Project title:	The role of entomopathogenic fungi in regulating aphid populations in field Brassicas
Project number:	CP 092
Project leader:	Dr. Dave Chandler, Warwick Crop Centre, University of Warwick, CV35 9EF
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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

Populations of cabbage aphid, feeding on brassica plants in a field experiment done over 2014, exhibited a sharp increase in numbers followed by a precipitous decline. This population decline, or "crash", occurred at the same time (in October) on plants of different physiological age and was associated with an increase in the population of natural enemies feeding on the aphids. The 2014 aphid "crash" occurred much later than a similar crash observed in 2013, which occurred in mid-summer. Laboratory experiments showed that the rate of population increase of aphids on brassica plants is affected by the age of the plant and its soluble nitrogen content; however this does not translate into observable effects on the timing of the population crash in the field. The entomopathogenic fungus *Pandora neoaphidis* is one of the key natural enemies effecting brassica aphid populations in field experiments, and detailed laboratory experiments have characterised how environmental temperature determines the ability of the fungus to kill aphids.

Background

This HDC PhD studentship is investigating the precipitous decline in populations of brassica aphids that occurs most years, which is referred to hereafter as the "aphid crash". The project focuses on the role of naturally occurring entomopathogenic fungi in the aphid crash, but includes studies of a range of factors that may impact on the timing and size of the crash including the presence of other natural enemy species and the role of plant age.

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 for a number of reasons: (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 in the summer, 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 the growing season (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 predicted accurately.

Many factors have been suggested for the mid-season crash, including plant age, the action of natural enemies and adverse weather conditions. These factors could affect population processes including birth, death and emigration. For example: a decrease in nitrogen content of older plants could result in a decrease in aphid birth rates and increased emigration rates as a result of intraspecific competition, while natural enemies – attracted to large aphid populations – could cause a large increase in mortality (Karley *et al*. 2004). Of the natural enemies, entomopathogenic fungi have been strongly implicated in the crash of aphid 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.

Thermal biology of entomopathogenic fungi

The activity of entomopathogenic fungi is dependent on temperature (Blanford & Thomas, 1999). However, there have been relatively few detailed studies of the effect of temperature on fungal infectivity to aphid hosts. Most of the research on the thermal biology of entomopathogenic fungi has looked at the effect of temperature on processes such as fungal growth and germination in the absence of the insect host. Until recently, entomopathogenic fungi were used mainly as biological control agents of protected crops where temperatures are stable and usually not limiting to fungal activity. However, as these fungi start to be investigated and exploited more as biocontrol agents in outdoor crops, where temperature conditions are more variable, there is an obvious need to understand in detail the effect of temperature on fungal performance (Blanford & Thomas, 1999).

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

Aim:

This project is investigating a hypothesis that fungal epizootics are one of the principle factors causing the mid-season crash in populations of aphids on horticultural brassicas. There are three main objectives as follows:

Objectives:

i. Monitor populations of healthy and fungus-infected cabbage aphids on sequentially planted brassicas and study the abiotic and biotic factors contributing to the mid-season population crash.

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

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

Summary

Below, summaries and main findings are discussed within the framework of the three main objectives:

i. Monitor populations of healthy and fungus-infected cabbage aphids on sequentially planted brassicas and study the abiotic and biotic factors contributing to the mid-season population crash.

Fieldwork 2014

A field experiment was done over 2014 to monitor populations of cabbage aphid and its natural enemies on plots of brassica plants at different stages of growth (= physiological age). It was found that the aphid population crashed in mid-October (figure 1). This was later than the crash observed in a similar experiment in 2013, which occurred at the end of July. However, in both years, plant age was shown to have no effect on the timing of the crash, that is all transplants responded in the same way at the same time (figure 1). Moreover, both years saw the establishment of a fungal epizootic and an increase in the population of other natural enemy species coinciding with the time of the crash

Quantification of the effect of density of apterous adult aphids on production of alate forms on brassica plants

One explanation put forward for the aphid crash is that an increase in the population of apterous (wingless) aphids on a particular brassica plant results in a sudden switch to the production of alate (winged) forms for emigration, resulting in a sharp population decline on the same plant. An experiment was done to monitor the production of alate forms in relation to the density of apterous forms. From this data it will be possible to calculate the density at which alate production begins. It should then be possible to manipulate aphid density and investigate its effect on natural enemy activity, in particular the epidemiology of *P. neoaphidis*. With this information it will be possible to build a simple epidemiological model to predict levels of infection/control in the field.

Preliminary investigation suggests the threshold for alate production to be approximately 100-150 individuals per plant. Based on observations in field experiments, a population of this size would not significantly damage brassica plants, and it is unlikely that the cue for alate production is related to a decrease in host plant quality caused by a large aphid population.

The effect of plant age on Brevicoryne brassicae fecundity

That soluble nitrogen effects fecundity in aphids is well documented (van Emden & Bashford, 1969). As a result it is not surprising that there is no significant difference in nymph production between plants of 'medium' and 'old' physiological ages as there was no significant different in soluble nitrogen. Nymph production declines significantly between the youngest and two older plant ages, but it is highly unlikely by itself to cause the sudden decline in aphid populations you observe in the field. For this to happen there would have to be no births. Field data also indicates an increase in mortality because the crash occurs over the course of a week. Plant age could be a contributory factor to the aphid population crash but is likely masked by other factors in the field.

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

Field experiments set up at Wellesbourne during the 2014 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) saw the establishment of a field epizootic which acted to reduce aphid infestations, as in 2013. Attempts were made to isolate the fungi and were successful. Morphological data, as in 2013, suggests that the epizootic was caused by *Pandora neoaphidis* (Commonwealth Mycological Institute, 1979). DNA identification confirmed that the pathogen isolated from *Brevicoryne brassicae* at Wellesbourne is *Pandora neoaphidis*.

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

The effect of temperature on the rate of colony extension of fungal isolates

Preliminary analysis suggests the optimal temperature for isolate NW420 is approximately 22^{o}C which is lower than the optima calculated for the Ascomycetes *B. bassiana* ATCC & GHA, *M. brunneum, I. fumosoros* and *L. longisporum* (25^{o}C) but higher than that of *L. muscarium* (Harvey, 2013). Data for *P. neoaphidis* isolate WELL1 is currently being collected.

Laboratory evaluation of the effect of temperature on the germination of fungal species from the phylum ascomycota

Germination times varied greatly depending on temperature with the slowest germination times for all isolates at 15^{o}C and increasing with increasing temperature.

Temperature dependence of the pathogenicity of P. neoaphidis

A series of bioassays were carried out at a range of temperatures from 12 to 28^{o}C and at varying spore showering times from 5-75 minutes. Death could be attributed to *P. neoaphidis* at all temperatures because spores were produced on cadavers i.e. individuals were mycosed. Dead but unmycosed individuals were only observed at 24^{o}C and 28^{o}C inferring that heat stress could be another source of mortality. Further investigation into this competing risks theory is to be carried out in 2015.

Temperature clearly affects the ability of the fungus to kill individuals, not surprising because ectothermic organisms need suitable environmental conditions to germinate and grow. These findings have important implications not only for pest management strategies involving the use of biopesticides i.e. spray windows, but also any conservation biocontrol strategy where the activity of enzootic fungal pathogens will be limited by the temperature of the environment.

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 elucidate the role entomopathogenic fungi play in the crash of aphid populations; as such there are no action points to growers at present.

SCIENCE SECTION

1.1 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).

1.2 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 for a number of reasons: (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.

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)

1.3 Aphid population dynamics

Aphids are r-strategist insects that reproduce parthenogenetically in the summer, 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 the growing season (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 predicted accurately.

Many factors have been suggested for the mid-season crash, including plant age, the action of natural enemies and adverse weather conditions. These factors could all affect population processes including birth, death and emigration. For example: a decrease in nitrogen content of older plants could result in a decrease in aphid birth rates and increased emigration rates as a result of intraspecific competition, while natural enemies – attracted to large aphid populations – could cause a large increase in mortality (Karley *et al*. 2004). Of the natural enemies, entomopathogenic fungi have been strongly implicated in the crash of aphid 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.

1.4 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.

1.5 Alternative control methods

1.5.1 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 agroecosystems 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.

1.5.2 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.

1.6 Entomopathogenic fungi

The two largest fungal phyla exhibiting entomopathogenicity are the Ascomycota and the Entomophthoromycota (Humber, 2008; Humber, 2012). The Ascomycota are considered to be generalist pathogens, causing death via toxin production (Pell *et al*. 2001), whereas, the Entomophthoromycota 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 utlisation of dead or decaying matter within the soil as a nutrient source. Epizootics are often caused by Entomophthoromycota 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 feeding 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).

1.6.1 Thermal biology of entomopathogenic fungi

The activity of entomopathogenic fungi is dependent on temperature (Blanford & Thomas, 1999). However, there have been relatively few detailed studies of the effect of temperature on fungal infectivity to aphid hosts. Most of the research on the thermal biology of entomopathogenic fungi has looked at the effect of temperature on processes such as fungal growth and germination in the absence of the insect host. Until recently, entomopathogenic fungi were used mainly as biological control agents of protected crops where temperatures are stable and usually not limiting to fungal activity. However, as these fungi start to be investigated and exploited more as biocontrol agents in outdoor crops, where temperature conditions are more variable, there is an obvious need to understand in detail the effect of temperature on fungal performance (Blanford & Thomas, 1999).

Below literature on the successful and unsuccessful use of entomopathogenic fungi (EPF) as BCAs is considered in relation to the three broad biological control approaches:

1.6.2 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).

1.6.3 Augmentation biological control

Natural enemies are generally too few in number within the crop to effectively control pest levels, 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 a

similar approach to how agrochemicals are applied (Shah & Pell, 2003). Indeed, the term ‘mycoinsecticide’ has been coined (Shah & Pell, 2003). At present there are a few commercial augmentation products available for the control of aphids including *Lecanicillium longisporum* and *Lecanicillium muscarium* (marketed as ‘Vertalec’ and ‘Mycotal’ respectively) (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 amount 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).

1.6.4 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.

1.6.5 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 hosts 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 for the mean time at least *P.neoaphidis* is best exploited through a CBC approach.

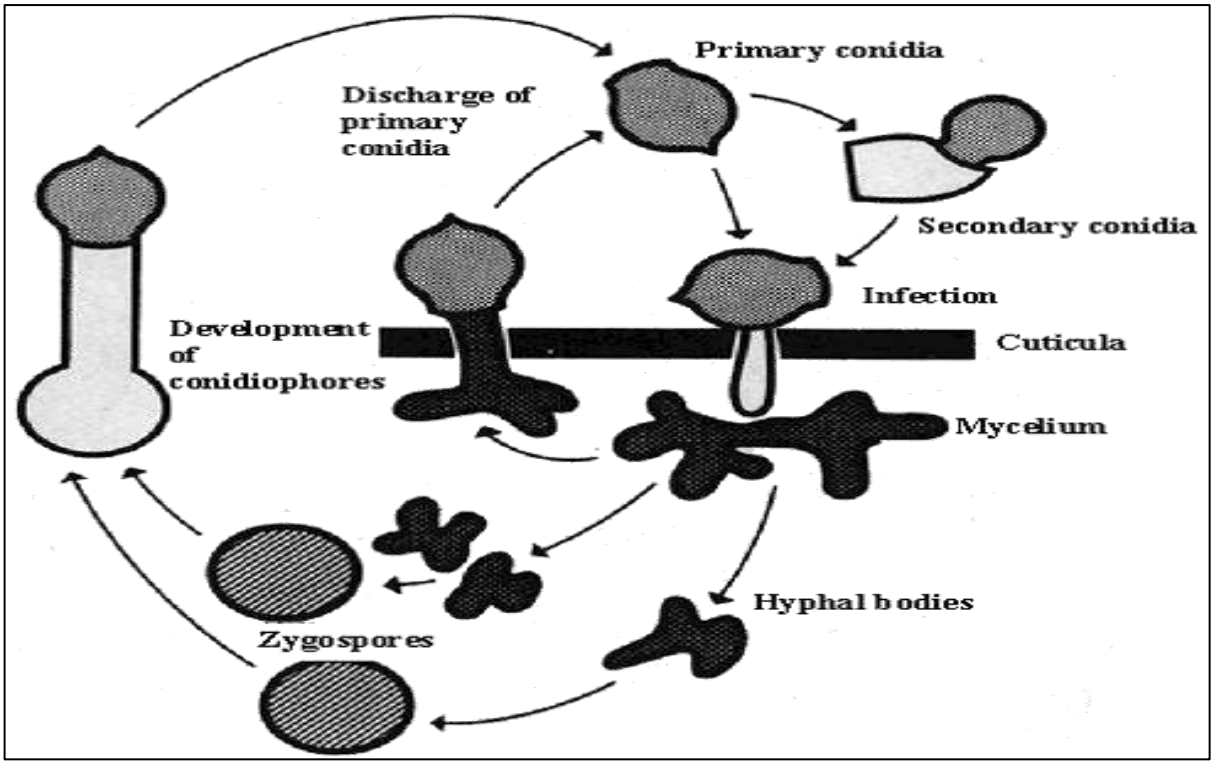

Figure 1a. Generalised lifecycle of Entomophthoralean fungi

(Danish Ministry of the Environment, 2013.

As result the aims and objectives of this project are:

Aim:

This project is investigating a hypothesis that fungal epizootics are one of the principle factors causing the mid-season crash in populations of aphids on horticultural brassicas. There are three main objectives as follows:

Objectives:

i. Monitor populations of healthy and fungus-infected cabbage aphids on sequentially planted brassicas and study the abiotic and biotic factors contributing to the mid-season population crash.

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

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

2 Materials and methods

2.1 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 in vermiculite until the cotyledons had unfolded. These seedlings were then transplanted singly into plastic 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 as required.

2.2 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 Previously: *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 27th 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).

2.3 Pandora neoaphidis

Isolates of *P.neoaphidis* were treated identically in storage and maintenance. One isolate (NW420) was donated to the Warwick Crop Centre collection by Rothamsted (Dr. Judith Pell) and the second was isolated from Wellesbourne at the Warwick Crop Centre, UK 2014 (Table 3).

For experiments, isolates were retrieved from long term liquid nitrogen storage and grown on Sabourand-dextrose agar supplemented with egg yolk and milk, Sabourand- dextrose egg and milk (SEMA) agar (Wilding & Brobyn, 1980). Petri dish cultures were maintained in darkness at 15-18°C inside humid plastic boxes. For all studies, isolates were not used after the third subculture from liquid nitrogen storage or if they had spent longer than 6 weeks at 15-18°C.

Table 3. *Pandora neoaphidis isolate*

Species	Isolate*	Original host	Source
Pandora neoaphidis	NW420	*Brevicoryne brassicae*	Rothamsted, UK
Pandora neoaphidis	WELL1	*Brevicoryne brassicae*	Wellesbourne, UK

*Isolate reference for the Warwick crop centre culture collection.

i. Monitor populations of healthy and fungus-infected cabbage aphids on sequentially planted brassicas and study the abiotic and biotic factors contributing to the mid-season population crash.

The following fieldwork was carried out on plots of Brussels sprout plants (*Brassica oleracea*) cv. Trafalgar (Tozer Seeds Ltd, Surrey, UK, KT11 3EH) at Warwick Crop Centre, Wellesbourne, CV35 9EF, UK from May to November 2014. It builds on findings in 2013 and aims to explore spatial differences in aphid population dynamics and associated natural enemies.

The fieldwork for 2014 consisted of two experiments as follows:

- **Experiment 1a**: Monitor the development of populations of *B. brassciae* within spatially separated plots that have been planted sequentially, on 4 separate occasions from May to August.
- **Experiment 1b**: Characterise the guild of natural enemies to species level.

Experiment 1a consisted of 16 plots (four planting occasions x 4 replicates) of 10 experimental plants arranged 2 plants x 5 plants, with 50cm spacing surrounded with a single guard row. There were four different locations separated by at least 80 meters, Little Cherry 'LC', Pump Ground 1 'PG1', Pump Ground 2 'PG2' & Long Meadow West 'LMW'. In each location there was one of each of the planting occasions 6th May, 3rd June, 3rd July & 1st August separated by at least 20 meters. Plants were grown for a month in Hassy trays in a glasshouse. Each of the 4 replicates for each transplant date were randomly assigned positions within locations. 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 as above. After 3-5 days in the field all experimental plants in experiment 1a 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. If aphids were found not to have established re-inoculation took place up until 7 days post initial inoculation.

Experimental Brussels sprout plants were inspected every 7-20 days until November 2014. 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. Three out of the ten plants per plot were randomly inspected to gain a plot mean.

Experiment 1b consisted of the identification down to species level where possible of all natural enemies seen on experimental plants.

Quantification of the effect of density of apterous adult aphids on production of alate forms on brassica plants

Brussels sprout plants (*Brassica oleracea* Gemmifera group) cv. 'Trafalgar' (Tozer seeds Ltd, Surrey, UK, KT11 3EH) were grown as above. Three plants at growth stage BBCH-15/16 were chosen and the experiment was repeated three times from October – December 2014. Experimental plants were inoculated with 10 adult (10-12 days) apterous *Brevicoryne brassicae* individuals. Plants were then caged and placed in a controlled environment room at 20±1^{o}C, 16:8 L:D light regime. Aphid population size and the number of alates produced were recorded every 3-4 days until aphid populations went into decline. In each cage there was a yellow and a blue sticky trap hanging from the roof in order to collect any alates.

The effect of plant age on* Brevicoryne brassicae *fecundity

Brussels sprout plants (*Brassica oleracea* Gemmifera group) cv. 'Trafalgar' (Tozer seeds Ltd, Surrey, UK, KT11 3EH) were grown as above and three different ages were chosen for experiments in accordance with the BBCH-scale for 'other brassica vegetables'. Young (y) plants were at growth stage BBCH-13/14 (third/fourth true leaf unfolded), medium (m) plants were at growth stage BBCH-16 (sixth/seventh true leaf unfolded) and old (o) plants were at growth stage BBCH-19 (twelfth true leaf unfolded). Three plants of each age were used during each repetition of the experiment and the experiment was repeated three times. Clip cages containing one, young apterous *Brevicoryne brassicae* adult (10-12 days) were attached to the abaxial side of the youngest and oldest leaves of each experimental plant. Clip cages were monitored daily for nymph production for seven days and any nymphs produced were removed.

After the experiment all young and old leaves inoculated with *B.brassicae* from each of the three different aged plants were taken for soluble nitrogen analysis.

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

Field experiments set up at Wellesbourne during the 2014 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) saw the establishment of a field epizootic which acted to reduced aphid infestations as in 2013. Attempts were made to isolate the fungi and were successful. Morphological data, as in 2013, suggests that the epizootic was caused by *Pandora neoaphidis* (Commonwealth Mycological Institute, 1979). DNA identification as confirmed that the pathogen isolated from *Brevicoryne brassicae* at Wellesbourne is *Pandora neoaphidis*.

Isolation techniques consisted of collecting individual aphids from the field, approx. 100. Some of which were yet to show signs of sporulation so they could be surface sterlised (an ethanol bath, followed by hypochlorite and finally two sterile water baths) and put on SEMA (Sabouraud dextrose agar supplemented with milk and egg yolk) (for method see appendix 2) in order to culture the fungus if sporulation occurred. Sporulating cadavers from the field were placed on infested Brussels sprout plant to encourage cross infection. In this way an *in vivo* culture was also established initially to ensure a continued supply of cadavers. Humidity was artificially raised by enclosing the infested plant with two plastic bags, after 24 hours one plastic bag was removed.

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

The effect of temperature on the rate of colony extension of fungal isolates

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. Petri dishes were then transferred to incubators set at the range of temperatures above. Assays were read every 3-4 days for a total of 22 days by measuring colony extension along 2 axes (mm).

Mycelial growth assays using *Pandora neoaphidis* (NW420) were conducted in the same way above except plugs were taken directly from growing plates that were 4-5 weeks old.

Mycelial growth assays with the newly isolated fungus (WELL1) are currently underway, running NW420 in parallel as for a control.

Laboratory evaluation of the effect of temperature on the germination of fungal species from the phylum ascomycota

Fungi were grown in the dark on SDA for 17 days at 20^{o}C $\pm$ 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.

Temperature dependence of fecundity and pathogenicity of P.neoaphidis

The method reported here to bioassay *Brevicoryne brassicae* was modified from techniques given by Feng & Johnson (1991), Papierok & Hajek (1997) and Sierotzki *et al*. (2000). Plugs of *P.neoaphidis* from petri dish cultures were used as *in vitro* sources of primary conidia because of convenience. Leaf discs (2cm in diameter) were placed abaxial side up in a 9cm plastic petri dish suspended in distilled water and held in place with a drawing pin. In order to expose aphids to conidia plugs of *P.neoaphidis* (size 5 corkborer, 7mm diameter) were taken aspetically, 16-24 hours prior to aphid exposure, from the growing edge of *in vitro* cultures and arraged in a triangle (three plugs) on moist filter paper in the centre of a petri dish lid and placed at 15 ^{o}C to encourage sporulation. For bioassays 15-20 *B.brassicae* adults (10-12 days old) were transferred with a fine camel hair brush and placed on leaf discs. The arrangement of plugs was suspended above groups of aphids with a palstic open ended cyclinder (25mm in lenngth x21mm internal diameter), one end of which was in contact with the base of the perti dish. Condial showering time varied as follows 5,15,25,35,45,55 & 75 minutes. Control aphids were treated in the same way as the longest condial shower except for exposure to the pathogen.

After innoculation 10-15 of the most active individulas from each showering time were placed on a single leaf of a 4-5 week old Brussels sprout plants (cv. 'Trafalgar') and subsequently encased in a 'Blackman boxes' (12.5cmx8cmx2cm) containing moist filter paper to maintain high humidity. All plants were then placed in controlled environment incubators at 12,15,20,24 or 28^{o}C at a 16:8 L:D photoperiod. Due to the availability of incubators the temperatures at which bioassays were run was randomised over time. Assays at each temperature were repeated three times.

An estimate of spore dose was obtained each time the experiment was conducted for each showring time by staining a cover slip (18mmx18mm) placed under the plugs post showering with 10% cotton blue in lactophenol. The stained spores were enumerated under a light microscope (125x magnification) in 20 random fields of veiw and converted to conidia mm^{-2}. Bioassay chambers were monitored daily for mortality and nymph production. The number of mycosed and unmycosed individuals was also noted as dead non-sporulating individuals were palced on 1.5% DWA.

3 Results

i. Monitor populations of healthy and fungus-infected cabbage aphids on sequentially planted brassicas and study the abiotic and biotic factors contributing to the mid-season population crash.

How natural enemies and plant age effect the population dynamics of the cabbage aphid (Brevicoryne brassicae) on Brussels sprout plants (Brassica oleracea cv. Trafalgar).

The fieldwork for 2014 consisted of two experiments as follows:

- **Experiment 1a**: Monitor the development of populations of B. brassciae within spatially separated plots that have been planted sequentially, on 4 separate occasions from May to August.
- **Experiment 1b**: Characterise the guild of natural enemies to species level.

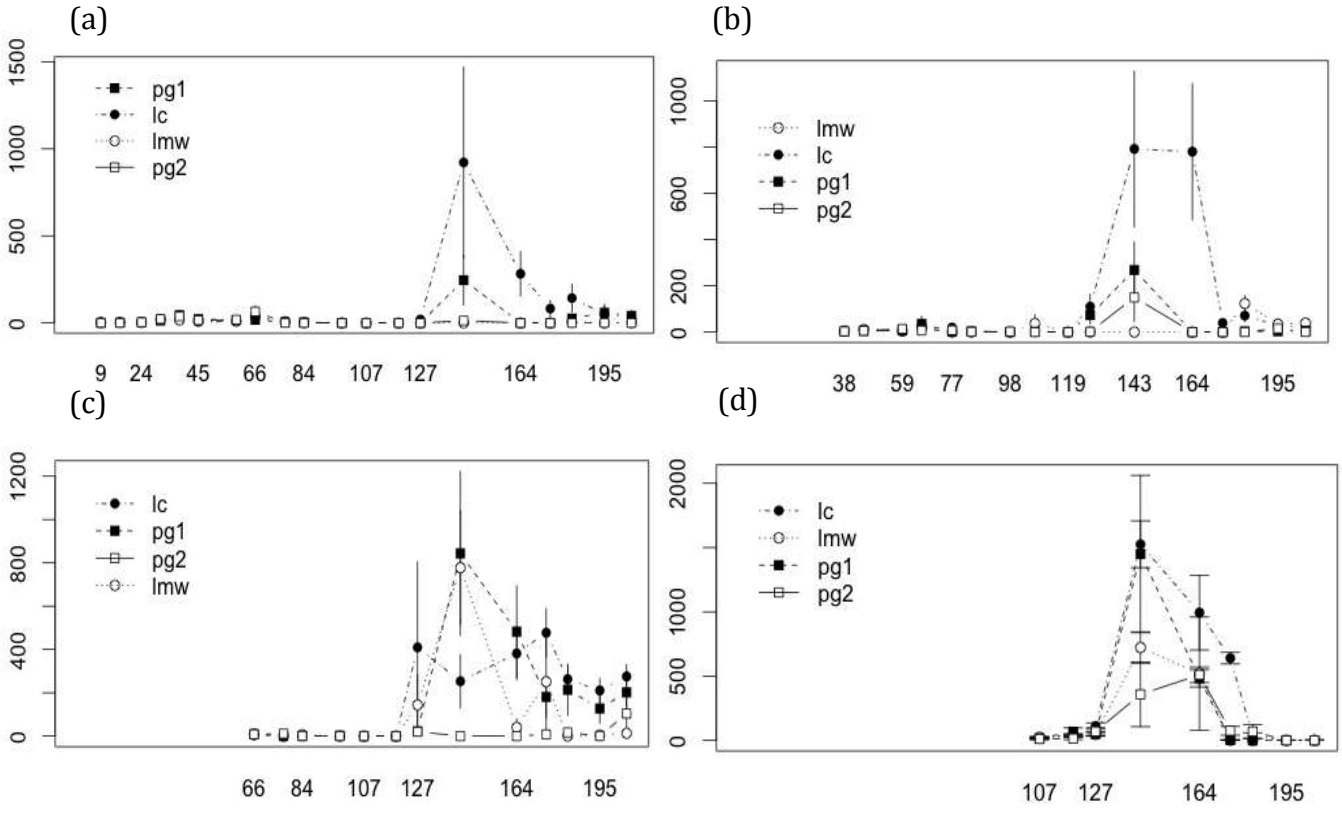

Fig 1. Changes in mean number of aphids per plant (±se) after first planting in each location Little Cherry 'lc', Pump Ground 1 'pg1', Pump Ground 2 'pg2' & Long Meadow West 'lmw' during May (a), June (b), July (c) & August (d).

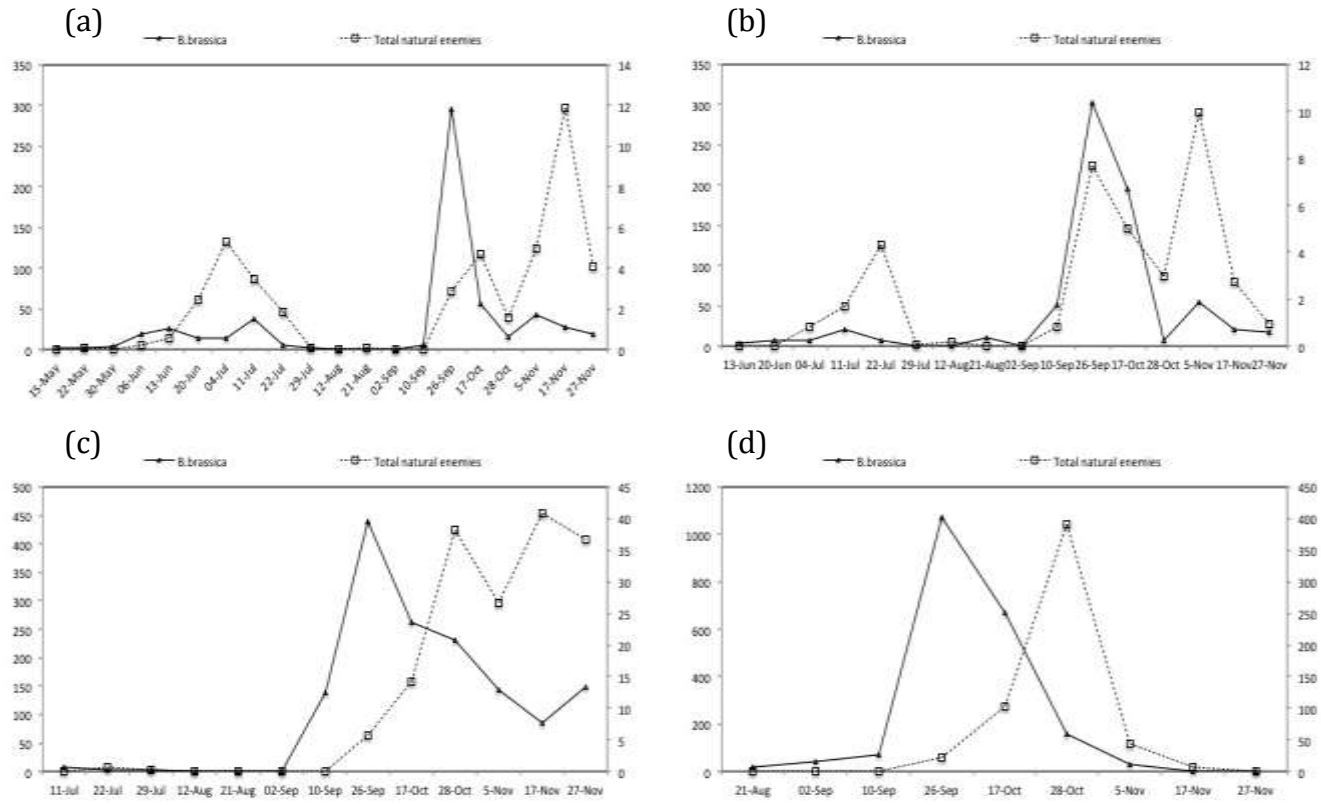

Fig 2. Changes in mean number of aphids per plant and associated natural enemies for plots of Brussels sprout plants planted in May (a), June (b), July (c) & August (d). Means grouped for location as no significant location differences were found. Solid lines indicate *B.brassicae* population, broken lines indicate natural enemy guild.

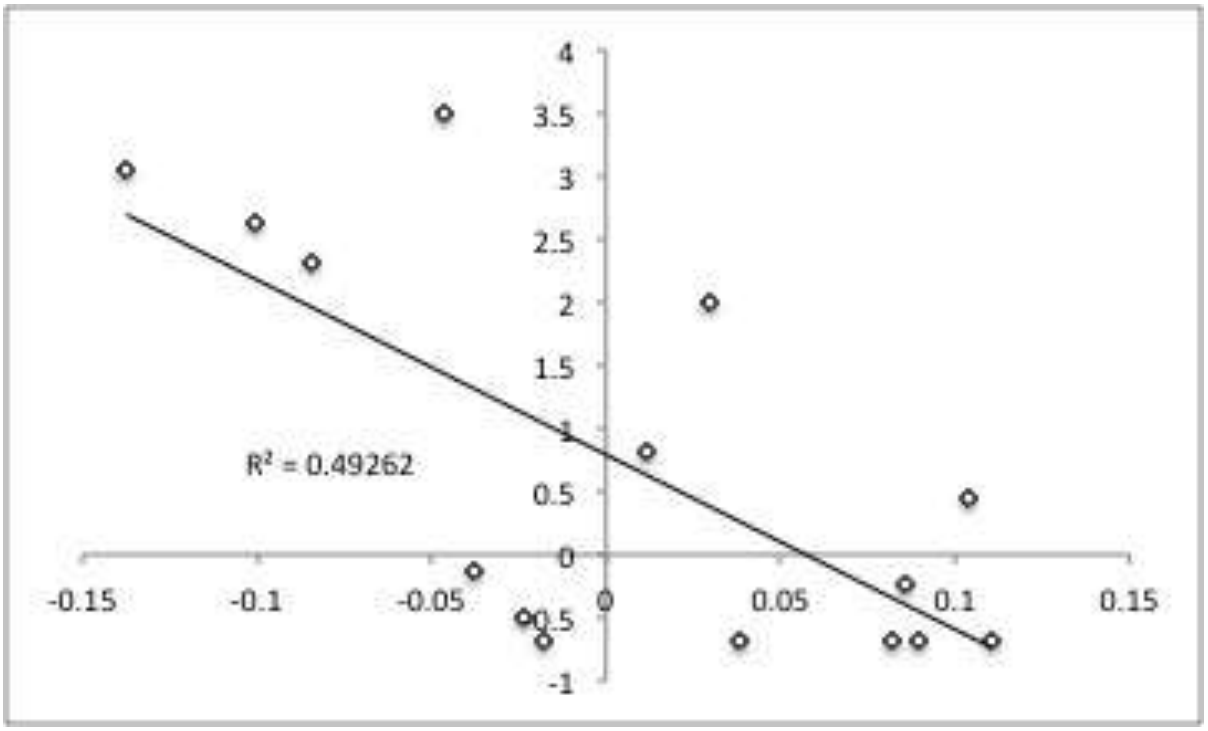

Fig 3. Mean number of natural enemies (y axis) against the instantaneous rate of increase (r_i) of aphid populations over time in the 2013 field season (x axis). (Linear regression, *P*=<0.001). All transplants grouped.

Instantaneous rate of increase (r_i) is a population growth parameter calculated by the natural log of the population size at time point b divided by the population at time point a divided by the amount of time elapsed between time point a and b. The value of the rate varies between -1 and 1, value below 0 and the population is said to be decreasing and vice versa for values above 0.

In all plots regardless of plant age and location the aphid population crash occurred circa mid October (figures 1 & 2). This observation is strengthened with analysis of r_i A significant day interaction was found as r_i does vary quite markedly over the course of the experiment (ANOVA, *P*=0.002). However, as suggested above this *change* over time is not significantly different for plant age (timing of transplantation) (ANOVA, *P*=0.413) or location (ANOVA *P*=0.848).

There was a significant negative relationship between the number of natural enemies observed and the aphid growth parameter r_i (Linear regression, *P*=<0.001) (figure 3). That is to say the greater the numbers of natural enemies present the lower the instantaneous rate of increase.

The most abundant natural enemies in the 2014 field season were parasitoid wasps and the

pathogen *P.neoaphidis*. The guild of natural enemies included: *Coccinella 7-punctata, Coccinella 5-punctata*, *Episyrphus balteatus*, *Aphidius ervi* & *Aphidius colemani*.

Quantification of the effect of density of apterous adult aphids on production of alate forms on brassica plants

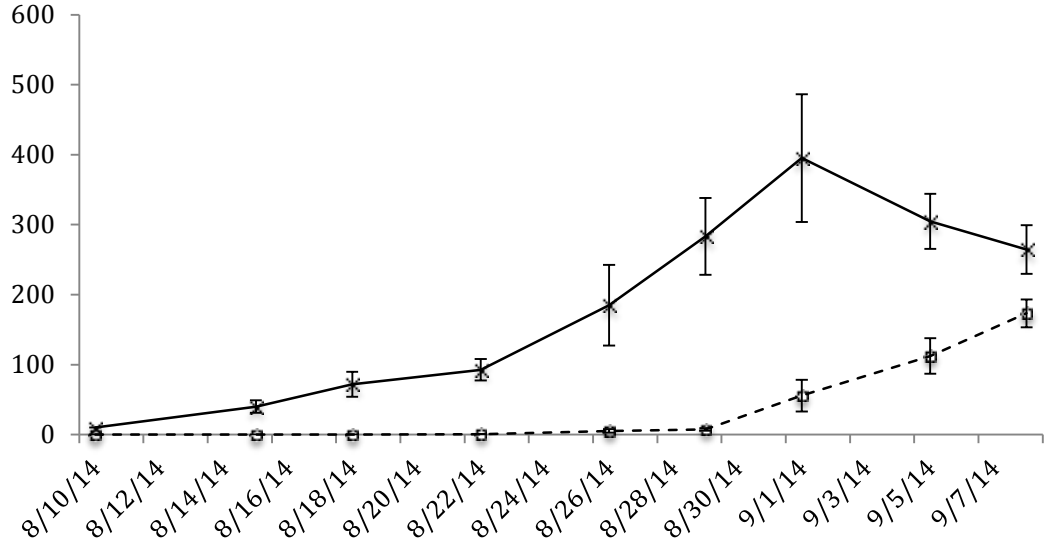

Fig 4. Aphid population dynamics over time. Apterous (solid line), alate (broken line).

In all replicates of this experiment alate production increased in response to increased density. The threshold for alate production was circa 100-150 individuals per plant.

The effect of plant age on Brevicoryne brassicae fecundity

Soluble nitrogen was significantly affected by plant age, a between plant difference, (ANOVA, *P*=<0.001) whereas soluble nitrogen did no vary with leaf age, a within plant measure. (ANOVA, *P*=0.914). Post hoc Tukey HSD analysis revealed significant differences in the soluble nitrogen content of young plants compared to medium (*P*=0.002) and old (*P*=<0.001) but no difference between medium and old (*P*=0.327) at the 5% level (figure 5).

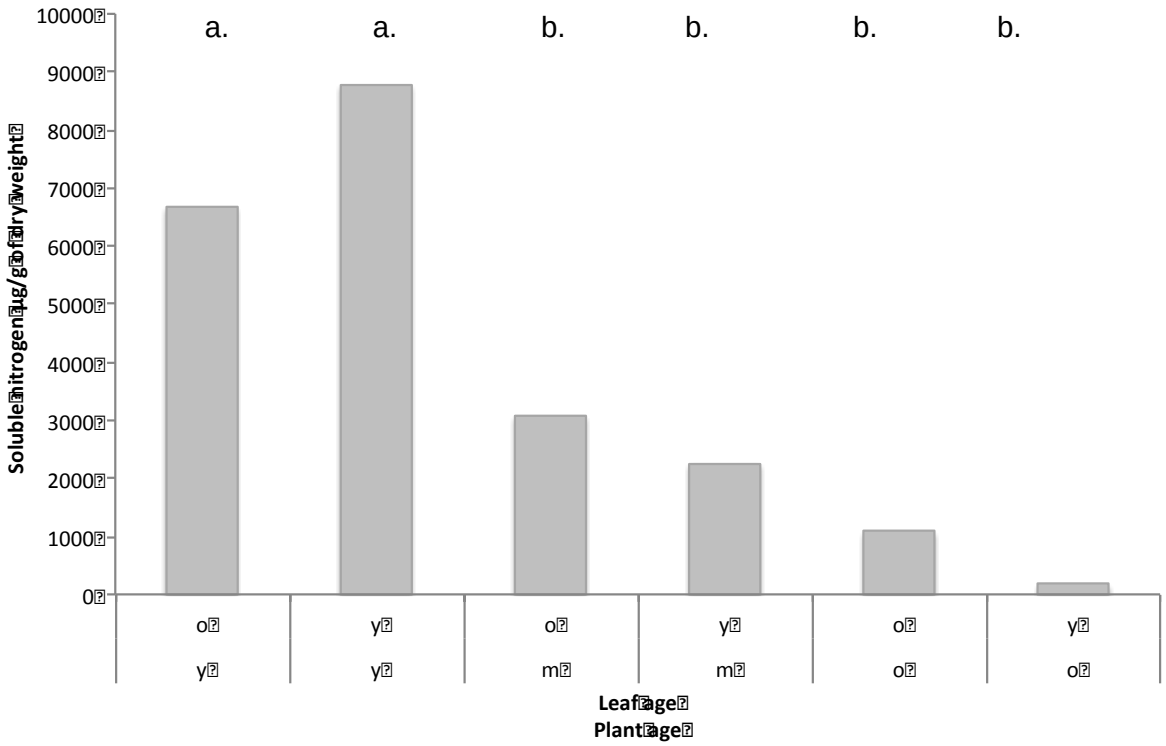

Fig 5. Average soluble nitrogen in dried leaf material for each treatment. Different letters indicate significant differences at the 5% level (Tukey's HSD, SPSS)

Table 4. Percentage mortality for each plant age and associated standard error

Plant age	Mortality after seven days (%)	Standard error
Young (y)	16.6	9.6
Medium (m)	44.4	5.5
Old (o)	72.2	14.7

Plant age significantly affected the number of nymphs produces (ANOVA, *P*=<0.001) whereas leaf age did not (ANOVA, *P*=0.436) (figure 6).

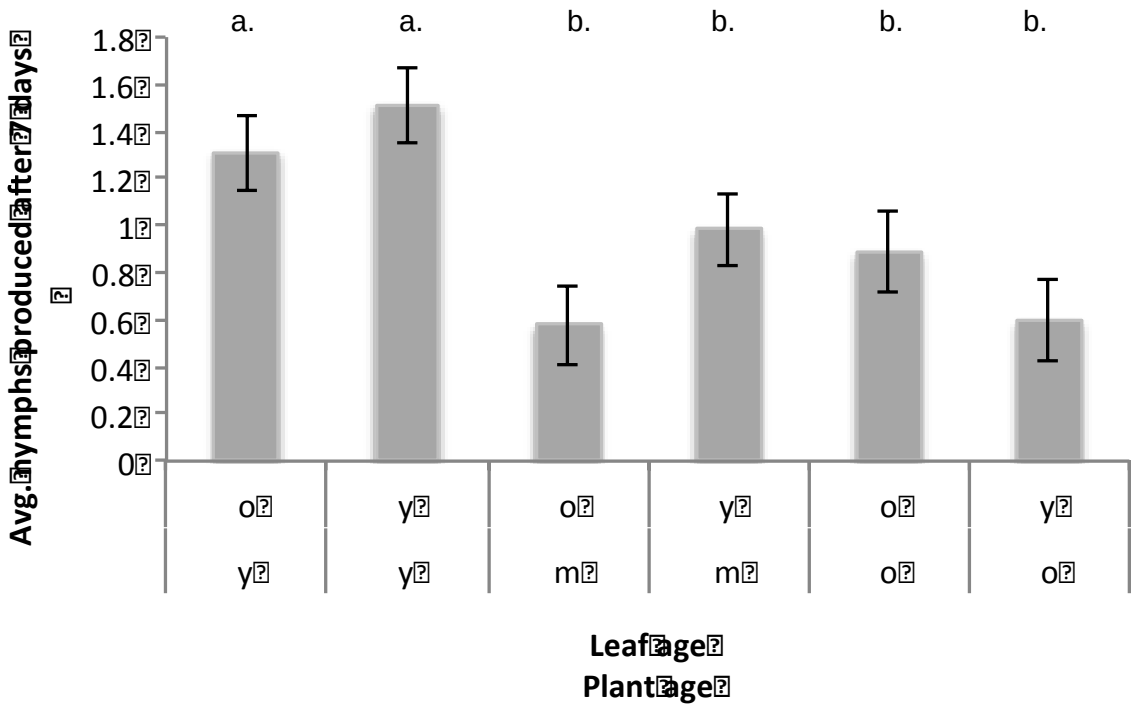

Fig 6. Average number of nymphs produced after seven days in each treatment (±se). Different letters indicate significant differences at the 5% level (Tukey's HSD, SPSS)

Mortality was greatest in aphids maintained on 'old' plants (72.2%) and decreased in line with decreasing plant age 44.4% and 16.6% for 'medium' and 'young' plants respectively.

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

DNA identification is currently underway for isolates NW420 & WELL1, results are expected beginning of February 2014.

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

The effect of temperature on the rate of colony extension of fungal isolates

Results for Ascomycota were reported in the annual report for 2013 (Harvey, 2013). Results for *P.neoaphidis* isolate NW420 are reported below, Data on isolate WELL1 are currently being collected.

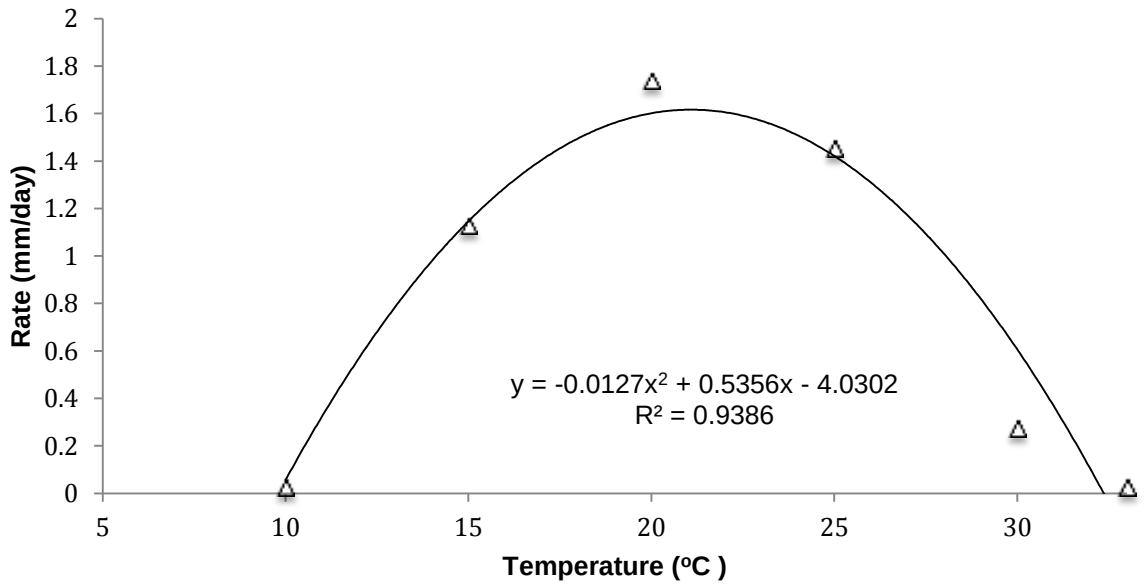

Fig 7. The effect of temperature on the growth rate of *Pandora neoaphidis* (NW420). Model fitted, polynominal quadratic (*P*=<0.05).

Preliminary analysis suggests the optimal temperature for growth of isolate NW420 is c.22^{o}C. This is lower than all the Ascomycetes tested in 2013 (Harvey, 2013) with the exception of *Lecanicillium muscarium*.

Laboratory evaluation of the effect of temperature on the germination of fungal species from the phylum ascomycota

The analysis below is preliminary and should be treated as such. Data for three of the six experimental temperatures are presented below, as models could not be applied to other temperatures at this time. Data isolate 1.72a is not reported here.

Isolate	Temperature (°C)	GT10	GT50	GT90	Model fit (lack of fit test)
432.99	15	15.88	23.05	33.47	0.31
433.99	15	13.40	21.54	34.63	
409.96	15	31.13	34.31	37.81	
19.79	15	16.84	21.66	27.86	
275.86	15	19.23	23.33	28.3	
432.99	20	10.42	15.25	22.32	0.20
433.99	20	9.33	14.20	21.60	
409.96	20	20.29	25.48	31.99	
19.79	20	9.72	14.86	22.69	
275.86	20	19.13	19.74	20.36	
432.99	25	8.80	11.31	14.54	0.99
433.99	25	8.35	10.99	14.46	
409.96	25	13.92	16.53	19.64	
19.79	25	8.76	13.91	19.85	
275.86	25	10.38	11.25	12.19	

Table 5. Relative germination times in hours for various Ascomycota. GT10 (time to 10% germination), GT50 (time to 50% germination) and GT90 (time to 90% germination).

There are substantial differences in germination times between species of Ascomycota but also between isolates 432.99 and 433.99 that are both Beauveria *bassiana* (table 5). Isolate 433.99 has GT50 times of 21.54, 14.20 & 10.99 at 15, 20 & 25 °C respectively, lower than the other isolates at these temperatures. Model fitted to data was log logistic, lack-of-fit test *P* has to be greater than 0.05 (*P*=1.00).

Temperature dependence of the pathogenicity of P.neoaphidis

The effect of exposure to *P.neoaphidis* is discussed in relation to lethal time; no reference is made about dose in this report other than Fig 8 as analysis is on-going. The analysis below is preliminary and should be treated as such. Fecundity data is currently being analysed. Data on lethal time 50 is presented for a showering time, 35 minutes.

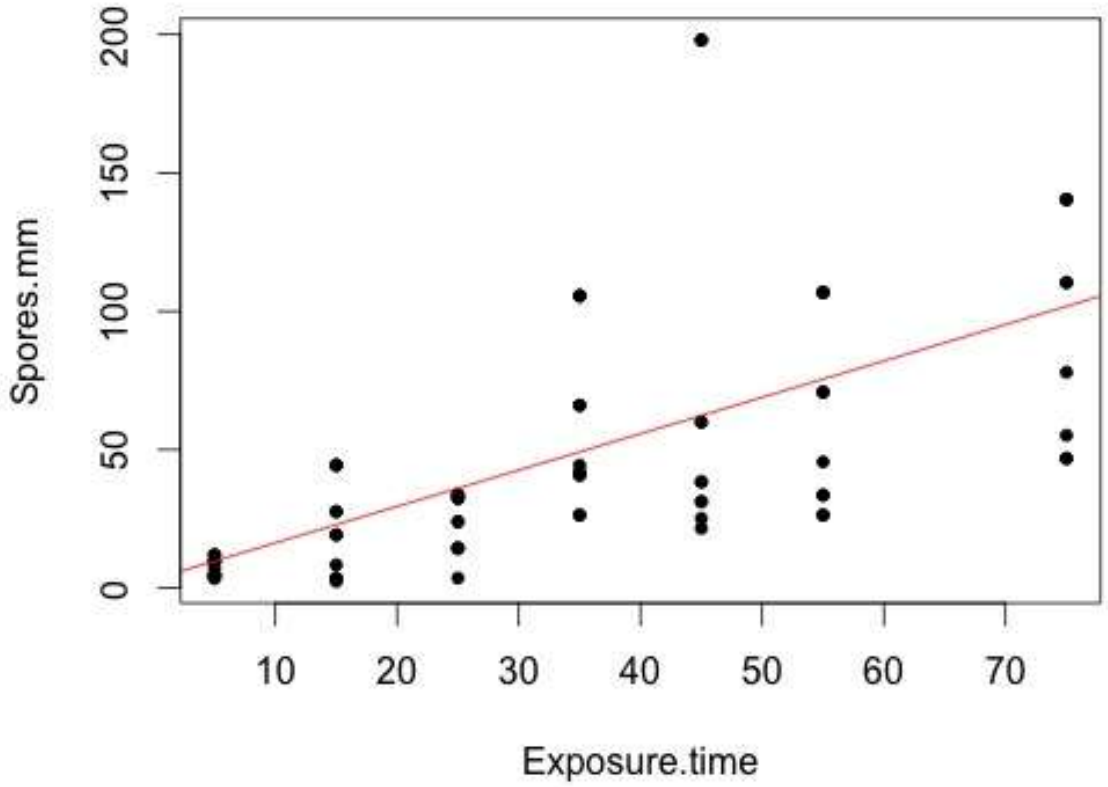

Fig 8. Exposure or showering time against the number of spores/mm^{-2} experimental populations of aphids would have received (linear regression, *P*=<0.001).

Exposure time can be used as a proxy for dose as although there is variation there is a significant linear relationship between exposure time and the number spores received by the aphids (figure 8). As such a single exposure time was used in the following analysis, 35 minutes.

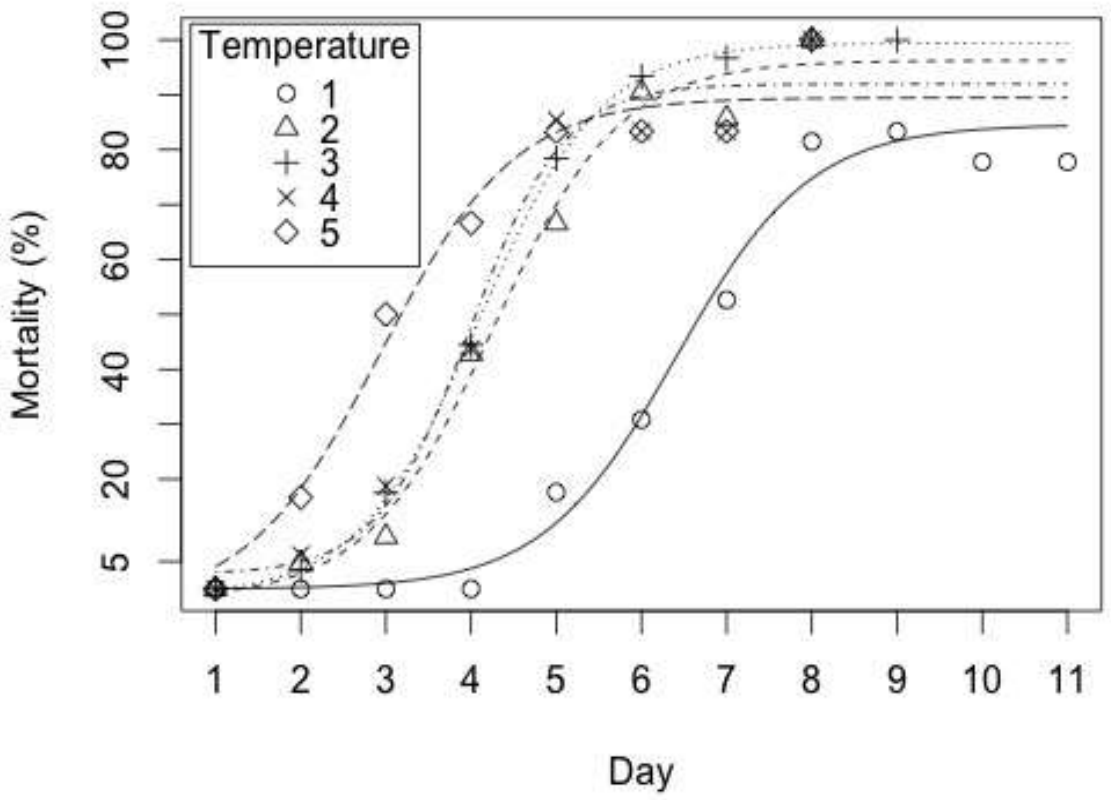

Fig 9. Percentage mortality over time (day) for each temperature 1=12 ^{o}C, 2=15 ^{o}C, 3=20 ^{o}C, 4=24 ^{o}C, 5=28 ^{o}C at a 35 minute spore showering duration. Model fitted to data was log logistic, lack-of-fit test *P* has to be greater than 0.05 (*P*=1.00).

Table 6. Lethal time to 50% mortality at experimental temperatures and associated standard error for exposure time 35 minutes giving an average dose of 60.9 spores/mm^{-2}.

*Numbers 1 to 6 refer to their respective temperatures in the Fig 9.

Temperature (^{o}C)*	LT50 (days)	St.err.
(1) 12	6.4	0.34
(2) 15	4.3	0.35
(3) 20	4.1	0.26
(4) 24	4.0	0.30
(5) 28	3.0	0.63

Preliminary analysis shows the lowest lethal time 50 (LT50) was at 28^{o}C and the highest was at 12^{o}C for an exposure time of 35 minutes.

4. Discussion

i. Monitor populations of healthy and fungus-infected cabbage aphids on sequentially planted brassicas and study the abiotic and biotic factors contributing to the mid-season population crash.

Fieldwork 2014

In experiment 1a the aphid population crashed around mid October (figure 1). This is much later than the crash in 2013; which occurred at the end of July. However there are similarities. For example in both years plant age was shown to have no effect on the timing of the crash, that is all transplants responded in the same way at the same time (figure 1). Moreover, both years saw the establishment of an epizootic around the timing of the crash strengthening a potential link between fungal entomopathogens and the population crash. However, with this epizootic we saw a corresponding increase in the entire guild of natural enemies suggesting (figure 2) they too might pay a role in the crash. Indeed figure 3 highlights this relationship, as when natural enemy numbers are high aphid population growth is at its lowest.

Quantification of the effect of density of apterous adult aphids on production of alate forms on brassica plants

One explanation put forward for the aphid crash is that an increase in the population of apterous (wingless) aphids on a particular brassica plant results in a sudden switch to the production of alate (winged) forms for emigration, resulting in a sharp population decline on the same plant. An experiment was done to monitor the production of alate forms in relation to the density of apterous forms. From this data it will be possible to calculate the density at which alate production begins. It should then be possible to manipulate aphid density and investigate its effect on natural enemy activity, in particular the epidemiology of *P.neoaphidis*. With this information it will be possible to build a simple epidemiological model to predict levels of infection/control in the field.

Preliminary investigation suggests the threshold for alate production to be approximately 100-150 individuals per plant. Based on observations in field experiments a population of this size would not significantly damage brassica plants, and it is unlikely that the cue for alate production is related to a decrease in host plant quality caused by a large aphid population.

The effect of plant age on Brevicoryne brassicae fecundity

That soluble nitrogen effects fecundity in aphids is well documented (van Emden, 1969). As a result it is not surprising that there is no significant difference in nymph production between plants of 'medium' and 'old' physiological ages as there was no significant different in soluble nitrogen. Nymph production does decline significantly between the youngest and two older plant ages, but it is highly unlikely by itself to cause the sudden decline in aphid populations observed in the field. For this to happen there would have to be no births. Field data also indicates an increase in mortality because the crash occurs over the course of a week. Plant age could be a contributory factor to the aphid population crash but is likely masked by other factors in the field.

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

Field experiments set up at Wellesbourne during the 2014 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 saw the establishment of a field epizootic which acted to reduced aphid infestations as in 2013. Attempts were made to isolate the fungi and were successful. Morphological data, as in 2013, suggests that the epizootic was caused by *Pandora neoaphidis* (Commonwealth Mycological Institute, 1979). DNA identification as confirmed that the pathogen isolated from *Brevicoryne brassicae* at Wellesbourne is *Pandora neoaphidis*.

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

The effect of temperature on the rate of colony extension of fungal isolates

Preliminary analysis suggests the optimal temperature for isolate NW420 is approximately 22^{o}C which is lower than the optima calculated for the Ascomycetes *B. bassiana* ATCC & GHA, *M. brunneum, I. fumosoros* & *L. longisporum* (25^{o}C) but higher than that of *L.muscarium* (Harvey, 2013). Data for *P.neoaphidis* isolate WELL1 is currently being collected.

Laboratory evaluation of the effect of temperature on the germination of fungal species from the phylum ascomycota

Germination times varied greatly depending on temperature with the slowest germination times for all isolates at 15^{o}C and increasing with increasing temperature.

Temperature dependence of the pathogenicity of P.neoaphidis

A series of bioassays were carried out at a range of temperatures from 12 to 28^{o}C and at varying spore showering times from 5-75 minutes. Death could be attributed to *P.neoaphidis* at all temperature because spores were produced on cadavers i.e. individuals were mycosed. Dead but unmycosed individuals were only observed at 24^{o}C and 28^{o}C inferring that heat stress could be another source of mortality. Further investigation into this competing risks theory is to be carried out in 2015.

Temperature clearly affects the ability of the fungus to kill individuals, not surprising because ectothermic organisms require suitable conditions to develop. These findings have important implications not only for pest management strategies involving the use of biopesticides i.e. spray windows, but also any conservation biocontrol strategies were the activity of enzootic fungal pathogens will be limited by the temperature of the environment. A better understanding of the effects of temperature on the biology of *P.neoaphidis* will allow accurate predictive models to be developed allowing growers to withhold pesticide applications.

5. Conclusions

- Although the aphid population crash occurred at a much later stage in the season in 2014 than in 2013, plant age was once again shown not to affect the timing of the crash. Moreover, there were no significant location variations in 2014 suggesting little spatial heterogeneity in the timing of the crash.
- Natural enemy abundance is reported to be significantly negatively associated with instantaneous rate of increase of aphid populations suggesting an antagonistic relationship.
- The 2014 field season also saw the establishment of a fungal epizootic around the timing of the crash as in 2013. This fungus was successfully isolated and is currently undergoing DNA identification, although morphological analysis suggests it to be *Pandora neoaphidis*.
- Alate production is linked to aphid population density.
- Plant age/soluble nitrogen effects *Brevicoryne brassicae* fecundity and mortality in the lab.
- Temperature has a significant impact on growth, germination and virulence further highlighting its importance in the biology of entomopathogenic fungi and consequences for any pest management strategy

6. Future work

- Further bioassays with Ascomycete fungal species used in previous bioassays at 20^{o}C (Harvey, 2013) will be carried out to observe the effect of temperature on their pathogenicity. These will provide a useful comparison to data already collected for *P.neoaphidis*.
- Investigation is warranted to look at the effect of temperature on *B.brassicae* survival and development in order to complete understanding of the host's thermal biology in addition to the pathogens.
- Whilst the mycelial growth assays of *P.neoaphidis* isolate WELL1 at different temperatures are underway experiments investigating germination are required to be conducted.
- Experiments investigating the compatibility of certain fungicides with *P.neoaphidis* will be carried out. This will involve lacing growth media with fungicide and measuring colony extension over time.
- Soluble nitrogen/plant age has been shown to effect aphid fecundity in the lab. This pest, plant interaction will be investigated further to include a third trophic level e.g. *P.neoaphidis*. Population parameters such as births, deaths and emigration will be monitored in order to quantify any effects.
- Aphid density will be manipulated and its effects on the spread/epidemiology of *P.neoaphidis* monitored.

7. Knowledge and Technology Transfer

The Society of Invertebrate Pathology. *Mainz, Germany. 2nd-8th August 2014*

The Association of Applied Biologists. *Lincolnshire. 19th-20th November 2014.*

HDC studentship conference. *York. 16th-17th September 2014.*

BGA conference. *Lincolnshire. 21st January 2014.*

Postgraduate Symposium. *University of Warwick. 24th-26th March 2014.*

8. References

Blackman, R.L. & Eastop, V.F., 1984. Aphids on the world's crops. *An identification guide*. Wiley & Sons, USA.

Blanford, S. & Thomas, M. B., 1999. Host thermal biology: the key to understanding host-pathogen interaction and microbial pest control. *Agricultural and Forest Entomology*, **1**, PP. 195-202.

Bruce, T.J.A., 2010. Tackling the threat to food security caused by crop pests in the new millennium. *Food Security*, **2**, pp. 133-141.

Chandler, D., 1994. Cryopreservation of fungal spores using porous beads. *Mycological Research*, **98**, pp. 525-526.

Commonwealth mycological institute., 1979. *Erynia neoaphidis* factsheet.

Danish Ministry of the Environment, 2013. miljoestyrelsen.dk. http://www.miljoestyrelsen.dk/common/Udgivramme/Frame.asp?http://www.miljoestyrelsen.dk/udgiv/publications/2001/87-7944-343-5/html/kap01_eng.htm accessed 22/05/2013.

Dedryver, C.A., Ralec, A.L. & Fabre, F., 2010. The conflicting relationships between aphids and men: A review of aphid damage and control strategies. *Comptes Rendus Biologies*, **333**, pp. 539-553.

Diaz, B.M., Legarrea, S., Marcos-Garcia. M.A. & Fereres, A., 2010. The spatio-temporal relationships among aphids, the entomophthoran fungus, *Pandora neoaphidis*, and aphidophagous hoverflies in outdoor lettuce. *Biological Control*, **53**, pp. 304-311.

van Emden, H, F. & Bashford, M. A., 1969. A comparison of the reproduction of *Brevicoryne brassicae* and *Myzus persicae* in relation to soluble nitrogen and leaf age (leaf position) in the Brussels sprout plant. *Entomologia Experimentalis et Applicata*, **12**, pp.351-364.

FAO, 2013. http://www.fao.org/agriculture/crops/core-themes/theme/pests/ipm/en/ accessed 11/09/2013.

Feng, M.G. & Johnsn, J.B., 1991. Bioassay of four entomophthoralean fungi (Entomophthorales) against *Diuraphis noxia* and *Metopolophium dirhodum* (Homoptera: Aphididae) *Environmental Entomology*, **20**, pp. 338-345.

Flint, M.L., 1985. Cabbage Aphid, *Brevicoryne brassicae*. Pp. 36-39. In: *Integrated pest management for Cole crops and Lettuce*. University of California publication.

Garthwaite, D.G., Thomas, M.R., Heywood, E., Battersby, A., Parrish, G. & Smith, L., 2007. PESTICIDE USAGE SURVEY REPORT 219:OUTDOOR VEGETABLE CROPS IN GREAT BRITAIN. FERA.

Hajek, A.E., McNeil, J.J. & Filotas, M.J., 2002. Using Entomophthorales for control: maturation, dormancy and survival of laboratory produced *Entomophaga maimaiga*. *Proceedings of the XXXIV annual meeting of the society for invertebrate pathology*, Noordwijkerhout, The Netherlands.

Hall, R.A., 1981. The fungus *Verticillium lecanii* as a microbial insecticide against aphids and scales. Pp. 483-498. In: *Microbial control of pests and plant diseases 1970-1980*. Academic Press, London/New York.

Harvey, L., 2013. The role of naturally occurring entomopathogenic fungi in regulating aphid populations on vegetable Brasica crops. *HDC Annual Report*, pp. 1-36.

Humber, R.A., 2008. Evolution of entomopathogenicity in fungi. *Journal of Invertebrate Pathology*, **98**, pp. 262-266.

Humber, R.A., 2012. Entomophthoromycota: a new phylum and reclassification for entomophthoroid fungi. *Mycotaxon*, **120**, pp.477-492.

Holland, J.M. & Oakley. J., 2007. Importance of arthropod pests and their natural enemies in relation to recent farming practice changes in the UK. *HGCA research review,* No. **6**, pp. 1-105.

IRAG (Insecticide Resistance Action Group)., 2012. Guidelines for controlling aphids in brassicae crops and combating insecticide resistance in the peach-potato aphid, *Myzus persicae*. pp. 1-8.

Isik, M. & Gorur, G., 2009. Aphidicidial activity of seven essential oils against the cabbage, *Brevicoryne brassicae* L. (Hemiptera: Aphididae). *Munis Entomology and Zoology*, **4**, pp. 424-431.

Karley, A.J., Parker, W.E., Pitchford, J.W. & Douglas, A.E., 2004. The mid-season crash in aphid populations: why and how does it occur? *Ecological Entomology*, **29**, pp. 383-388.

Karley, A.J., Pitchford, J.W., Douglas, A.E., Parker, W.E. & Howard, J.J., 2003. The causes and processes of the mid-summer crash of the patio aphids *Macrosiphum euphorbiae* and *Myzus persicae* (Hemiptera: Aphididae). *Bulletin of Entomological Research*, **93**, pp. 425-437.

Lacey, L.A., Frutos, R., Kaya, H.K. & Vail, P., 2001. Insect pathogens as biological control agents: do they have a future? *Biological Control*, **11**, pp. 230-248.

Maddock, J.V., McManus, M.L., Jeffords, M.R. &Webb, R.E., 1992. Exotic insect pathogens as classical biological control agents with an emphasis on regulatory considerations. Pp. 27-39. In: *Selection criteria and ecological consequences of importing natural enemies*. Thomas Say publications entomology society, USA, Lanham, MD.

McCoy, C.W., 1981. Pest control by the fungus *Hirsutella thompsonii*. Pp. 499-512. In: *Microbial control of pests and plant diseases 1970-1980*. Academic Press, London/New York.

Oerke, E.C., 2006. Crop losses to pests. *Journal of Agricultural Science*, **144**, pp. 31-43.

Papierok, . & Hajek, A.E., 1997. Fungi: Entomophthorales. In: Lacey, L.A. (Ed.), Manual of Techniques in Insect Pathology. Academic press, San Diego, pp. 187-212.

Pell, J.K., Eilenberg, J., Hajek, A.E. & Straus, D.C., 2001. Fungi as Biocontrol Agents. Chapter 4. Biology,Ecology and Pest Management Potential of Entomophthorales.: CAB International.

Razaq, M., Aslam, M., Amer, M. & Shad, S.A., 2011. Insect pest status of aphids on oilseed brassica crops and need for chemical control. *Crop and Environment*, **2**, pp. 60-63.

Rehner, S.A. & Buckley, E., 2005. A *Beauveria* phylogeny inferred from nuclear ITS and EFI-alpha sequences: evidence fro cryptic diversification and links to *Cordyceps* teleomorphs. *Mycologia*, **97**, pp.84-98.

Royal Society Report, 2009. *Reaping the benefits: Science and the sustainable intensification of global agriculture.*

Scorsetti, A.C., Maciá, A., Steinkraus, D.C. & López Lastra, C.C., 2010. Prevalence of Pandora neoaphidis (Zygomycetes: Entomophthorales) infecting Nasonovia ribisnigri (Hemiptera: Aphididae) on lettuce crops in Argentina. *Biological Control*, **52**, pp. 46-50.

Shah, P.A., Tymon, A. & Pell, J.K., 2001. Conservation biocontrol with mycopathogens: *Erynia neoaphidis* and agricultural field margins. *Proceedings of the XXXIV annual meeting of the society for invertebrate pathology*, Noordwijkerhout, The Netherlands.

Shah, P.A. & Pell, J.K., 2003. Entomopathogenic fungi as biological control agents. *Applied Microbiological Biotechnology*, **61**, pp. 413-423.

Sierotzki, H., Camastral, F., Shah, P.A., Aebi, M. &Tuor, U., 2000. Biological charateristics of selected *Erynia neoaphidis* isolates. *Mycoogical Research*, **104**, pp. 213-219.

Steinkraus, D.C., Hollingsworth, R.G.& Slaymaker, P.H., 1995. Prevalence of *Neozygites fresenii* (Entomophthorales: Neozygitaceae) on cotton aphids (Homoptera: Aphididae) in Arkansas cotton. *Environmental Entomology*, **24**, pp. 465-474.

Sullivan, D.J. & Völkl, W., 1999. Hyperparasitism: Multitrophic ecology and behaviour. *Annual Review of Entomology*, **44**, pp. 291-315.

Thacker, J.R.M., 2002. *An introduction to arthropod pest control*. Cambridge University Press, Cambridge, United Kingdom.

Vega, F.E., Goettel, M.S., Blackwell, M., Chandler, D., Jackson, M.A., Keller, S., Koike, M., Maniania, N.K.., Monzon, A., Ownley, B.H., Pell, J.K., Rangel, D.E. & Roy, H.E., 2009. Fungal entomopathogens: new insights on their ecology. *Fungal Ecology*, **2**, pp. 149-159.

Wiliding, N. & Brobyn., 1980. Effects of fungicides on the development of Entomophthora aphidis. Transactions of the British Mycological Society, **75**, pp. 297-302.

