. While this is not a drastic increase in development, those larvae in heat wave treatments had mass 44% greater than control suggesting that those larvae in the heat wave treatment were near the end of their 4th instar while those on control plants were either in their early stages of the 4th instar or late stages of their 3rd instar. Larval mass however, increased by 16% by feeding on blighted plants but the heat wave conditions seem to cancel out the benefits of feeding on blighted plants as larvae feeding on blighted plants in the heat wave treatment had similar larval mass as larvae feeding on healthy plants in the heat wave treatment. This increase in larval mass and instar is evidence that heat waves may boost larval development cycles allowing for faster turnover of generations especially if there are more than one heat waves in a season (Pulatov et al. 2016).

Potato yield is a key response variable from a production perspective; it was most influenced by combinations of stressors but not by any stressor alone. Plants in the early blight and heat wave treatments had similar yields to that of the control, but when combining the stress of a heat wave with beetle damage there was around a 20% decrease in total yield. This suggests that potato plants are capable of managing one stressor with minimal effect on yield, but when adding an additional stressor that requires a different defense pathway, resources normally used for reproduction now have to be allocated to defend against the second stressor (Züst & Agrawal, 2017). Plants that were exposed to all three stressors had similar yields to those that were exposed to any two stressors, suggesting that the effects of this set of stressors saturate at two and that adding a third stressor does not reduce potato growth any further. These yield reductions would likely be much worse if the plant was exposed to earlier inoculation of insect and disease pests, but the yield reduction seen here is evidence that heat waves during early reproductive stages of potato paired with simultaneous herbivore or disease pressure can have drastic impacts on yield.

In this study, I provided evidence that a 5-d heat wave can have major consequences for the interactions of two pests and their crop host plant. Results indicated that heat waves can slow the infection rate of disease that thrive in humid conditions and accelerate herbivore development. The accelerated development of the herbivore and slowed progression of disease will potentially allow for sufficient potato foliage for an extra generation of herbivores to feed on. The likelihood of an additional generation of herbivores may be higher if farmers plant later into the year taking advantage of the extended growing season. Heat waves caused insect and disease stressed plants to have reduced yields. Therefore, it is important to control insect pest infestations and disease infection prior to a forecasted heat wave event. The yield losses are large enough that they may warrant applying a pesticide prior to reaching the recommended economic threshold. While yield is important these results show that multi-species interactions are heavily influenced by short duration, high intensity heat waves and justifies the attention of more ecological studies, especially since the frequency intensity and duration of heat waves continues to rise (Meehl and Tebaldi 2004; Rahmstorf and Coumou 2011; Fischer and Knutti 2015; Perkins-Kirkpatrick and Gibson 2017). Future climate warming scenarios on plant, herbivore, and disease interactions should include the consequences of brief but extreme stress events, such as heat waves.

APPENDIX

Table 3.1 Mean ± SEM temperatures (°C) for field heat wave experiments in Michigan in an experimental potato field in 2019. Heat waves were created using open top chambers and ceramic heaters. Day means are calculated during a three-hour interval from (14:00-16:00 h), night from (4:00-6:00 h) on all five days of the heat wave treatment. Means were calculated from 5 Hobo data loggers in control and 5 data loggers in heat wave treatments. Also showing Relative humidity for the same time frames (no standard error only one logger per treatment).

	August 5-10 2019			
	Control		Heat wave	
	Day	Night	Day	Night
Air temperature	32.1±0.5	17.2±0.1	38.1±0.6	21.1±0.2
Leaf surface	27.2±0.5	13.1±0.3	32.3±0.8	22.7±0.2
Soil surface	39.2±1.1	15.4±0.2	43.6±1.1	24.4±0.2
Soil sub-surface	25.6±0.3	22.9±0.1	28.7±0.2	26.6±0.1
% Rel. Humidity	48.6	93.6	41.3	76.9

Table 3.2 Differences between heat wave and control treatments in open top chambers for heat waves created in an experimental potato field in Michigan in 2019.

	Difference HW-Control	
	Day	Night
Air temperature	6	3.9
Leaf temp	5.1	9.6
Soil surface	4.4	9
Soil sub-surface	3.1	3.7
Humidity	-7.3	-16.7

CHAPTER 4: Conclusions and future directions

The goal of this research was to improve our understanding of the direct and indirect effects of heat waves on insect, plant, and disease interactions including the role of heat wave timing and plant ontogeny, with the end goal of improving future crop management. In chapter 2 I found direct negative effects of heat waves on herbivore survival as well as indirect effects on survival for larvae feeding on a plant that was previously exposed to heat wave conditions one week earlier. The field experiment in 2018 showed the timing of heat waves and herbivory is important for yield, with plants in early reproductive stages tuber initiation and early tuber bulking being the most affected by heat stress and herbivory. Insects feeding on 8-week-old plants and 12-week-old plants during a heat wave had the lowest survival rate of any treatments. A potential explanation for the reduced survival on younger plants is that the younger plants' defenses may have been concentrated by water loss during the heat wave reducing palatability for the herbivore. Larvae likely performed worse on older plants due to lower nutritional value in their foliage as resources are being directed towards tuber bulking and maturation. Although larval mass was consistently higher on heat wave treated plants, regardless of plant age, the only significant increase in larval mass occurred in larvae feeding on those 8-week-old plants. CPB are specialist herbivores that have evolved to cope with Solanaceous defensive compounds which likely explains the higher mass achieved by these larvae (Groden & Casagrande, 1986).

Marketable yield was most effected by the treatments compared to total yield in our 2018 field experiment with smaller but still significant reductions in marketable yield in the 2019 experiment suggesting that heat wave and beetle treatments significantly alter tuber quality not just tuber production. This could result in major losses of revenue for farmers by having to sell their crop to a different market and or lower price as it does not meet quality standards. With heat waves in the Midwest occurring most commonly from early June to mid-August the risk of these sensitive life stages of potato being exposed to stressful temperatures is high, especially for later crop plantings (Anderson et al., 2011).

The third chapter investigated the influence of heat waves on multi-species interactions. While no treatment combinations influenced larval survival, I observed an increase in larval mass and development rate for those exposed to heat wave treatments. This suggests that heat waves may be beneficial to specialist insect herbivores which could lead to outbreaks. The increased development rate may also increase the likelihood of there being an additional generation of herbivores prior to what is predicted in gradual warming models (Pulatov et al. 2016). This may be facilitated by farmers taking advantage of the longer growing season and planting crops later in the year. We also found that heat waves decreased the infection by early blight likely because the temperatures and humidity achieved during the heat wave are outside the optimal range for early blight sporulation (Chaerani & Voorrips, 2006). This delayed infection rate may prolong the availability of edible leaf tissue for herbivores furthering the possibility of an additional generation of insect pests.

The impact of heat waves on the interaction of potato plants with an associated specialist herbivore and fungal pathogen showed minimal effects on yield from any single stressor. The evolutionary relationship between these or similar organisms means that the plant likely has well developed defense strategies for protection. While heat waves slightly increased total yield, the addition of beetle or early blight damage decreased yield by 24% and 21% respective to plants in the heat wave treatment. While yield is important for the bottom line, these results show that multi-species interactions are heavily influenced by short duration, high intensity heat waves and justifies the attention of more ecological studies, especially since the frequency, intensity and duration of heat waves continues to rise (Meehl and Tebaldi 2004; Rahmstorf and Coumou 2011; Fischer and Knutti 2015; Perkins-Kirkpatrick and Gibson 2017). Scientists investigating future climate warming scenarios on either plant, herbivore, or disease interactions should include the consequences of extreme stress events, such as heat waves. These future studies could be used to create a more intelligent pest prediction model that would inform growers of optimal times to control pest populations to protect yield. While the duration of a heat wave may be short, the ecological and agronomic impacts are large and lasting.

APPENDIX

RECORD OF DEPOSITION OF VOUCHER SPECIMENS

The specimens listed below have been deposited in the named museum as samples of those species or other taxa, which were used in this research. Voucher recognition labels bearing the voucher number have been attached or included in fluid preserved specimens.

Voucher Number: 2020-08

Author: Joshua S Snook

Title of thesis: EFFECTS OF HEAT WAVE TIMING ON PLANT, HERBIVORE, DISEASE INTERACTIONS

Museum(s) where deposited:
Albert J. Cook Arthropod Research Collection, Michigan State University (MSU)

Specimens:

Family	Genus-Species	Life Stage	Quantity	Preservation
Chrysomelidae	*Leptinotarsa decemlineata*	adult	20	pinned

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