



Grower Summary

CP 092

The role of entomopathogenic fungi in regulating aphid populations in field Brassicas

Final 2016

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GROWER SUMMARY

Headline

Populations of cabbage aphid, feeding on brassica plants in a field experiment, 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 “crash” has been observed in experiments in the previous year of this project, but it 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 affecting brassica aphid populations in field experiments, and detailed laboratory experiments have characterised how environmental temperature influences the speed of kill of the fungus against 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). Reliance on a small number of insecticides for *B. brassicae* control increases the chances of this pest evolving insecticide resistance. Because of the need to prevent over-reliance on synthetic chemical pesticides, there is a requirement to develop alternative forms of aphid management that can be used as part of Integrated Pest 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 briefly discussed.

Spatial heterogeneity in population fluctuations of *Brevicoryne brassicae* and their natural enemies on sequentially planted Brussels sprout plants in 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.

Effect of *B.oleracea* plant growth stage and leaf age on the reproduction of adult aphids (Experiment 5.2.1)

That soluble nitrogen affects 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 difference 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 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.

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*.

The effect of temperature on the growth, germination and pathogenicity of entomopathogenic fungi

Experiments were conducted to elucidate the effect temperature has on the growth, germination and pathogenicity of 7 species on entomopathogenic fungus. The details of which can be found in table 2.3.1 and 2.3.3. The effect of various durations of high relative humidity on the ability of *P.neoaphidis* to kill *B.brassicae* was also investigated. Additionally, the effect of temperature on *Brevicoryne brassicae* was explored to gain a deeper understanding of host-pathogen interactions. Experiments conducted were:

- The effect of temperature on the growth of 6 selected Ascomycetes from four different genera and *Pandora neoaphidis*
- The effect of temperature the germination of 6 selected Ascomycetes from four different genera and *Pandora neoaphidis*
- Temperature effects on *Brevicoryne brassicae* development (time to adulthood)
- Temperature effects on the performance of *Brevicoryne brassicae* (fecundity & mortality)
- Influence of various constant temperatures on the virulence of three biopesticides to *Brevicoryne brassicae*
- Influence of various constant temperatures on the virulence of *Pandora neoaphidis* to *Brevicoryne brassicae*
- Effect of relative humidity on the virulence of *Pandora neoaphidis* to *Brevicoryne brassicae*

The effect of temperature on the rate of colony extension of entomopathogenic fungal species from the phylum Ascomycota and *Pandora neoaphidis* = Entomophthoromycota.

. Analysis suggests the optimal temperature for isolate *P. neoaphidis* NW420 is approximately 22°C which is lower than the optima calculated for the Ascomycetes *B. bassiana* ATCC &

GHA, *M. brunneum*, *I. fumosoros* & *L. longisporum* (25°C) but higher than that of *L. muscarium* (Harvey, 2013).

Laboratory evaluation of the effect of temperature on the germination of entomopathogenic fungal species from the phylum Ascomycota and Pandora neoaphidis = Entomophthoromycota.

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

Temperature dependence of the pathogenicity of entomopathogenic fungal species from the phylum Ascomycota and P.neoaphidis = Entomophthoromycota.

A series of bioassays to measure the effect temperature has on the speed of kill of entomopathogenic fungi against *Brevicoryne brassica* were carried out at a range of temperatures from 12 to 28°C. Various spore showering times from 5-75 minutes were used for *Pandora neoaphidis* isolates, whereas LD95's were chosen for the Hypocrealean fungal species used in bioassays. 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°C and 28°C inferring that heat stress could be another source of mortality.

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

The research eludes to the role entomopathogenic fungi and other natural enemies play in the crash of aphid populations. Using the knowledge of temperature- insect-pathogen interactions accumulated in this project work can begin on a predictive model to determine to timing of the aphid population crash.

