The effect of temperature on the survival of potential biological control agents for release in glasshouses.

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T.urticae and Biological control agents

- T. urticae also known as the spider mite is a common pest that feeds on crops. It feeds by sucking cell contents from the cells and preventing the crops from photosynthesising. This means less yield and as the population increases and yield decreases demands may not be met.
- The mite breeds rapidly as the female will lay a male egg without a male and then this will hatch and the male will reproduce sexually with the female producing more offspring. The female can lay around 500 eggs in a lifetime.
- This leads to resistance to pesticides and spraying harmful pesticides on crops is not safe for the consumer and also eutrophication is a problem that is increasing.
- Therefore another organism such as a predator or parasite is introduced to control the population of the pest, this is a biological control agent.
- Biological control agents are often from foreign countries so must be tested to see their survival in the British winter. This is because if the agent escaped from the glasshouse and was able to survive and reproduce it could damage the ecosystem by displacing a species.
- The agent may not also target the organism it is supposed to deeming it unusable.
- My study tests the cold tolerance of the pest so an appropriate predator can be selected with similar physiology as the pest as an agent. It is also useful to understand what makes this species able to invade the UK.
- My tests focus on the conditions the Spider mite is able to survive in and therefore the most suitable biocontrol agent for the pest and if conditions in glasshouses need to be altered to kill all spider mites at the end of the season. Spider mites are the species used in my experiments.

My Hypothesis

- I thought as the temperature decreased and as the speed of the decrease in temperature slowed the mortality rate of the mites would increase.
- My objective was to see how different temperatures and how different speeds in the change in temperature affected the mites.

Experiment 1 – Super cooling point (SCP)

- Firstly I prepared a leaf by pulling it from the plant and then wrapping tissue dipped in distilled water around the stem to keep the leaf fresh.
- Then I used one paint brush to apply the Oceotak to the end of each thermocouple and then the other paintbrush to transfer mites from the leaf to the top of each thermocouple and then placed a cap on the top.
- I used a microscope to make the mites easier to see and placed the leaf on white tissue paper to make them clearer.
- Then I set the alcohol bath to the starting temperature of 25^0C and placed the thermocouples in the test tubes in the bath ensuring they were beneath the alcohol and secured with sponge segments.
- The lid was then placed on the bath to avoid burns or chemical injury.
- The thermocouples were connected to a data logger which was connected to a computer which I then ran the program to record the temperature.
- I ramped down to -30^0C at different speeds to prolong exposure. The speeds were 0.5^0C/min, 1^0C/min and 2^0C/min.
- The data was recorded on a graph which I could interpret and the peaks for each line representing each thermocouple and arthropod represents the exothermic release of temperature as the arthropod instantly freezes. This is the super cooling point.

SCP Results

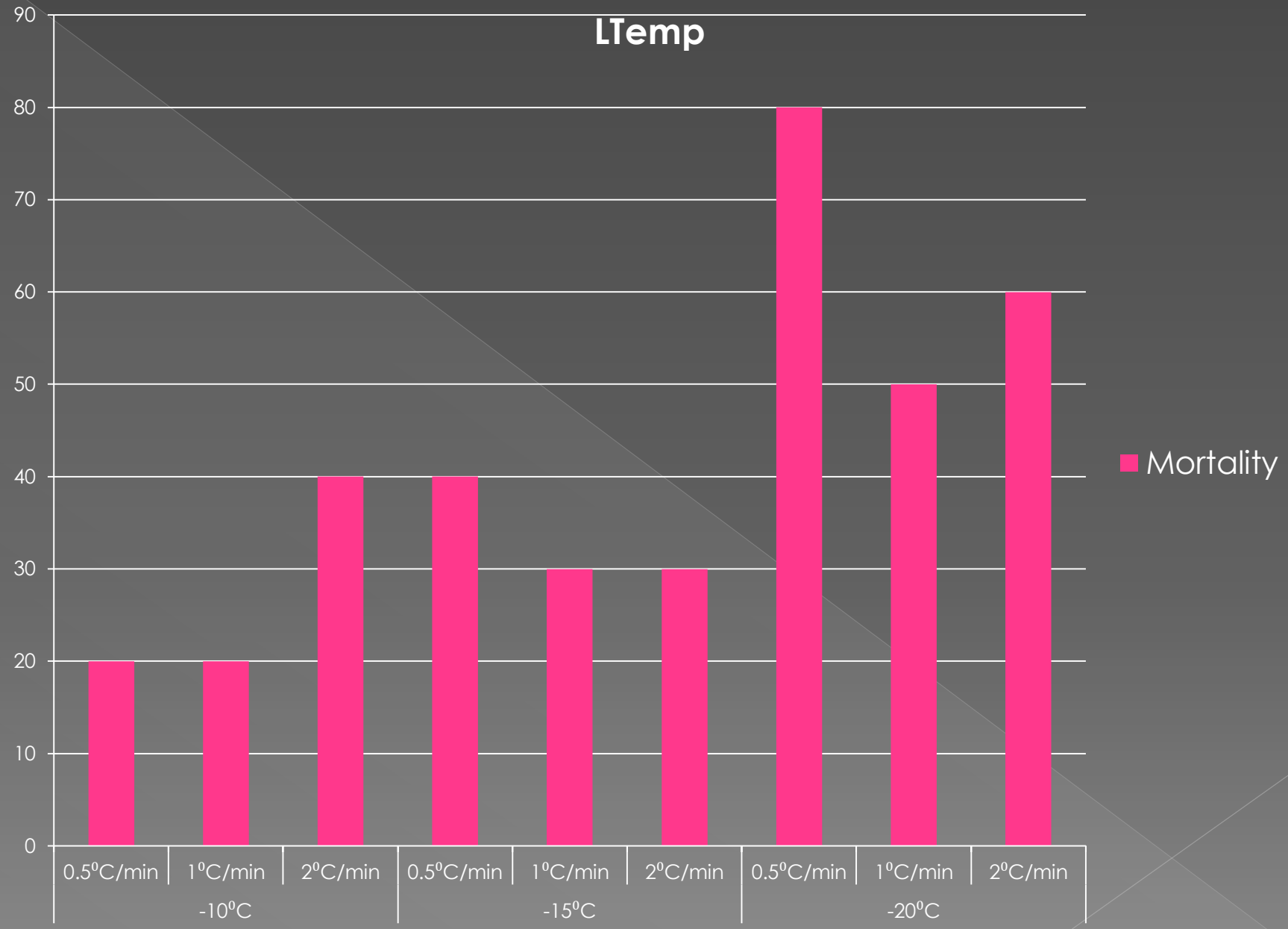
	SCP (°C)		
	2°C/min	1°C/min	0.5°C/min
	-15.40	-16.50	-17.75
	-16.18	-16.64	-23.40
	-19.16	-20.62	-25.35
	-20.16	-22.86	-26.19
	-21.64	-23.35	-26.41
	-24.44	-23.42	-27.18
	-24.45	-23.60	-28.40
Mean	-20.20	-20.10	-24.95

Experiment 2 – Lethal temperature (Ltemp)

- Firstly I transferred 10 mites using a paint brush from the populated leaf into the 10 beem capsules.
- Then I programmed the alcohol bath to 25^{0}C and put the beem capsules in to the test tube within the bath.
- I set a ramp down to -10^{0}C a minute at 2^{0}C/min.
- This was repeated for -10^{0}C, -15 ^{0}C and -20^{0}C at speeds of 0.5^{0}C/min, 1^{0}C/min and 2^{0}C/min.
- The lid was placed on the bath during the ramps to make sure no hot alcohol spilled out or I was not burnt.
- Then the beem capsules were removed from the alcohol bath and the mites were transferred onto a new leaf in a plastic container and sealed using parafilm around the lid.
- Then the mites were left for 24 hours in a rearing room at 23^{0}C.
- I then examined them to see which ones were alive and dead therefore seeing the mortality rate, showing if the spider mites had survived the temperature drop before the SCP.
- The temperature at which 50% of the mites are killed is the lethal temperature. The mortality percentage of the mites decreased as the temperature decreased.

Ltemp Results

Ltemp experiment									
	-10°C			-15°C			-20°C		
	0.5°C/min	1°C/min	2°C/min	0.5°C/min	1°C/min	2°C/min	0.5°C/min	1°C/min	2°C/min
Alive	8	8	6	6	7	7	2	5	4
Dead	2	2	4	4	3	3	8	5	6


LTemp
90
80
70
60
50
40
30
20
10
0
0.5⁰C/min
1⁰C/min
2⁰C/min
0.5⁰C/min
1⁰C/min
2⁰C/min
0.5⁰C/min
1⁰C/min
2⁰C/min
-10⁰C
-15⁰C
-20⁰C
Mortality

Experiment 3- Lethal time (Ltime)

- Firstly I prepared a leaf by pulling from the plant and wrapping tissue paper dipped in distilled water around the stem to keep the leaf fresh.
- Then I used an infected leaf and paintbrush to transfer 10 mites onto a clean leaf in a plastic container and sealed this with parafilm around the lid.
- The pot was then placed in a 10^0C room for one hour to avoid cold shock in the mites which would kill them.
- After the hour I placed the mites in the incubator at -5^0C for both 4 days and 6 days with separate experiments.
- After the time had passed, I removed the pot from the incubator and kept it at 10 ^{0}C for one hour to prevent cold shock then at 23^0C for 24 hours.
- Then I used a microscope to see how many of the mites were still alive. This shows me a comparison of the mites killed at 4 days and 6 days at -5^0C .

Ltime Results

Ltime		
	4 Day	6 Day
Alive	0	2
Dead	3	8

Conclusion.

- I came to the conclusion that as the temperature cooled to decreases the mortality percentage increases. And as the speed of cooling decreases the mortality percentage increases. This is despite the opposite in the SCP experiment because as the speed increased the SCP decreased causing the mites to freeze faster.
- As there was a trend in the Itemp experiment that as the temperature decreases mortality increases. It is also shown in the Itime experiment that 6 days is a sufficient time to kill 80% of the population.
- Therefore it seems that the most effective speed is 2^0C/min as shown in the SCP experiment.

Application

- Applying this to the real world would be applying it to glasshouses.
- If a crop was infected with mites the glasshouse environment could be adjusted accordingly to kill the mites.
- A temperature of -20^{0}C from 25 at 0.5^{0}C/min for 4 days should kill the population as shown by the results of my experiments.
- This can be used to save crops in glasshouses from spider mite infections instead of using pesticides which can harm humans.
- This method is more environmentally friendly also because it doesn't involve pesticides at all and this reduces air pollution and the amount of pesticides in the soil and water meaning that there is less eutrophication.
- This is also cheaper for farmers and industry as it doesn't require them to buy expensive pesticides and only requires them to adjust their conditions for 6 days. This should kill 80% of the population as shown in my results.
- This would result in the yield of crops increasing as fewer crops are being eaten by the mites therefore global demand is more likely to be met.
- This would also mean that the resistance in the spider mites would not evolve any further meaning that if a pesticide did need to be used in a crisis it would prove to be effective.

Questions?