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

Introduction:

Tetranychus urticae is also known as the "Spider mite". The arthropod is around 0.4 mm long. This arthropod is a pest that is common in most crops as it feeds on plants such as peppers, tomatoes, potatoes and strawberries.

Female *T.urticae* lays transparent eggs on the plant and a female can lay around five hundred eggs in a lifetime, this means the species reproduces rapidly and can cause major damage to plants and leaves especially. The female *T.urticae* are able to reproduce asexually to lay a male egg and this will then hatch and reproduce sexually with the female to lay more eggs.

An egg will hatch in 2 days and become mature in 5 days (ref. Wikipedia and talk from Miss White). This is another problem as this develops resistance by mutation rapidly due to sexual reproduction and also only one female is required to survive to repopulate a leaf (ref. Wikipedia).

The problems commercially as the spider mites are damaging crops that are widely used by sucking the cell contents from the leaves and leaving scars on the leaves.

The spider mite only causes a small lesion to the cell but many lesions caused by many of the mites damage the cell significantly restricting the photosynthetic ability of the cell restricting growth and destroying cells. The result is the crops not growing because the cells of the leaf are heavily damaged and are not able to perform photosynthesis therefore the fruit or vegetables are not able to be harvested.

Spraying the crops with pesticides is not an ethical or viable option because the food is so widely used it would be dangerous to spray toxic pesticides onto the food as this may harm humans. This is mainly due to the arthropods' resistance to pesticides because of the sexual reproduction.

An Ecological problem concerning pesticides is eutrophication, the growth of plant life on the surface of a water body due to pesticides entering the water though the soil or sewer system. The result is the water becoming anoxic and the life in the water will be killed due to lack of oxygen as there are no pests to control to plant life growing over the surface as the pesticides have killed them (ref. talk from Dr. Hayward).

This is also a problem economically as the yield of crops is decreasing whereas the population is increasing and this is a concern as there is no control over the pests affecting crops and there will eventually be a high demand for food which we might not be able to meet. As by 2050 the population of the earth is expected to reach 9.7 billion and the food demands may not be able to be met (ref. http://www.fao.org/fileadmin/templates/wsfs/docs/Issues_papers/HLEF2050_Global_Agriculture.pdf).

There are many ways to attempt to stop the spider mite damaging a crop. GM crops are one; this involves causing the crop to express a gene to naturals stop the pest. The bacteria *Bacillus thuingiensis* is used to cause the crops to express a toxin which is known to kill the mite and should not result in the development of resistance so far however there are many disagreements about the ethical use of these crops and their effect on human consumption in the long term and if there would be any potential dangers.

Another way which is the basis of my investigation is the use of biocontrol agents, these are organisms that are able to destroy or control the population of a pest in a crop. The biocontrol agent may be a predator or a parasitoid but either will kill the pest.

A biocontrol agent needs to be tested for the conditions of the winter in the UK as if it were to leave the glasshouse, would it be able to colonise and populate the UK. If this event occurred would it cause a problem to the surrounding environment such as displacing a species by attacking the other species and reducing biodiversity in the area .The biological control agent may not also target the species that it is supposed to meaning it is unusable. If a species is removed from the area the biocontrol agent moved to this will damage the ecosystem.

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 aim for this project is to see the conditions the mites are least likely to survive in and for which periods of time so this may be applied to glasshouses to kill pests killing crops. This will be done through a series of experiments that test the mites' survival at:

- Different time periods at set temperature below 0^oC (-5^oC)
- Different rates at which the temperature is altered
- Different temperatures below 0^oC including -10^oC,-15^oC and -20^oC

The mites were cultures on a French bean plant at 23^oC. Each mite colony I used was 6-7 days old to allow a large population.

My objectives are to see how these experiments affect the mites and how this can be taken further, how it can be applied to industry and used as an alternative to pesticides. This will be shown by the mortality percentage in the mites in the lethal temperature experiment.

My hypothesis for this research was as the temperature is decreased and the exposure time increases the mortality rate would increase. This is because as the temperature decreases the mites will become colder and eventually freeze. As exposure time increases, as the rate of a ramp decreases the mites would be exposed to the cold for a longer period slowing them down and their processes down causing them to slowly shut down and freeze and eventually die.

Each one of these points will be investigated by different experiments. Temperature and time were the best factors to investigate because they are the most applicable to the environment the mites live in and therefore the results may be used to adjust glasshouses for select amounts of time. The predators could have been tested however the effects on the mites themselves seem more relevant. Furthermore my experiments have not been done before so the results may show new information due to the originality of the experiments.

My project will be presented to industrial representatives and at an open day showing the public what I have learned from my experiments and how it could be applied to glasshouses as the experiments were original and provided new information.

The first experiment I conducted was the super cooling point experiment.

My hypothesis for this experiment is that I believe in this in experiment the lower ramping speed will increase the value for SCP.

Equipment:

- Programmable alcohol bath
- Oceotak
- Leaf populated with mites that is 6-7 days old from 23°C storage
- Microscope
- Paint brushes
- Thermocouple
- Tissue paper
- Distilled water

Method:

- 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^{o}C 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 ran the relevant program to record the temperature.
- I ramped down to -30^{o}C at different speeds of 0.5^{o}C/min, 1^{o}C/min and 2^{o}C/min to prolong exposure.
- 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.

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

The second experiment I performed was the lethal temperature experiment.

I believe in this experiment that as the temperature of the ramp decreases more insects will be killed and as the rate of the ramp increases the mortality rate will increase also.

Equipment:

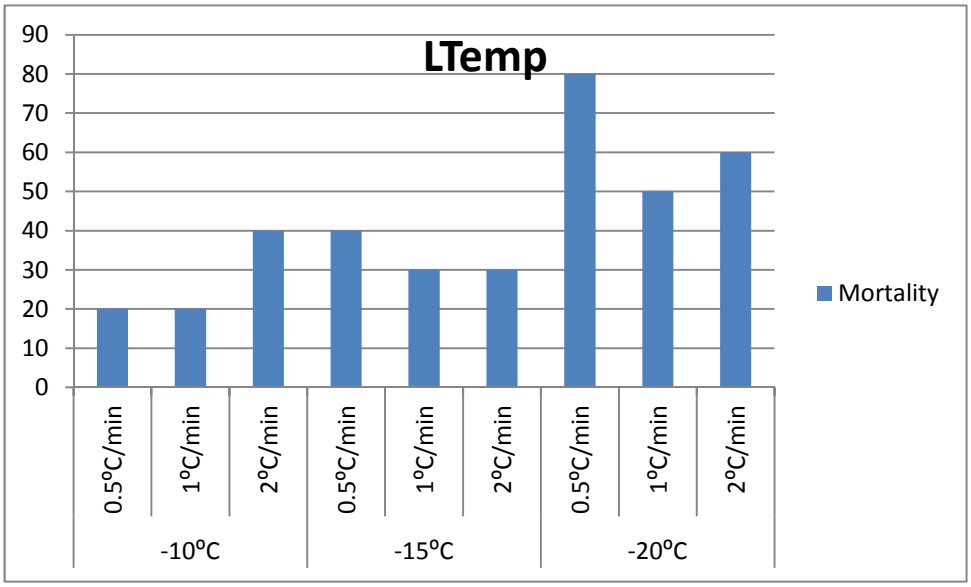
- Programmable alcohol bath
- Leaf populated with mites from French bean plant that is 6-7 days old from 23°C storage
- Microscope
- Paint brushes
- Tissue paper
- 10 Beem capsules
- Plastic container
- Parafilm

Method:

- 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^{o}C and put the beem capsules in to the test tube within the bath.
- I set a ramp down to -10^{o}C a minute at 2^{o}C/min.
- This was repeated for -10^{o}C, -15 ^{o}C and -20^{o}C at speeds of 0.5^{o}C/min, 1^{o}C/min and 2^{o}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^{o}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.

The results for the experiment are as follows (absolute values):

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



The third experiment I performed was a lethal time experiment.

Equipment:

- A leaf populated with spider mites that is 6-7 days old from 23 °C storage
- A clean leaf from French bean plant
- Paint brush
- Incubator
- Plastic container
- Parafilm
- Microscope
- Distilled water
- Tissue paper

Method:

- 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^{o}C 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^{o}C 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 ^{o}C for one hour to prevent cold shock then at 23^{o}C 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^{o}C.

Results:

Ltime		
	4 Day	6 Day
Alive	0	2
Dead	3	8

Discussion:

In conclusion I can see that the SCP decreases as the cooling speed increases which is the opposite of my original hypothesis as I thought the mortality rate and hence the SCP would increase as the cooling speed decreased. The mortality rate also increased as the cooling temperature decreased as shown by the lethal temperature experiment however the speed results seem to be varied and no distinct pattern can be seen to which speed gives the highest mortality in all 3 temperatures. The speed of 0.5^{o}C/min at -20^{o}C had the highest mortality percentage of 80% which agrees with my hypothesis and overall -20^{o}C seems to be the most lethal temperature as it had the highest mortalities overall. The ltime experiment showed me that the mites cannot survive 6 days at -5^{o}C.

The SCP value decreased from -20.10 to -25.00 and I believe this was due to the length of time allowed in 0.5^{o}C/min allowing the mites to produce the chemicals the mites needed to keep themselves warm such as glycerol and provided more time for reactions to occur meaning the mites will survive for a longer period of time as they have the chemical compounds available. This results in the SCP being a more negative number as the mites are surviving to lower temperatures.

In the Ltime experiment ice formed in the 4 day ltime arena meaning that some mites could not be found therefore the experiment for 4 day is a failure. So 6 day at -5^{o}C is sufficient to kill 80% of the population.

There was a trend in the Ltemp as the temperature decreased the mortality percentage increased for example at -10^{o}C at 0.5^{o}C/min the mortality was 20% however at -20^{o}C at 0.5^{o}C/min the mortality was 80% showing an increase and the increase is visible through the dead row in the table as it increased as the temperature decreased.

To continue this project further I would test lower temperatures and different times, both at different intervals meaning I could find the ideal conditions to kill a mite population. The intervals could be halved or even a quarter of what they were in this project.

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 -30^{o}C from 25 at 0.5^{o}C/min for 6 days should kill 80% of 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 4 days. 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.

Overall I believe my hypothesis to have been partially proven as the mortality percentage increased as the temperature decreased and although the SCP decreased as the speed increased the mortality percentage increased as the speed decreased meaning more died before the SCP value.

Problems I encountered:

During the SCP experiment the recording instrument had an error and I attempted to fix it myself then found my mentor who fixed it for me and then I continued with the experiment. If I ran this experiment again I would check the equipment beforehand setting up the mites to ensure it was all functioning properly.

During the SCP experiment one of the thermocouples was broken so I disconnected it and continued with 7 results. This still provided me with a valid set of results that could be used to calculate a mean and showed a pattern. To rectify this next time I would use a thermocouple with 10 fully functioning plugs for more accurate and valid means.

Due to sub-zero temperatures ice formed in the lethal time 4 day experiment plastic containers meaning this experiment was technically a failure as I lost several of the mites. If I ran this experiment again I would use the method that I changed to in the 6 day experiment which was to not allow the leaf to defrost and to move the mites to a new leaf whilst in the 10^{o}C room straight after removing them. This proved to work as I was able to find all 10 mites as the leaf did not curl up due to water.

Green boxes indicate time spent on my report and presentation.

Date	Time									
	09:00	10:00	11:00	12:00	13:00	14:00	15:00	16:00	17:00	18:00
04/08/2014		Introduction to Equipment	Intro to Project	CREST award introduction	Background research on Spider mites	Planning for my experiments				
05/08/2014		SCP 1°C/min	SCP 2°/min Take 1°C/min out		SCP 0.5°C/min Take 2°/min out			Take 0.5°C/min out		
06/08/2014	Ltemp -10°C 2°C/min	Ltemp -10°C 1°C/min Take 2°C/min out			Take 1°C/min Out					
07/08/2014	Ltemp -10°C 0.5°C/min			Ltemp -15°C 0.5°C/min Examine Ltemp -10°C 2°C/min and 1°C/min Take -10°C 0.5°C/min out				Ltemp -15°C 1°C/min and 2°C/min Take -15°C 0.5°C/min out		Take -15°C 1°C/min and 2°C/min out
08/08/2014	Ltime 4 day and 7 day at 10°C prep	Ltime 4 day and 6 day moved to -5°C		Examine Ltemp -10°C 0.5°C/min -15°C 1°C/min, 2°C/min and 0.5°C/min						
09/08/2014										
10/08/2014										
11/08/2014					Ltemp -20°C 1 °C/min			Ltemp -20°C 2°C/min Take -20°C 2°C/min Out	Take -20°C 2°C/min Out	
12/08/2014	Ltemp -20°C, 0.5°C/min 4 day Ltime prep. 1 hour at 10°C and 24 hours in 23°C room to rear.					Examine -20°C 1°C/min and 2°C/min				
13/08/2014				Examine 4 day Ltime Examine Ltemp -20°C, 0.5°C/min						
14/08/2014			6 day Ltemp prep hour at 10°C 6 day Ltemp moved to 23°C for 24 hours							
15/08/2014	Examine 6 day Ltemp									

References to human resources and materials:

http://en.wikipedia.org/wiki/Tetranychus_urticae

http://en.wikipedia.org/wiki/Biological_pest_control

http://www.fao.org/fileadmin/templates/wsfs/docs/Issues_papers/HLEF2050_Global_Agriculture.pdf

and www.scieb.br

provided me with the background research to give me a more in depth knowledge on the spider mites and the methods behind biocontrol to help me in my experiments and understand and explain things.

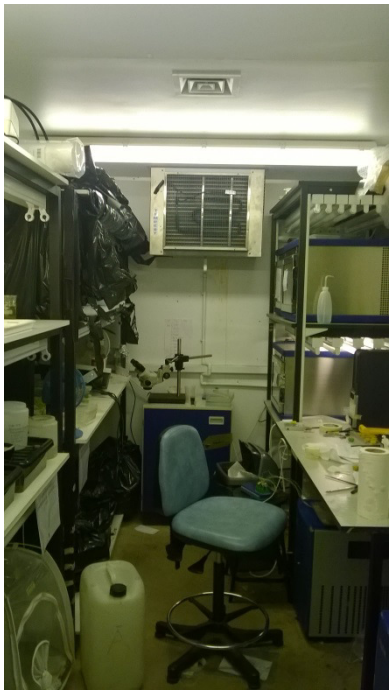
Acknowledgements.

Miss. White and Dr. Hayward; They assisted me by showing me how to perform the experiments and then allowing me to do it independently and also provided me with any other additional information that I required on the species I was working on.

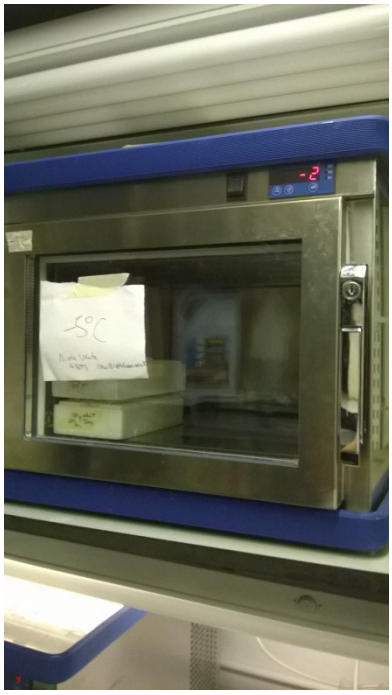
Equipment and machines I used with notes:

Programmable alcohol bath:

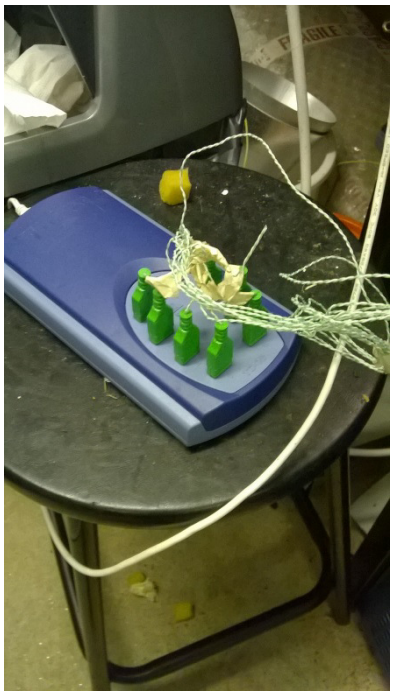
Temperature controlled room:



Incubator:



Thermocouple:



Oceotak: