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| **Project title:** | The role of naturally occurring entomopathogenic fungi in regulating aphid populations on vegetable Brassica crops |
| **Project number:** | CP 092 |
| **Project leader:** | Dr. Dave Chandler, Warwick Crop Centre, University of Warwick, CV35 9EF |
| **Report:** | Annual report, February, 2014 |
| **Previous report:** | n/a |
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| **Location of project:** | Warwick Crop Centre, University of Warwick, CV35 9EF |
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| **Date project commenced:** | 01/02/2013 |
| **Date project completed (or expected completion date):** | 30/04/2016 |

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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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## *CONTENTS*

# GROWER SUMMARY

## Headline

Brassica aphid populations are charaterised by a sharp population decline around July in the UK. At present this population 'crash' cannot be accurately predicted. Initial fieldwork results suggest the aphid population crash occurs regardless of plant age and that a guild of natural enemies are involved.

## Background

### *Aphids as crop pests*

Aphids (Hemiptera, Aphididae) are one of the most serious pests of vegetable brassica crops (Blackman & Eastop, 1984; Dedryver *et al.* 2010). Among the aphid species colonizing Brassica, *Brevicoryne brassicae* and *Myzus persicae* are the most economically important (Blackman & Eastop, 1984). Plant damage is caused directly via aphid feeding action on foliage and in the case of *B.brassicae* severe leaf fouling due to its tendency to form dense colonies, or indirectly through the transmission of plant pathogenic viruses including, turnip and cauliflower mosaic virus and cabbage black ring spot virus (Blackman & Eastop, 1984; Flint, 1985). Annual brassica yield losses due to aphid infestations range from 30% to 80% in developed and developing countries respectively (Razaq *et al.* 2011; Dedryver *et al.* 2010; Isik & Gorur, 2009). At present, aphid management in brassica crops is heavily reliant on the use of synthetic chemical insecticides and aphicides account for 39% of all insecticide applications (Garthwaite *et al.* 2007). Current chemical control methods of aphids include neonicotinoids, pyrethroids, pirimicarb, chlorpyrifos and pymetrozine (IRAG, 2012). However, growers are under pressure to reduce their reliance on insecticides: (a) consumer concerns (and by extension retailer concerns) over pesticide residues in food; (b) effective insecticides declining in number as a result of product withdrawals linked to new, more stringent health and safety criteria as part of European pesticides legislation (Directive EC1107/09); and (c) excessive use of insecticides resulting in control failure through the evolution of heritable resistance (IRAG, 2012). Whilst there is currently no evidence to suggest *B. brassicae* is resistant to insecticides *M.persicae* has three known resistance mechanisms, esterase, MACE and kdr rendering certain organophosphates, carbamates, and pyrethroids ineffective (IRAG, 2012). As a result, there is an urgent requirement to develop alternative forms of aphid management.

### *Aphid population dynamics*

Aphids are r-strategist insects that reproduce parthenogenetically meaning they are capable of producing significant amounts of biomass in a short period of time (Blackman & Eastop, 1984; Karley *et al*. 2004). However, the exponential growth seen during spring and early summer does not continue. During mid-summer (usually July) many aphid species exhibit a sharp population decline to apparent local extinction (Karley *et al.* 2003). This mid-season 'crash' occurs in the absence of insecticide in both agricultural and natural landscapes and populations generally remain low or undetectable for at least 6-8 weeks post crash (Karley *et al*. 2003; Karley *et al*. 2004). At present the timing of this crash cannot be accurately predicted.

Many factors have been suggested for the mid-season crash, ranging from ecological factors such as plant age, natural enemies and adverse weather conditions to population process including decreased birth rates (decreased performance), increased death rates (increased natural enemies action) and increased alate production (emigration) (Karley *et al*. 2004). Of the natural enemies, entomopathogenic fungi have been strongly implicated in the crash of populations but little is known of their biology (Karley *et al*. 2003; Karley *et al*. 2004). A better understanding of the role of natural enemies in aphid population dynamics might enable the mid-season crash to be forecast, which would give growers the option of withholding pesticide sprays. Particularly effective natural enemy species may also be worth considering as augmentation biocontrol agents.

As a result the aims and objectives of this project are:

Aim:

The proposed work will test the hypothesis that fungal epizootics are one of the principle factors causing the mid-season crash in populations of aphids on horticultural brassicas.

Objectives:

i. Monitor populations of healthy and fungus-infected cabbage aphids on sequentially planted brassicas and study the link between the mid-summer population crash and epizootics of insect pathogenic fungi.

ii. Identify insect pathogenic fungi associated with the cabbage aphid *Brevicoryne brassicae* on field brassicas.

iii. Use laboratory bioassays to measure the susceptibility of the cabbage aphid to fungi collected from the field and compare to commercial mycopesticides.

iv. Model the effect of temperature and moisture on the pathogenicity of fungi to the cabbage aphid to forecast the outbreak of fungal epizootics.

## Summary

Work began on Ascomycetes as fungi in this order are 'easier' to work with, which allowed time to get acquainted with the techniques required for working with *Pandora neoaphidis*. Moreover, the data collected will provide a useful comparison with *P.neoaphidis.*

### *Objective 1- Monitor populations of healthy and fungus-infected cabbage aphids on sequentially planted brassicas and study the link between the mid-summer population crash and epizootics of insect pathogenic fungi.*

Fieldwork for 2013 took place from May-October 2013 and consisted of two separate experiments as follows:

- Experiment 1: Monitor the development of populations of *B. brassicae* on plants of two different ages transplanted into the field at the same time.
- Experiment 2: Monitor the development of populations of *B. brassciae* within plots that have been planted sequentially, on 4 separate occasions from May to August.

Aphid populations on field brassicas increased until mid to late July when they crashed regardless of plant age. Associated with the timing of the crash was a fungal epizootic and a guild of other natural enemies suggesting a potential link. The composition of the guild of natural enemies changed throughout the course of the field season with parasitoid mummies being the most common. As the season progressed primary parasitoids became less frequent implying a potential fourth trophic level interaction that will be investigated further during the field season of year 2.

### *Objective 2 - Identify insect pathogenic fungi associated with the cabbage aphid Brevicoryne brassicae on field brassicas.*

Field experiments set up at Wellesbourne during the 2013 growing season in order to monitor aphid populations on brassicas and study the link between the mid-season crash and epizootics of insect pathogenic fungi (objective 1) did indeed see the establishment of a field epizootic which acted to reduced aphid infestations. Attempts were made to isolate the

fungi but were unsuccessful, however, morphological data suggests that the epizootic was caused by *Pandora neoaphidis* (Commonwealth Mycological Institute, 1979).

### *Objective 3 - Use laboratory bioassays to measure the susceptibility of the cabbage aphid to fungi collected from the field and compare to commercial mycopesticides.*

A series of bioassays were conducted in controlled environment rooms set at 20$^{o}$C and a 16:8 L:D photoperiod. Bioassays were repeated three times in a block design allowing flexibility in scheduling as fieldwork took over in May 2013. Replicates were completed in November 2013.

Initial analysis of results suggest the cabbage aphid, *Brevicoryne brassicae*, is more susceptible than the peach potato aphid, *Myzus persicae*, to the products Naturalis L (*Beauveria bassiana* (ATCC strain)) (Troy Biosciences Inc., 113 South 27$^{th}$ Ave. Phoenix, AZ 850433, USA), Mycotal (*Lecanicillium muscarium*) (Koppert B.V., Unit 8, 53 Hollands Road, Haverhill, Suffolk, CB9 8PJ, UK) and Vertalec (*Lecanicillium longisporum)* (Koppert B.V., Unit 8, 53 Hollands Road, Haverhill, Suffolk, CB9 8PJ, UK).

In bioassays at a dose of 1x10$^{8}$ml$^{-1}$ *L.longisporum* was the most effective control against *M.persicae* (LT$^{50}$ 4.67 days); conversely, it was the least effective against *B.brassicae* (LT$^{50}$ 4.67 days). The most effective control of *B.brassicae* was *L.muscarium*, LT$^{50}$ 3.33, however, this EPF proved to be least effective against *M.persicae* (LT$^{50}$ 5.33 days).

### *Objective 4- Model the effect of temperature and moisture on the pathogenicity of fungi to the cabbage aphid to forecast the outbreak of fungal epizootics.*

There are three processes vital to the infection and subsequent spread of infection within a host population; growth, germination and sporulation, each have been or will be considered in this project. Since fungi are ectothermic organisms their biology is largely driven by external temperature. Mycelial growth and germination assays were conducted during February-May 2013 & October-December 2013 respectively. Experimental treatment consisted of 6 different temperatures as to investigate and eventually model the influence temperature exerts on these processes.

Five of the six Ascomycetes included in mycelial growth experiments (*B. bassiana* ATCC & GHA, *M. brunneum, I. fumosoros* & *L. longisporum*) seemed to respond to temperature in a similar way with the optimum for growth approximately 25$^{o}$C. One interesting point to make is the difference for *L.muscarium* it seems to have a lower optimum for growth around 20$^{o}$C.

## Financial Benefits

It is difficult to comment on the financial benefits given that this work is in its infancy. However any new method that would allow growers to reduce their reliance on synthetic chemical would clearly be financially beneficial.

## Action Points

Experiments are still underway to elude the role entomopathogenic fungi play in the crash of aphid populations, as such there are no action points to growers at present.

## *SCIENCE SECTION*

### Introduction

Global population increase and climate change have brought to the forefront the need to increase food production whilst at the same time reducing the adverse environmental impacts of agriculture (Vega *et al.,* 2009). Crop losses due to pests, disease and weeds represent a major constraint to global food productivity. These losses account for 40% of potential production (Thacker, 2002). Despite a marked increase in the use of pesticides since the 1960s crop losses have not decreased (Bruce, 2010; Vega *et al.,* 2009; Oerke, 2006).

#### *Aphids as crop pests*

Aphids (Hemiptera, Aphididae) are one of the most serious pests of vegetable brassica crops. Among the aphid species colonizing Brassica, *Brevicoryne brassicae* and *Myzus persicae* are the most economically important. Details of their distribution and pest status are summarised (Table 1). Plant damage is caused directly via aphid feeding action on foliage and in the case of *B.brassicae* severe leaf fouling due to its tendency to form dense colonies, or indirectly through the transmission of plant pathogenic viruses including, turnip and cauliflower mosaic virus and cabbage black ring spot virus (Table 1). Annual Brassica yield losses due to aphid infestations range from 30% to 80% in developed and developing countries respectively (Isik & Gorur, 2009; Dedryver *et al*. 2010; Razaq *et al*. 2011).

**Table 1** Pest status of *B.brassicae* & *M.persicae*

| Species | Appearance | Status[3] & Host | Secondary impacts | Distribution |
|---|---|---|---|---|
| *B.brassicae*[1] | Grayish-green, waxy covering gives them a grayish-white to powdery blue appearance. Short dark siphunculi. Length 1.8-2.3 mm. | A problem after mild winters. Oligophagous on *Crucifers.* | Vector of 23 viruses of the *Cruciferae* family. | Native to Europe. Abundant worldwide (Inc. anterior and mid Asia, North America, North Africa, Australia and New Zealand). |
| *M.persicae*[2] | Varying shades from yellow, green to pink, red and almost black. Length 1.2-2.3 mm. | A problem spreading viruses during mild winters but less so due to widespread insecticide use. Considered a problem on a range of crops; potatoes, sugar beet, lettuce, brassicas and legumes. | Virus vector responsible for the transmission of over 100 plant viruses. Amoung the most important are Potato leaf roll virus, Beet western yellows virus and lettuce mosaic virus. | Worldwide. |

[1]Flint (1985)
[2]Blackman & Eastop (1984)
[3]Holland & Oakley (2007)

## *Aphid population dynamics*

Aphids are r-strategist insects that reproduce parthenogenetically, meaning they are capable of producing significant amounts of biomass in a short period of time (Blackman & Eastop, 1984). However, the exponential growth seen during spring and early summer does not continue. During mid-summer (usually July) many aphid species exhibit a sharp population decline to apparent local extinction (Karley et al. 2003). This mid-season 'crash' occurs in the absence of insecticide in both agricultural and natural landscapes and populations generally remain low or undetectable for at least 6-8 weeks post crash. At present the timing of this crash cannot be accurately predicted.

Many factors have been suggested for the mid-season crash, ranging from ecological factors such as plant age, natural enemies and adverse weather conditions to population process including decreased birth rates (decreased performance), increased death rates (increased natural enemies action) and increased alate production (emigration) (Karley et al. 2004). Of the natural enemies, entomopathogenic fungi have been strongly implicated in the crash of populations but little is known of their biology (Karley et al. 2003; Karley et al. 2004). A better understanding of the role of natural enemies in aphid population dynamics might enable the mid-season crash to be forecast, which would give growers the option of withholding pesticide sprays. Particularly effective natural enemy species may also be worth considering as augmentation biocontrol agents.

## *Current control methods*

At present aphid management in Brassica crops is heavily reliant on the use of synthetic chemical insecticides with aphicides account for 39% of all insecticide applications (Garthwaite *et al*., 2007). Current chemical control methods of aphids include neonicotinoids, pyrethroids, pirimicarb, chlorpyrifos and pymetrozine (IRAG, 2012). However, growers are under pressure to reduce their reliance on insecticides: (a) consumer concerns (and by extension retailer concerns) over pesticide residues in food; (b) effective insecticides declining in number as a result of product withdrawals linked to new, more stringent health and safety criteria as part of European pesticides legislation (Directive EC1107/09); and (c) excessive use of insecticides resulting in control failure through the evolution of heritable resistance (IRAG, 2012). Whilst there is currently no evidence to suggest *B. brassicae* is resistant to insecticides *M.persicae* has three known resistance mechanisms, esterase, MACE and kdr rendering certain organophosphates, carbamates, and pyrethroids ineffective (IRAG, 2012). As a result, there is an urgent requirement to develop alternative forms of aphid management making it more sustainable by reducing reliance on synthetic chemical pesticides.

## *Alternative control methods*

### *Integrated pest management (IPM)*

Integrated pest management (IPM) is accepted as the most expedient way to make crop protection more sustainable. IPM refers to the combined and coordinated use of chemical, cultural and biological control measures to minimise economic injury to crop plants (Garthwaite *et al*., 2007; FAO, 2013). Implementation relies upon close crop monitoring and surveys to determine infestation and economic injury levels for specific crops, which in turn inform action thresholds. Whilst in reality agrochemicals are still the cornerstones of many

pest management strategies, IPM aims to minimise “possible disruption to agro-ecosystems and encourage natural pest control mechanisms” (FAO, 2013).

With this in mind, much emphasis is now being placed on other components of IPM such as biological control.

### *Aphid biological control*

Aphid infestations are predated by a guild of aphidophagous natural enemies including true predators, parasitoids and pathogens i.e. entomopathogenic fungi (Diaz et al. 2010). This guild of natural enemy species has the potential to be exploited for use in three broad biological control strategies: classical, augmentation and conservation biological control (CBC). A good understanding of the ecological dynamics of aphid pest populations and their guild of natural enemies is required both to develop biological control strategies and to develop individual natural enemy species as useful biocontrol products for augmentation. CBC and/or augmentation control could be a useful approach for the control of aphids in Brassica crops in light of new EU legislation restricting the use of many agrochemicals. (Royal Society, 2009)

The most abundant native natural enemies in Brassica agroecosystems that share aphids as an extraguild prey type and considerably reduce aphid populations are *Pandora neoaphidis*, syrphids (*Episyrphus balteatus*), Aphidiidae and Aphelinidae (Karley et al. 2003; Karley et al. 2004). Natural enemies can be used simultaneously as part of a CBC strategy, although an increase in the richness of natural enemies used for pest control does not necessarily lead to a corresponding increase in CBC efficacy (Diaz *et al.* 2010), largely because intraguild predation and competitive exclusion will act to decrease the diversity of natural enemies within cropping systems. Natural enemies that co-occur naturally or that are introduced to a cropping system may have additive or synergistic effects should their feeding niches (realized niches) complement each other i.e. minimise exploitation competition (Diaz *et al.* 2010).

Thus, in order to conserve a diversity of natural enemies to give additive or synergistic effects, a good understanding of their ecology is required. This understanding is currently lacking in the brassica, *B.brassicae* system. In many situations natural enemies are present in agroecosystems, but are either too few or active too late to limit crop damage (Bruce, 2010). This provides opportunity to augment their contribution to biocontrol.

*Entomopathogenic (EPF) fungi*

The two largest fungal orders exhibiting entomopathogenicity are the Hypocreales and the Entomophthorales. The Hypocreales are considered to be generalist pathogens, causing death via toxin production (Pell *et al*. 2001), whereas, the Entomophthorales are considered to have evolved into higher parasite forms leading to narrow host ranges, forming close biotrophic associations with their insect hosts and seldom engaging in saprotrophic growth (Shah & Pell, 2003). In this context saprotrophic growth refers to the utilisation of dead or decaying matter within the soil as a nutrient source. Epizootics are often caused by both fungal orders because the host is comparatively motile when infected allowing for the spread of the pathogen (Shah & Pell, 2003). Individuals only become incapacitated upon death and sporulation of the fungus.

Little is known about the role entomopathogenic fungi play in natural population regulation of aphids, although data collected in HDC sponsored research at the Warwick Crop Centre indicates that they are associated with the mid season aphid crash on brassica and lettuce crops. The use of pathogens as biocontrol agents has lagged considerably behind that of predators and parasitoids (Lacey *et al*. 2001; Maddox *et al.* 1992). However, entomopathogenic fungi exhibit certain ecomorphological adaptations in their potential as biological control agents (BCAs) for sucking pests i.e. aphids, where the stylet feeing mechanism prevents the transmission of other entomopathogens via ingestion, as they invade through the hosts cuticle or exoskeleton thereby circumventing the need to be ingested (Scorsetti *et al.*, 2010). Their considerable potential is reflected in the large amount of literature concerning their use as BCAs, however problems in mass production and a lack of knowledge on how abiotic factors influence efficacy in field situations have hampered their widespread use (Shah & Pell, 2003; Hajek *et al*. 2002). Literature on the use of entomopathogenic fungi (EPF) as BCAs is considered below in relation to the three broad biological control approaches:

**Classical biological control**

Classical biological control states that a pest species is exotic to an area and has been able to establish in the absence of its guild of natural enemies. Larvae of the gypsy moth, *Lymantria dispar*, feed on the leaves of many trees including oaks and aspen. It was accidentally introduced to the USA in the 1860s; control with *Entomopthora maimaiga* is now widespread in the United States through a combination of releasing infected cadavers, collecting resting spores from the soil and through wind dispersal. Work is currently underway investigating mass production methodologies to reduce the labour requirements

associated with using *E. maimaiga* in the above strategies (Hajek *et al*. 2002; Shah & Pell, 2003).

**Augmentation biological control**

Natural enemies are generally too few in number within the crop to effectively control pest levels and augmentation biological control aims to enhance their control efficacy through two strategies. Either in an inoculative capacity as with the EPF *Hirsutella thompsonii* (McCoy, 1981) or *Verticillium lecanii* (Hall, 1981), often inoculative releases are repeated during a season as it is not expected that the epizootic will persist, or in an inundation capacity, in an approach similar to that used for agrochemical application. (Shah & Pell, 2003). Indeed, the term 'mycoinsecticide' has been coined (Shah & Pell, 2003). At present there are a few commercial augmentation products for the control of aphids including *Verticillium lecanii* (marketed as 'Vertalec' and 'Mycotal') (Table 2). A prerequisite of this approach demands that any species that is to be used in this way can be grown in an economic manner in order to produce the large mount of inoculum required during application. As a result there are currently no examples of the use of Entomophthoralean fungi in an augmentation approach (Shah & Pell, 2003).

**Conservation biological control**

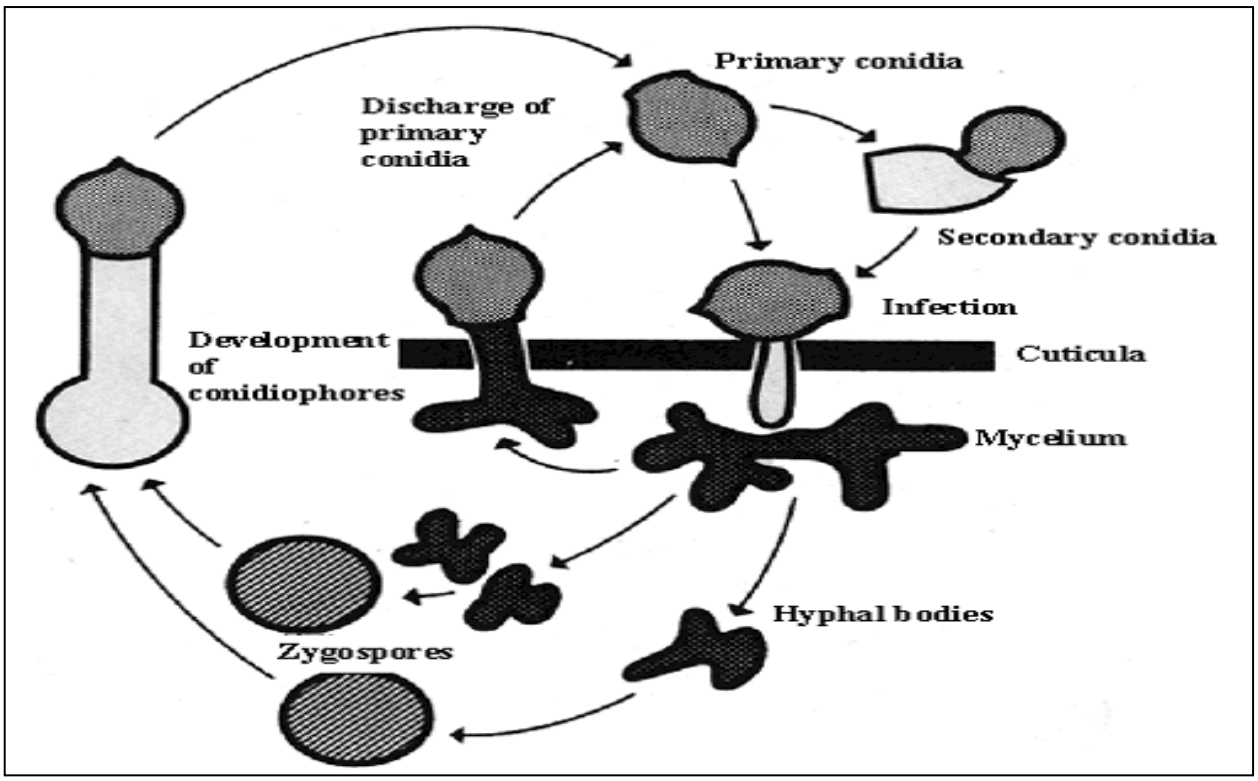
CBC requires the manipulation of the environment to enhance the activity of the enzootic natural enemies, including fungal pathogens, adapting farming practices to enhance their control potential in the field. Such manipulations have proved to be successful in systems that are well understood such as with *Neozygites fresenii* and *Aphis gossypii* on cotton in the United States (Shah & Pell, 2003; Steinkraus *et al,* 1995). This example has proved to be a particular success and now covers a large portion of the USA and has it own dedicated website: (http:// www.uark.edu/misc/aphid/) to keep growers up to date (Shah & Pell, 2003). Shah *et al*. 2001 suggest the use of field margins as refugia for *Pandora neoaphidis* by allowing aphids to persist in the environment on secondary hosts within the margin once the crop has been removed.

### *Pandora neoaphidis*

*Pandora neoaphidis* is the commonest entomopathogen causing epizootics in aphid pest species. It has a wide distribution, recorded from Europe, Asia, Africa, North and South America and Australia (Shah & Pell 2003) and is a highly specific, obligate parasite of aphids, presenting no risk to other natural enemies (Diaz *et al.* 2010). As a result it has received much attention as a biological control agent. Conidia attach to the external surface of a host and under permissible conditions of temperature and humidity the conidia germinate, penetrating the host's cuticle and colonizing the body cavity or hemocoel. Death in some species is attributed to toxin production. Again under permissible conditions conidiophores develop and in the case of *P.neoaphidis* primary conidia are actively discharged creating the characteristic 'halo' of spores around the infected cadaver. Its lifecycle is summarised in figure 1a, *P.neoaphidis* exists as protoplasts rather than zygospores (Shah & Pell 2003). Issues with mass culturing Entomophthorales on a commercial scale mean that at present *P.neoaphidis* is best exploited through a CBC approach.

**Figure 1a. Generalised lifecycle of Entomophthoralean fungi**

(Danish Ministry of the Environment, 2013.



Aim:

The proposed work will test the hypothesis that fungal epizootics are one of the principle factors causing the mid-season crash in populations of aphids on horticultural brassicas.

Objectives:

i. Monitor populations of healthy and fungus-infected cabbage aphids on sequentially planted brassicas and study the link between the mid-summer population crash and epizootics of insect pathogenic fungi.

ii. Identify insect pathogenic fungi associated with the cabbage aphid *Brevicoryne brassicae* on field brassicas.

iii. Use laboratory bioassays to measure the susceptibility of the cabbage aphid to fungi collected from the field and compare to commercial mycopesticides.

iv. Model the effect of temperature and moisture on the pathogenicity of fungi to the cabbage aphid to forecast the outbreak of fungal epizootics.

## Materials and methods

### *Insect rearing & plant maintenance*

Populations of *B.brassicae* and *M.persicae* were reared on 5\6 week old Brussels sprout plants (*Brassica oleracea* Gemmifera group) cv. 'Trafalgar' (Tozer seeds Ltd, Surrey, UK, KT11 3EH) grown singly in pots (5cmx5cmx8cm) filled with soil (F2+S, Levington compost, Surrey, UK, GU7 1XE). Infested plants were placed in bugdorms (30x30x30) (BugDorm store, MegaView Science Co., Ltd, Taiwan, 40762) to prevent cross contamination and ensure adequate ventilation. Infested and 'clean' plants were stored in separate controlled environment rooms, however, conditions remained the same at 20±2$^{o}$C, 16L: 8D photoperiod, airflow 0.31m$^{3}$s$^{-1}$. Colonies were 'refreshed' with the introduction of a new plant every 2 weeks and the oldest colony removed. Plants were watered *ad libitum* and watering trays were cleaned when required.

### *Ascomycota fungal isolates*

Six EPF Ascomycota from 4 different genera were used in the study. All isolates are currently utilised in various mycopesticide products (Table 2). Stock cultures of the isolates were stored on porous plastic beads in cryotolerant plastic tubes (Merick) over liquid nitrogen vapour (Chandler 1994). Laboratory cultures were grown on Sabouraud dextrose agar (SDA) slopes (universal tubes) (Starlab UK, Milton Keynes, MK14 5NA) and maintained in darkness at 20±1$^{o}$C for 10 days before being transferred to cold storage (4±2$^{o}$C, darkness). Working cultures were obtained from these slopes as required and grown on 90mm triple vented petri dishes (Fisher Scientific). Laboratory culture slopes were replaced every 3-4 months. The above procedure minimised the potential attenuation of fungal cultures.

**Table 2. Fungal isolates**

| Species | Isolate* | Original host | Source |
|---|---|---|---|
| *Beauveria bassiana* (ATCC strain) [1] | 432.99[(a)] | *Anthonomus grandis* | USA |
| *Beauveria bassiana* (GHA strain) [1] | 433.99[(b)] | *Bemisia* spp. | USA |
| *Lecanicillium muscarium* *Verticillium lecanii* | 19.79[(c)] | *Trialeurodes vaporariorum* | UK |
| *Metarhizium brunneum* | 275.86[(d)] | *Cydia pomonella* | Germany |
| *Isaria fumosoroseus* | 409.96[(e)] | *Phenacoccus solani* (Hemiptera: Pseudococcidae) | USA |
| *Lecanicillium longisporum* | 1.72a[(f)] | Vertalec | |

Isolate reference for the Warwick crop centre culture collection.

[1]Rehner & Buckley (2005)

[(a)] Isolate forms the active ingredient in the proprietary mycopesticide 'Naturalis L' (Troy Biosciences Inc., 113 South 27$^{th}$ Ave. Phoenix, AZ 850433, USA).

[(b)] Isolate forms the active ingredient in the proprietary mycopesticide 'BotaniGard' (Mycotech, 117 South Parkmont, Butte, MT, 59702-4109, USA).

[(c)] Isolate forms the active ingredient in the proprietary mycopesticide 'Mycotal' (Koppert B.V., Unit 8, 53 Hollands Road, Haverhill, Suffolk, CB9 8PJ, UK).

[(d)] Isolate forms the active ingredient in the proprietary mycopesticide 'Met52' (Novozymes Biologicals Inc., 5400 corporate circle, Salem, VA 24153, USA).

[(e)] Isolate forms the active ingredient in the proprietary mycopesticide 'PFR97' (ThermoTrilogy Corporation, 9145 Guildford Road, Suite 175, Columbia, MD 21046, USA).

[f] Isolate forms the active ingredient in the proprietary 'Vertalec' (Koppert B.V., Unit 8, 53 Hollands Road, Haverhill, Suffolk, CB9 8PJ, UK).

### *Objective 1- Monitor populations of healthy and fungus-infected cabbage aphids on sequentially planted brassicas and study the link between the mid-summer population crash and epizootics of insect pathogenic fungi.*

The following fieldwork was carried out on plots of Brussels sprout (*Brassica oleracea*) cv. Trafalgar (Tozer Seeds Ltd, Surrey, UK, KT11 3EH) at Warwick Crop Centre, Wellesbourne, CV35 9EF, UK from May to October 2013. The field area was orientated NNE to SSW and was composed of two separate experiments. The first experiment consisted of 24 plots (12 'young' plots & 12 'old' plots) of 10 experiment plants (arranged 2 plants x 5 plants, with 50cm spacing) separated by a single guard row ordered in three beds of equal length (8 plots). All plants were transplanted to the field on the 15th June 2013 after experimental treatment. 'Young' plants were grown for 4 weeks in a glasshouse and 'old' plants were grown in the glasshouse for 8 weeks prior to transplanting. Experiment 2 consisted of 16 plots (4 planting occasions x 4 replicates) of 10 experimental plants (arranged as in experiment 1) ordered in a randomized complete block design. Plots were separated by 'double' guard rows meaning the total size of the experiment covered an area of 8m x 14m. Plots of Brussels sprout plants were transplanted sequentially: on 2nd May, 3rd June, 3rd July and 5th August 2013 after a month of growth in Hassy trays in a glasshouse. Each of the 4 replicates for each transplant date were randomly assigned plots in a 4 x 4 plot grid. Tracer (Dow AgroSciences) was used as a module drench to protect all plants against cabbage root fly damage prior to field transplantation; no additional agrochemicals were applied.

*Brevicoryne brassicae* colonies for the initial infestation of field plots were maintained in a controlled environment room at 20°C, 16: 8h L: D on 5-6 week old *B. oleracea* plants within 'bug-dorm' cages (30 x 30 x 30cm) (MegaView Science co Ltd, Taichung, Taiwan). Plants were "refreshed" every 2-3 weeks to maintain the culture. After a week in the field all experimental plants in experiment 1 were inoculated with 5 adult, apterous *B.brassicae* adults using clip cages; these cages were removed after approx.. 4 hours to ensure establishment of individuals. In experiment 2, May and June transplants were inoculated with 5 *B.brassicae* apterous adults per plant. Subsequent transplants (July & August) were colonised naturally.

The experimental Brussels sprout plants were inspected every 7-14 days until October 2013. An entire Brussels sprout plant constituted a single sample unit. Plants were

examined visually to record aphid density, fungus-infected cadavers, syrphid larvae, parasitoid mummies, Coccinellidae and anthocorids. Fungi infected individuals were identified as sporulating cadavers. Due to time constraints as aphid population density grew, three out of the ten plants per plot were randomly inspected.

### *Objective 2- Identify insect pathogenic fungi associated with the cabbage aphid Brevicoryne brassicae on field brassicas*

Field experiments set up at Wellesbourne during the 2013 growing season in order to monitor aphid populations on brassicas and study the link between the mid-season crash and epizootics of insect pathogenic fungi (objective 1) did indeed see the establishment of a field epizootic which acted to reduced aphid infestations. Attempts were made to isolate the fungi but were unsuccessful; however, morphological data suggests that the epizootic was caused by *Pandora neoaphidis* (Commonwealth Mycological Institute, 1979).

Isolation techniques consisted of collecting individual aphids from the field approximately 100. Some of which that were yet to show signs of sporulation so they could be surface sterilised (an ethanol bath, followed by hypochlorite and finally two sterile water baths) and put on SEMA in order to culture *P.neoaphiids* if sporulation occurred. Attempts were also made to establish an *in vivo* culture. Sporulating individuals were collected from the field and placed on infested Brussels sprout plant to encourage cross infection. Humidity was artificially raised by enclosing the infested plant with two plastic bags, after 24 hours one plastic bag was removed. Lastly healthy *B.brassicae* individuals were placed in close proximity to sporulating cadavers in a petri dish with moist filter paper in order to maintain high humidity. After 4-5 hours the healthy individuals were placed onto a ‘clean’ Brussels sprout plant and double bagged for 24 hours, as above.

### *Objective 3 - Use laboratory bioassays to measure the susceptibility of the cabbage aphid to fungi collected from the field and compare to commercial mycopesticides.*

Work began on Ascomycetes as fungi in this order are ‘easier’ to work with, which allowed time to get acquainted with the techniques required for working with *Pandora neoaphidis*. Moreover, the data collected will provide a useful comparison with *P.neoaphidis.*

To determine the lethal concentration ($LC_{50}$) of *Beauveria bassiana* (432.99), *Lecanicillium muscarium* (19.79) and *Lecanicillium longisporum* (1.72a) to *Brevicoryne brassicae* and *Myzus persicae* fixed aged populations of *B.brassicae & M.persicae* were reared by

infecting ‘clean’ 4-5 week old Brussels sprout plants with 20 apterous adults and placing a bread bag over the potted plant. After 48 hours all adults were removed from the plant and the nymphs allowed to mature to adulthood; 10 days and 8 days for *B. brassicae* and *M. persicae* respectively. Spore suspensions of EPF were prepared by harvesting conidia in 0.05% Triton X-100 from Petri dishes (90mm) grown for 10-12 days in darkness at 20±1$^{o}$C. Suspensions were enumerated using an Improved Neubauer haemocytometer and were adjusted to concentrations of $3x10^{5}ml^{-1}$, $1x10^{6}ml^{-1}$, $1x10^{7}ml^{-1}$ and $1x10^{8}ml^{-1}$. The procedure remained identical for each isolate. Cohorts of approx. 20 adult aphids were exposed to the above suspensions using a Potter tower (2ml at 5lbs/inch$^{2}$); control aphids were sprayed with 0.05% triton X-100. Effective dose was calculated from a cover slide placed in the Potter tower at each exposure. After an hour the 10 most active individuals were placed on a single leaf of a 4-5 week old Brussels sprout plant and subsequently encased in a Blackman box (12.5cmx8cmx2cm) containing moist filter paper. All plants were then placed in a controlled environment room at a temperature of 20$^{o}$C and a 16:8 L:D photoperiod. Mortality was monitored daily for up to 10 days, any dead aphids were placed in a marked petri with moist filter paper to encourage sporulation and checked after 24 hours to attribute death to mycosis.

Regarding *P.neoaphidis* bioassays, this is a second year aim. With various *P.neoaphidis* isolates now stored in liquid nitrogen work can begin developing bioassay in order to manipulate temperature and humidity in the second year. Work on a method is underway and preliminary bioassays are to take place in February 2014.

## *Objective 4- Model the effect of temperature and moisture on the pathogenicity of fungi to the cabbage aphid to forecast the outbreak of fungal epizootics.*

There are three processes vital to the infection and subsequent spread of infection within a host population; growth, germination and sporulation, each have been or will be considered in this project. Since fungi are ectothermic organisms their biology is largely driven by external temperature. Mycelial growth and germination assays were conducted during February-May 2013 & October-December 2013 respectively. Experimental treatment consisted of 6 different temperatures as to investigate and eventually model the influence temperature exerts on these processes.

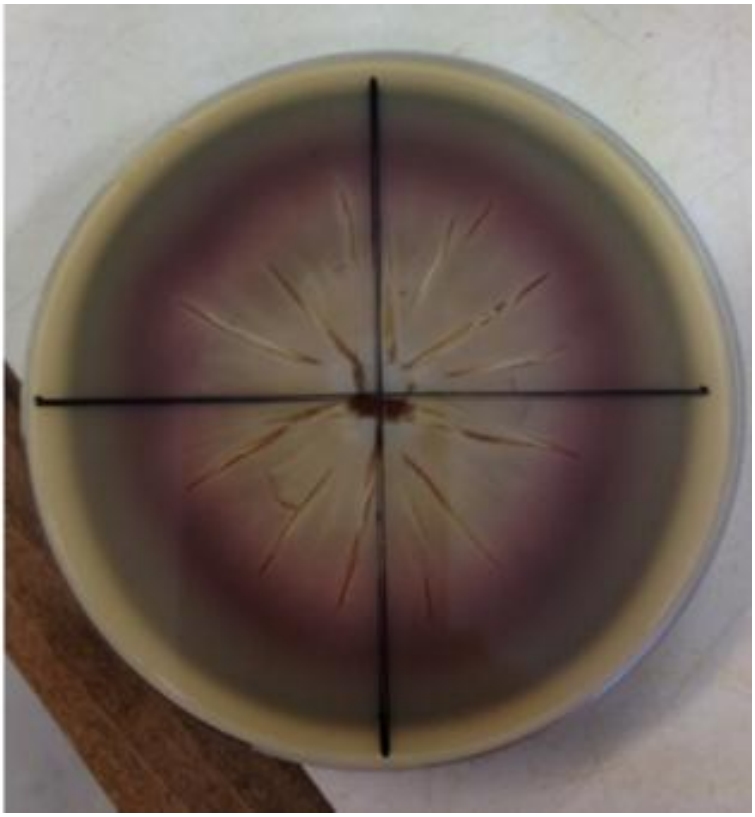
**Mycelial growth assays under different temperatures**

Mycelial growth assays were carried out between February and May 2013 on all 6 species of Ascomycota (Table 2) at various temperatures (10, 15, 20, 25, 30 and 33$^{o}$C) on two

different growth media; SDA (Sabouraud dextrose agar) and SEMA (Sabouraud dextrose agar supplemented with milk and egg yolk) (for method see appendix 2). Fungal species for experimental use were taken from slope cultures (see Ascomycota fungal isolates) and grown on 90mm petri dishes (Fisher Scientific) containing SDA in darkness at 20$^{o}$C for 15 days to ensure enough conidia could be harvested. Conidial suspensions were made by agitating the mycelium with an 'L-shaped' (Fisher scientific) spreader in 0.05% Triton. Suspensions were enumerated using an Improved Neubauer haemocytometer and diluted (in 0.05% Triton) to 1x10$^{7}$ml$^{-1}$. Then 0.1ml of the 1X10$^{7}$ml$^{-1}$ suspension for each species was spread over a fresh plate (SDA) and left for 48 hours. Seven mm plugs were taken and inverted in the centre of a 90mm petri dish containing either SEMA (Sabourads-Egg-Milk-Agar) or SDA marked with an x/y axis on the base for experimental treatment (Picture 1). The above was carried out in sterile conditions to prevent contamination. Assays were read every 3-4 days for a total of 22 days by measuring colony extension along 2 axes (mm) (Picture 1).

Experiments with *P.neoaphidis* are yet to be conducted.

**Picture 1.** Mycelial growth assay. Underside of Petri showing the 2 axes, *Beauveria bassiana* on SEMA growth media.

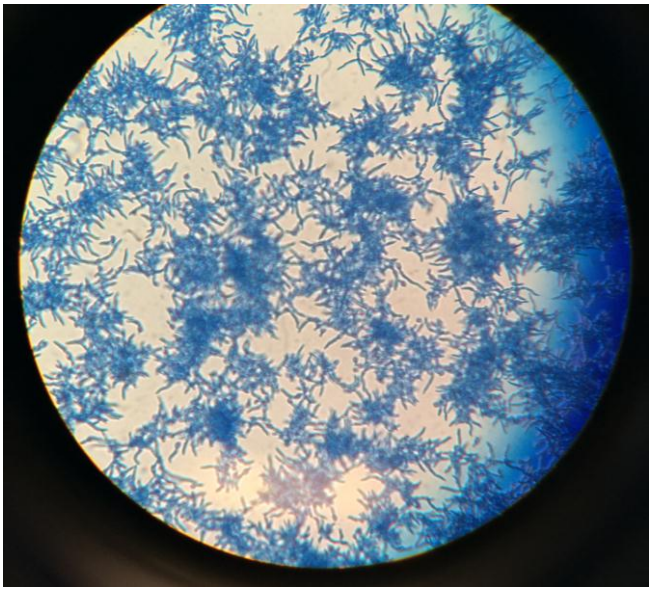


### *Germination assays under different temperatures*

Experiments investigating the effect of temperature on germination of the Ascomycota fungal species (Table 2) are currently underway. Experiments with *P.neoaphidis* are yet to be done. The method is as follows:

Fungi were grown in the dark on SDA for 17 days at 20$^{o}$C ± 1$^{o}$C. Conidia were harvested in 0.05% Triton X-100, enumerated using an Improved Neubauer haemocytometer, and adjusted to a concentration of 1X10$^{7}$ conidia ml$^{-1}$. Aliquots of 20µl were pipetted onto three previously marked circles (approximately 1cm diameter) on the germination media, plates were sealed with parafilm and incubated in the dark at a range of temperatures; 10, 15, 20, 25, 30 and 33$^{o}$C. Sampling was carried out destructively at regular time intervals (every four hours for 48 hours) by pipetting a drop of lactophenol methylene blue inside each circle. Plates were sealed and stored at 4$^{o}$C, before examination under the light microscope (magnification x200). Incidence of germination was recorded for approximately 100 conidia per circle. Germination was defined as the point when an emerging germ tube was equal to, or larger than, the length of the conidium.

**Picture 2.** Germination plate showing *Beauveria bassiana* stained with lactophenol



### *Sporulation*

Other than a review of the literature no work has been done regarding the effects of temperature or humidity on sporulation, this will be reviewed in year 2.

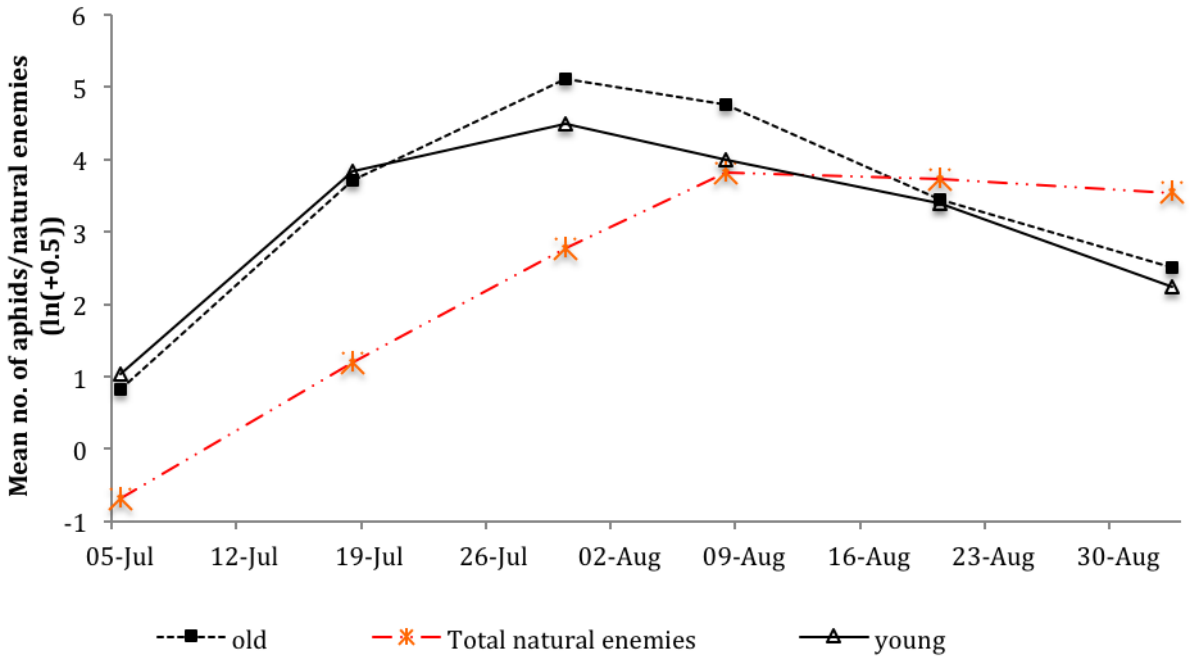
## Results

### *Objective 1- Monitor populations of healthy and fungus-infected cabbage aphids on sequentially planted brassicas and study the link between the mid-summer population crash and epizootics of insect pathogenic fungi.*

Fieldwork for 2013 consisted of two separate experiments as follows:

- Experiment 1: Monitor the development of populations of *B. brassicae* on plants of two different ages transplanted into the field at the same time.
- Experiment 2: Monitor the development of populations of *B. brassciae* within plots that have been planted sequentially, on 4 separate occasions from May to August.

***Figure 1. Results from experiment 1.*** *Aphid and natural enemy poplation dynamics on 'young' (4 weeks of age on trasplant date) and 'old' (8 weeks of age on transplant date) plants.*




Aphid population decline occurred around the end of July regardless of plant age (figure 1). This is true of experiment 2 also (figure 2). Plant material was collected from experiment 2 in order to quantify the differences in plant age; HPLC analysis will be conducted December 2013. Soluble Nitrogen is linked to plant age, decreasing, as plants get older. Soluble

Nitrogen is also an important factor in the fecundity of aphids. HPLC analysis will highlight these plant age effects on soluble Nitrogen levels and reinforce findings from the field that aphid population crash regardless of plant age and regardless of the effects that plant age may indirectly have on aphid population dynamics through soluble Nitrogen,

In both experiments we see a corresponding increase in the population size of the guild of natural enemies, alluding to a clear density dependent relationship. Formal modeling of the population dynamics is to be looked at in more detail in year 2. In experiment 2 we don't see a corresponding increase in natural enemies as the aphid population begins to build post crash (figure 2). Potentially these natural enemies are themselves being 'controlled' by hyperparasites and/or hyperpredators as their numbers build throughout the course of the field season. Indeed, the notion of a fourth trophic level interaction is supported by the literature (Sullivan & Völkl, 1999) and by preliminary qualitative data collected this year by myself as I have managed to identify hyperparasites of primary parasitoids obtained from aphid mummies collected from experiments 1 and 2. Examining and quantifying this interaction will be an aim of year 2 fieldwork.

***Figure 2. Results from experiment 2.*** *Aphid and natural enemy population dynamics on sequentially transplanted plots.*

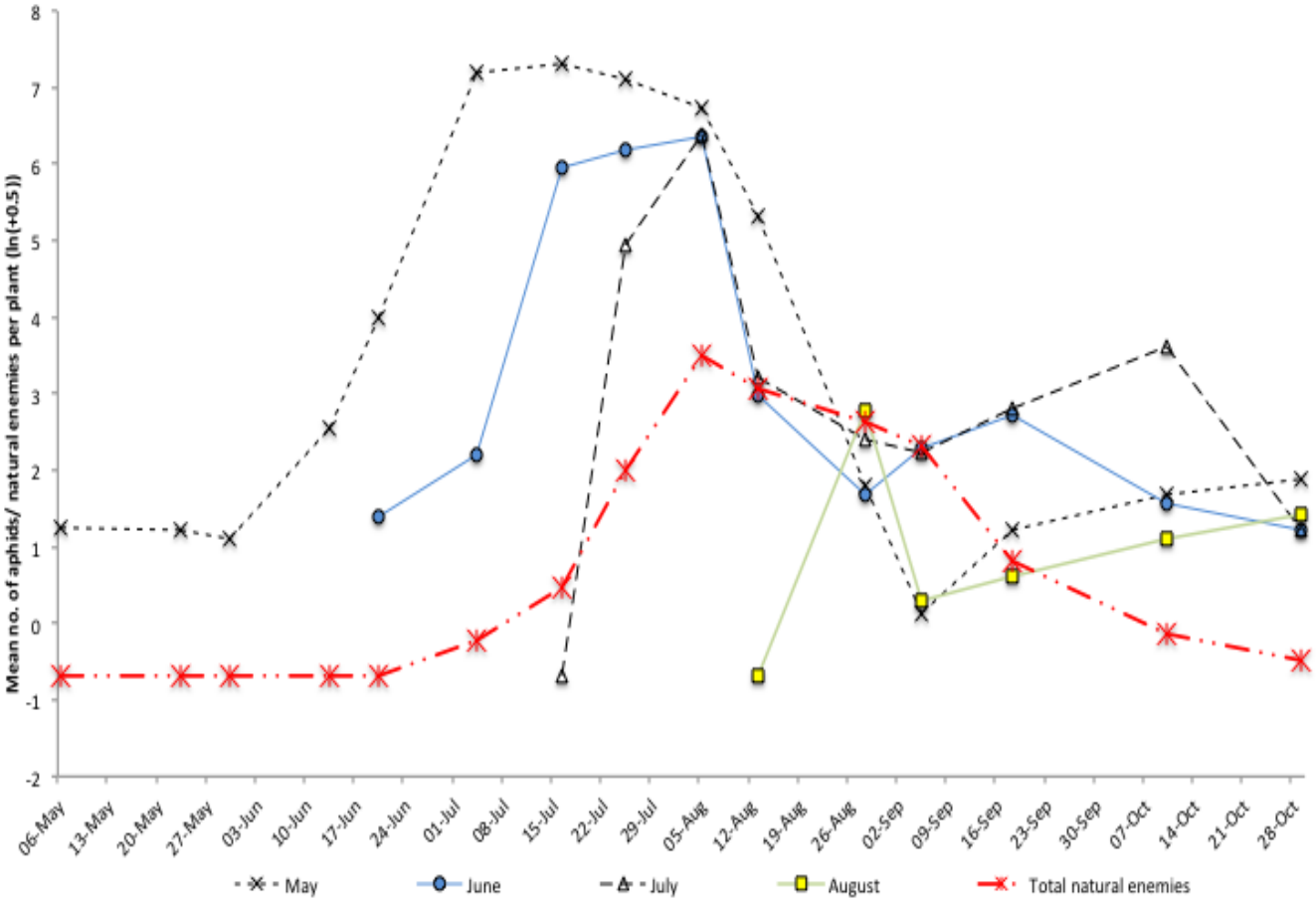


***Figure 3. Natural enemy community structure***. *The relative number of each clade of natural enemy associated with B.brassicae in experiment 2.*

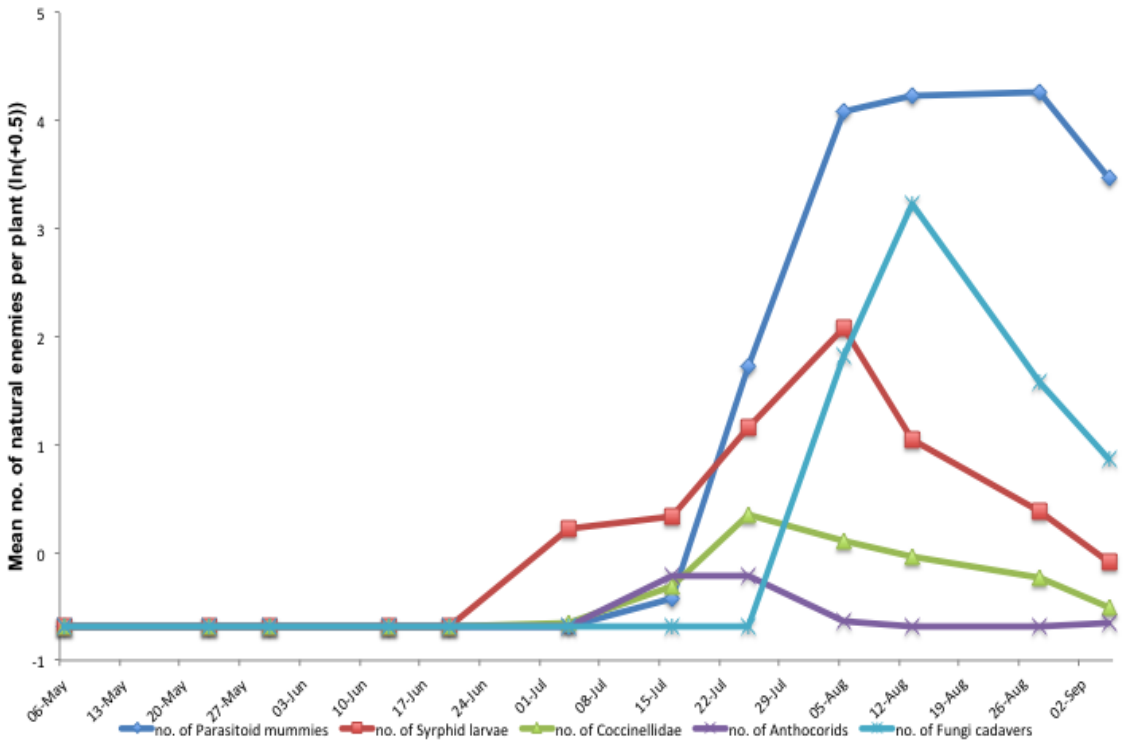



Figure 3 elucidates the guild of natural enemies associated with *B.brassicae* in experiment 2 and their relative numbers over time. More importantly it highlights the timing of the fungal epizootic, which occurs at the end of July when aphid population density is at it's highest and during which we see the aphid population in both experiments begin to decline. While cause and effect is difficult this shows a strong experimental association between the timing of the fungal epizootic and the aphid population crash. Temperature and humidity data for each experiment was collected and will be used in conjunction with year 2 and 3 field data to develop a forecast of the epizootic.

## *Objective 2- Identify insect pathogenic fungi associated with the cabbage aphid Brevicoryne brassicae on field brassicas.*

The 2013 field season at Wellesbourne saw the establishment of a fungal epizootic, during which attempts were made to isolate the fungi from the field. Morphological data suggests the epizootic was caused by *Pandora neoaphidis*. Unfortunately none of the isolation techniques resulted in successful isolation of the fungi. As a result sporulating individuals were collected from the field and placed in eppendorfs with 10% glycerol for -80$^{o}$C storage.

It is hoped that isolation will be possible from these cadavers. Failing this the techniques described above will be used in 2014.

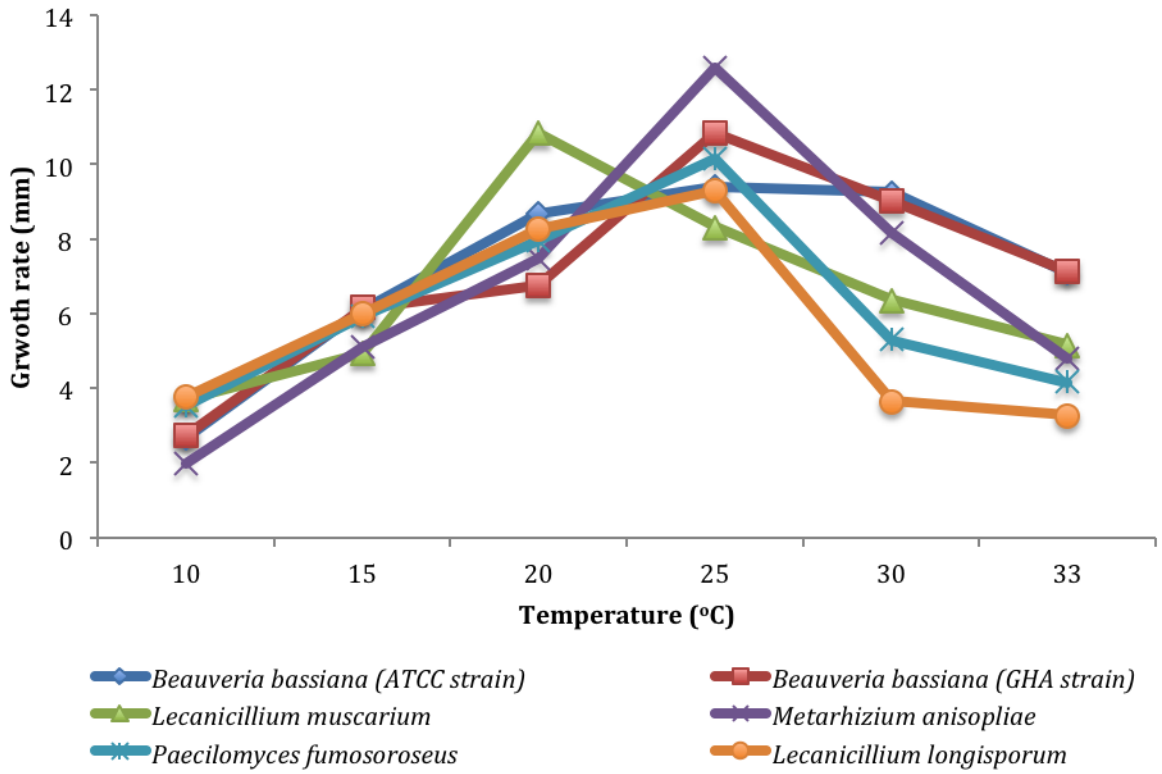
### *Objective 3 - Use laboratory bioassays to measure the susceptibility of the cabbage aphid to fungi collected from the field and compare to commercial mycopesticides.*

Initial analysis of results suggest the cabbage aphid, *Brevicoryne brassicae*, is more susceptible than the peach potato aphid, *Myzus persicae*, to the products Naturalis L (*Beauveria bassiana* (ATCC strain)), Mycotal (*Lecanicillium muscarium*) and Vertalec (*Lecanicillium longisporum)*.

In bioassays at a dose of $1x10^{8}ml^{-1}$ *L.longisporum* was the most effective control against *M.persicae* ($LT^{50}$ 4.67 days); conversely, it was the least effective against *B.brassicae* ($LT^{50}$ 4.67 days). The most effective control of *B.brassicae* was *L.muscarium*, $LT^{50}$ 3.33, however, this EPF proved to be least effective against *M.persicae* ($LT^{50}$ 5.33 days).

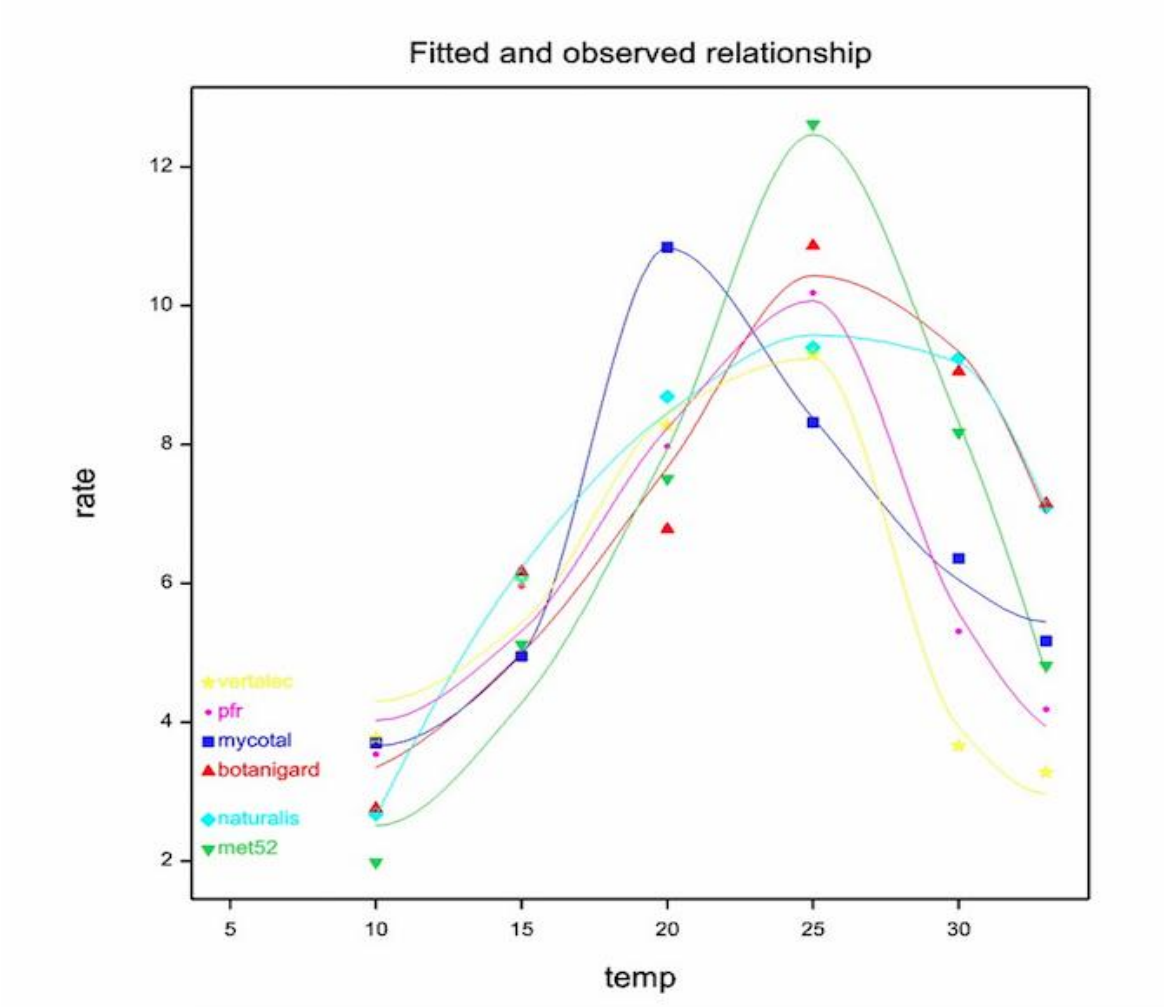
### *Objective 4 - Model the effect of temperature and moisture on the pathogenicity of fungi to the cabbage aphid to forecast the outbreak of fungal epizootics.*

***Figure 4. Results from mycelial growth assay***. *The following results are based on the radial growth (mm/day) and corresponding rates of each species on SDA (sabouraud dextrose agar). Data on SEMA are not graphically shown.*




Five of the six Ascomycetes included in mycelial growth experiments (*B. bassiana* ATCC & GHA, *M. brunneum, I. fumosoros* & *L. longisporum*) seemed to respond to temperature in a similar way with the optimum for growth approximately 25°C. One interesting point to make is the difference for *L.muscarium* it seems to have a lower optimum for growth around 20°C (figure 4).

***Figure 5. Quadratic model applied to observed relationship in figure 4.*** *Growth rate (mm/day).*




The most appropriate regression applied to the above data is polynomial of which quadratic regression is a special case (see figure 5). The assumptions of polynomial regression are the same as for linear regressions except the relationship is assumed to be non-linear. The quadratic curve is often used to fit a 'humped-shaped' curve to data.

The quadratic model fits all species significantly ($p$=0.001, se: 1.43, MS residual: 2.06) and accounts for 70.7% of variance in the observed relationship.

Germination data is still being collected.

## Discussion

The results obtained during the first field season suggest that the aphid population crash occurs regardless of plant age and that a guild of natural enemies are associated with aphid populations in the field. The community of natural enemies can change throughout the course of the field season. While cause and effect are difficult there is a clear density dependence between the guild of natural enemies and aphid populations. As such the relationship warrants further investigation. Moreover hyperparasitoids of primary parasitoids and of hoverfly larvae were identified. As the season progresses the influence of the *fourth* trophic level interaction will increase as their numbers build and primary natural enemies are 'controlled' themselves. Indeed the notion of a fourth trophic level interaction is well supported in the literature.

With regards to the potential use of enzootic natural enemies ('native' natural enemies) for use as biological control agents it is important to consider the use of any agrochemicals applied as they may have detrimental effects on the guild. Clearly then any approach to control aphid population within a conservation biological control strategy must be considered in an integrated way, with all other farm/pest management approaches.

Initial results from growth assays of the ascomycetes suggest that control failures might be seen at the lower and upper cardinal temperatures, 10$^{o}$C and 30$^{o}$C. This is because growth ceases or continues at a significantly reduced rate as temperatures reach these limits. These preliminary findings will be important when considering application times of these products as biopesticdes. As research continues to manipulate humidity these data will also be useful in determining the timing of myco-insecticde applications.

## Conclusions

### *Objective 1- Monitor populations of healthy and fungus-infected cabbage aphids on sequentially planted brassicas and study the link between the mid-summer population crash and epizootics of insect pathogenic fungi.*

Aphid populations on field brassicas increased until mid to late July when they crashed regardless of plant age. Associated with the timing of the crash was a fungal epizootic suggesting a potential link. The composition of the guild of natural enemies changed throughout the course of the field season with parasitoid mummies being the most common.

As the season progressed parasitoid mummies became less frequent implying potential fourth trophic level interaction that will be investigated further during the field season of year 2.

### *Objective 2 - Identify insect pathogenic fungi associated with the cabbage aphid Brevicoryne brassicae on field brassicas.*

The 2013 field season at Wellesbourne saw the establishment of a fungal epizootic, during which attempts were made to isolate the fungi from the field. Morphological data suggests the epizootic was caused by *Pandora neoaphidis*.

### *Objective 3 - Use laboratory bioassays to measure the susceptibility of the cabbage aphid to fungi collected from the field and compare to commercial mycopesticides.*

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### Objective 4 - Model the effect of temperature and moisture on the pathogenicity of fungi to the cabbage aphid to forecast the outbreak of fungal epizootics.

Five of the six Ascomycetes included in mycelial growth experiments (*B. bassiana* ATCC & GHA, *M. brunneum, I. fumosoros* & *L. longisporum*) seemed to respond to temperature in a similar way with the optimum for growth approximately 25$^{o}$C. One interesting point to make is the difference for *L.muscarium* it seems to have a lower optimum for growth around 20$^{o}$C.

## Knowledge and Technology Transfer

**The University of Warwick Postgraduate Symposium.** Coventry, UK. 24th- 26th March 2013. *Abstract.*

**HDC (Horticultural Development Company) studentship conference.** Gloucester, UK. 9th- 10th September 2013. *Poster presentation.*

**The Royal Entomological Society Aphid Special Interest Group.** *"Exploring new ways to manage aphids in crops"* Leamington Spa, UK. 11th September 2013. *Oral presentation*.

**Warwick Crop Centre Open Day.** Wellesbourne, UK. 18th September 2013. *Poster presentation.*

**IOBC (International Organisation for Biological and Integrated Control)/ WPRS (West Palaearctic Regional Section).** *"Integrated protection in field vegetables"* Bergerac, France. 21st – 26th September 2013. *Oral presentation.*

**HDC (Horticultural Development Company) R&D Technical Conference.** Lincolnshire, UK. 9th October 2013. *Oral presentation.*

**AAB (Association of Applied Biologists).** "*IPM: Pushing back the frontiers conference"* Lincolnshire, UK.15th- 16th October 2013. *Oral presentation.*

**IOBC (International Organisation for Biological and Integrated Control)/ WPRS (West Palaearctic Regional Section).** *"The role of naturally enemies in cotrolling aphid populations in field Brassicas." Article publication.* **In press**

**BGA (British Growers Association) Conference.** Lincolnshire, UK. 21st January 2014.

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