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| **Project title:** | Pests, Plants and Parasitoids: how does climatic variability affect tritrophic interactions in apple orchards? |
| **Project number:** | **CTP_FCR_2017_5** |
| **Project leader:** | Dr.Jake Bishop, University of Reading<br>Dr. Robbie Girling, University of Reading<br>Dr.Glen Powell, NIAB EMR |
| **Report:** | Annual report, October 2019 |
| **Previous report:** | NA |
| **Key staff:** | Stuart Edwards |
| **Location of project:** | University of Reading |
| **Industry Representative:** | National Association of Cider Makers<br>Worldwide Fruit Ltd |
| **Date project commenced:** | October 1st 2017 |

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*The results and conclusions in this report are based on an investigation conducted over a one-year period. The conditions under which the experiments were carried out and the results have been reported in detail and with accuracy. However, because of the biological nature of the work it must be borne in mind that different circumstances and conditions could produce different results. Therefore, care must be taken with interpretation of the results, especially if they are used as the basis for commercial product recommendations.*

## AUTHENTICATION

We declare that this work was done under our supervision according to the procedures described herein and that the report represents a true and accurate record of the results obtained.

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# GROWER SUMMARY

## Headline

Climate change threatens species interactions in economically important crops leading to potential pest outbreaks.

## Background

Our current understanding is that species are likely to respond to temperature changes at different rates. This has implications for the control of aphid pests of apple in the future. For instance, aphids may be able to reproduce faster than their natural enemies in warmer conditions and escape control by natural means. The effectiveness of biological control may also change making them more or less efficient for pest control in the future. Understanding these changes will be crucial for pest control under future climes.

## Summary

Ever tightening restrictions of pesticide application in conventional production combined with an increasing demand for organic produce has increased the necessity of understanding the intricacies of pest control under predicted future climate scenarios. This project aims to quantify and model the responses of two apple pests (Woolly Apple Aphid and Green Apple Aphid) and their interactions in both plant and biological control in an attempt to influence future Integrated Pest Management (IPM) programmes.

## Financial Benefits

Due to the constraints of legislation and the potential for financial deficit through damaged crop yields, understanding the effects of climatic variability on pest-parasitoid interactions is key to all crop producing practices. Understanding trophic interactions in future climate scenarios will inform future IPM thus ensuring an acceptable level of pest control whilst potentially saving money by not applying unnecessary treatments. For example, should the ratio of pest to parasitoid exist at a level controllable by a parasitoid then it makes sense not to spray pesticides which will risk damaging the biological control population, such damage to the population risks a rebound behaviour in the pest species.

 

## Action Points

Whilst there are no grower action points stemming directly from this project at such an early stage. Early indications from a literature review suggest that supporting communities of natural enemies via increased habitat complexity and through provision of additional resources such as nectar will be crucial in ensuring optimal pest regulation by natural enemies in future climate scenarios.

## SCIENCE SECTION

### Introduction

A recent Meteorological Office study reported that UK temperature increases of between 2.3°C-5.8°C could be expected by the end of the century (Lowe *et al.*, 2018). To accompany this, extreme temperature events such as heatwaves are expected to increase in frequency (IPCC, 2014). Changing climate is proving to be detrimental for horticultural and agricultural practices. The focus of this project is to investigate the effects of changing climate on the ecosystems that occur within horticultural and agricultural systems. Legave *et al*. (2013) has documented advances in blooming of Cv. Golden Delicious by on average 10 days in northern Europe since 1965, similar advances have been documented in many other apple varieties coinciding with increased temperatures due to climate change (Chmielewski, Müller and Bruns, 2004). Phenological advancements increase the risk of damage from late frost and also effect quality of fruit forming, ultimately leading to reduced grower profits.

Organic apple orchards provide complex ecosystems for arthropods with reports of up to 2000 species being reported in UK orchards (Bleicher *et al.*, 2010; Kondorosy, Markó and Cross, 2010). Of these, up to 25% are considered pests of varying severity (Cross *et al.*, 2015). Arthropod life history traits are directly linked to their thermal environment and as such, their response to climate change brings uncertainty over pest control practices.

A study from Eastern Hungary (Korösi *et al.*, 2018) experimentally manipulated budbreak of apple trees in organic orchards creating three treatments, an advanced budbreak group, a control group and a delayed budbreak group. The advanced group documented lower number of the Green Apple aphid (*Aphis pomi*) later in this season, and this is attributed to the level of maturation of the tree shoots upon *A.pomi* emergence, *A.pomi* preferring young growing shoots. This also corresponded with lower diversity and abundance of aphidophagous beetle assemblages, reducing the efficacy of biological control of other pests within the orchard. Coevolution of specialist species such as *A.pomi* is likely to lead to traits capable of phenological advancement with their host (Berkowic *et al.*, 2015), a trend documented in a recent study in Britain revealing 55 aphid species had advanced first flight dates over the last 50 years (Bell *et al.*, 2015). Uncoupling of such relationships is likely to occur at higher trophic levels, for instance the Harlequin ladybird (*Harmonia axyridis*) a species whose distribution is spreading with climate change, was the most abundant natural enemy in a study by Korosi *et al.* This species is a generalist and as such is less likely to respond to the cues of one prey species.

The predicted increase in extreme weather events such as heatwaves is a prevalent feature of current climate change studies. How pests and natural enemies respond to such events is of primary concern to horticultural practitioners. Studies to date which have considered pest and natural enemy responses to temperature have often focused on constant temperature experiments and as such have little relevance to the fluctuating environment of real-world situations. Meanwhile, results from fluctuating temperature studies are somewhat inconclusive. Satar *et al.* (2005) for example showed that Cabbage aphid (*Brevicoryne brassicae*) reared at 12 hour alternating temperatures of 25°C/30°C had a significantly larger intrinsic rate of increase, that is to say reproductive rate, compared to those reared at 30°C, however not significantly different from what is considered the optimum temperature of 25°C. This provides evidence that in some instances, heatwaves can be beneficial to pest species life-history traits and asks the question as to what frequency and amplitude of heatwave events the pest species can withstand.

The current study documented herein is the first of many looking to untangle the potential effects of heatwave events on pest-parasitoid population dynamics. Using *B. brassicae* as a model system, this study looks to quantify the effects on life-history traits of heatwave events of varying length at temperatures above the species optimum. It is predicted that short-term heatwave events will be beneficial to the life-history traits of *B. brassicae* whereas longer events will have adverse effects.

## Materials and methods

This study was conducted at the Crop and Environment Laboratory at the University of Reading, UK. *B. brassicae* were cultured at 20°C on cabbage plants of the 'greyhound' variety provided by Molesseeds ([insert address]). Cabbage Cv. greyhound was used as the host in all the experiments due to this variety being susceptibile to *B. brassicae* (Simpson, Jackson and Grace, 2012). Plants were grown to the 10-leaf stage (approx. 6 weeks) at 20°C, 70% relative humidity and a 16/8 light/dark cycle in controlled environment rooms.

To investigate the effects of heatwave events of differing length on the life-history traits of *B.brassicae* with potential plant mediated effects two heatwave length treatments were chosen. Aphid/plant model were subjected to either 24hrs or 144hrs at 30°C. 30°C represents a temperature above optimum for *B. brassicae* where detrimental effects begin to occur, a treatment maintained at 20°C represented a control group. 10 adult apterous aphids were placed upon each plant 24 hours prior to the experiment within 30cm cubed net cages (Watkins & Doncaster), the following morning all adults were removed, and the number of nymphs counted to represent a starting value. Cages were randomly assigned to one of the three treatments and placed within controlled temperature cabinets at 30°C, 70%RH, 16/8 L:D, for their designated amount of time. Due to space limitations only 3 replicates of each were treatment were setup. In addition to aphid/plant combinations, the same amount of plants were placed without aphids into the respective treatments to be inoculated with aphids post-heatwave event. Microcosms were returned to the controlled temperature room at 20°C following the heat treatment and life-history traits monitored until the final aphid in the initial introduced cohort died.

Aphids were checked daily throughout the experiment recording; aphid mortality by noting any deaths and at which stages they occurred; rate of development as the time between each nymph stage; and reproductive rates by removing any nymphs produced daily. Survival rates and net reproductive rates were calculated in life-table format (table 1).

 

**Table 1**. A standard life table used for calculating net reproductive rate and survival rates (*Townsend et al.(2008)*

| Age interval (x-x') | Number surviving to stage x ($a_x$) | Proportion of original cohort surviving to stage x ($l_x$) | Nymphs produced in each stage ($F_x$) | Nymphs produced per individual in each stage ($M_x$) | Nymphs produced per original in each stage ($l_xM_x$) |
|---|---|---|---|---|---|
| 1st instar | | | | | |
| 2nd instar | | | | | |
| 3rd instar | | | | | |
| 4th instar | | | | | |
| adult | | | | | |
| Total | | | | | $R_0 = \sum l_x m_x = \frac{\sum F_x}{a_0}$ |

Statistical analysis analysing the data was conducted using Analysis of Variance (ANOVA) tests in the statistical package R (R Core Team, 2013).

## Results

A two-way ANOVA revealed no significant differences between the number of aphids produced per replicate and heatwave treatments (P= 0.355), similarly between the number of aphids produced and whether the aphids were subjected to heatwaves or not during the experiment (P= 0.685). Likewise, no significant difference considering the interaction effect of heatwave treatment and when the aphids were applied (P= 0.559).

Interactions utilising the independent variable of nymphs produced per surviving individual yielded insignificant results considering heatwave treatment (P= 0.442), aphid treatment (P=0.524) and the interaction between aphid and heatwave treatment (P=0.932).

No significant results were found utilising a two-way ANOVA to test for differences of net reproductive rate between the groups; Heatwave (P=0.668), aphid treatment, (P=0.479), or the interaction effect between heatwave and aphid treatments (P=0.866).

A two-way ANOVA comparing differences between total development time with heatwave and aphid treatment revealed a significant difference between the total development time and the heatwave treatment (P=0.003). A post-hoc analysis in the form of a Tukey test revealed significant differences to lay between 0-24hrs (P= 0.106) and the 144-24hr treatment (P=0.007) whilst no significant difference was found between the 0hr and the 144hr treatment (P=0.893). A significant difference was found between total development time and whether aphids were expose to the heatwave treatment or not, (P=0.007) (Illustrated in fig.2), and finally the interaction effect between heatwave and aphids yielded significant results of (P=0.034).

 

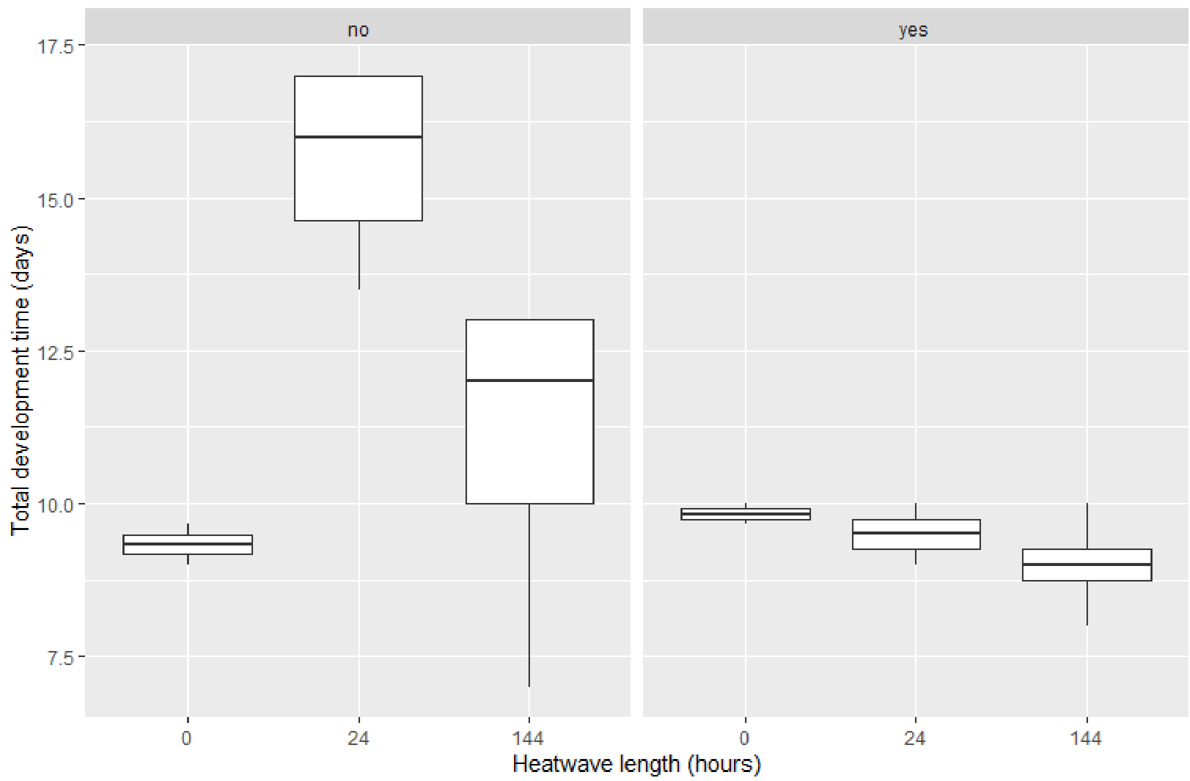


*Figure 1: The effect of heatwave and aphid treatment on the total development time of B.brassicae.* Aphids were either exposed ('yes') or not exposed ('no') to a heatwave event.

No significant difference was found when comparing the instantaneous mortality rate with heatwave treatment (P=0.717), aphid treatment, (P=0.848), or the interaction between heatwave and aphid treatment (P=0.802).

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## Discussion

This experiment set out to examine the effects of heatwaves of varying lengths on the life-history parameters of the cabbage aphid *Brevicoryne* whilst also taking ino consideration possible plant effects. Developmental rates showed statistically significant differences among temperature groups and whether the aphids were present before or after the heatwave period. No statistical differences were observed between the reproductive parameters of the aphids at different temperature treatments.

The fact that no statistical difference was found between the reproductive traits is inconsistent with the literature for *B.brassicae*. Referring back to Satar *et al.* (2005) where the study showed a significant difference between net reproductive rate at 20°C compared to the fluctuating 30/25°C. One possible explanation for this is that only the nymphal phases of the aphids lifecycle are exposed to the heatwave treatments in the Satar study whilst in the current study the whole lifecycle is exposed, potentially resulting in the aphids reproductive development being unphased by heat shock. Zang *et al.* (2015) noted that the effects of 'hot days' during Diamond-back moth (*Plutella xylostella)* development were entirely dependent on which stage were affected, additionally the effects are not always additive throughout the continued development of the insect. Further experiments in the current study could subject aphids at different developmental stages to heatwave events and observe such effects on life-history traits.

Significant differences between development time and treatments were to be expected with regards to temperature. Development time is commonly quicker with exposure to increased temperature due to increased metabolic rate (Angilletta, 2009; Kingsolver, 2009). Additionally the presence of a plant effect could be attributed to an increase in nitrogen concentration in plant tissue (Bezemer and Jones, 1998). This is consistent with the findings in this experiment with differences highlighted between the 0 and 24hr treatments and the 24 and 144hr treatments, however it is currently unclear why no significant difference was observed between the 0hr and 144hr treatments. It is possible that exposure to higher temperatures above optimum for a prolonged period of time could negate the accelerated development of *B.brassicae* at high temperatures for a shorter period of time, however further experimentation would be needed to investigate this and shall be considered later in the project.

Finding no significant differences between mortality rates and the treatments was another surprise result. One would expect greater mortality to occur at higher temperatures above the optima and this has been found in a multitude of studies (for examples see Asante and Danthanarayana, 1992; Morgan, Walters and Aegerter, 2001; Satar, Kersting and Ulusoy, 2005; Satar, Kersting and Uygun, 2008; Eben *et al.*, 2017; Kekeunou *et al.*, 2018). A potential explanation for this is the extremely high mortality rate at 1$^{st}$ instar naturally occurring across all treatments including the control, in combination with a lack of sufficient replicate numbers for all treatments.

Whilst the only significant effects highlighted from this experiment was within the developmental time, this still has implications for agricultural practitioners. A faster development time in periods with short heatwaves could lead to an overall increase in the number of generations of aphid pests throughout the year leading to large numbers of pest outbreaks.

This experiment utilised plants and aphids which had been subjected to a series of temperature treatments out of line with the original context of the experiment. Due to technical faults the cabbages and initial aphid colony were exposed to temperatures ranging from constant 20°C to fluctuating day/night temperatures of 24°C/14°C, additionally, cabinets used to provide heatwave treatments had issues in maintaining humidity and these were significantly different between the cabinets. As such, the results of this experiment are to be utilised as preliminary results subjecting both plant and aphids to heatwave treatments above their mean before dropping to fluctuating lower temperatures. This experiment had too few replicates per treatment whilst showing a large degree of variation and as such it is clear that more replicates are needed. Nonetheless, trends in the data has begun to unravel the effects of high temperatures on aphid life-history traits and as to the potential plant effects which can mediate these. Future experiments will separate the heatwave treatments with and without aphids in order to increase the number of replicates within each treatment.

## Knowledge and Technology Transfer

To date the most beneficial knowledge and technology transfer activities engaged with have been the NACM funded trips to H Weston and Sons Ltd, Heineken UK and the NACM Orchard and Machinery day 2019. In particular during the Orchard and Machinery day I was able to engage directly with growers conveying my research and also asking for advice regarding the pressing matters of apple growing.

 

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