Project number:	FV 221 (SAPPIO LINK Project LK 0908)
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Lead partner:	Rothamsted Research (Dr Judy Pell)
Scientific partners:	University of Newcastle
Co-sponsors:	BBSRC, HDC, PGRO, BPC, Sainsburys, NIAB
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The following are members of the Consortium for LINK project LK0908.

Scientific Partners
Rothamsted Research (Prof. Wilf Powell)
University of Newcastle

Co-sponsors
BBSRC
HDC
PGRO
BPC
Sainsburys
NIAB

GROWER SUMMARY

Key messages emerging from the project:

The aphid-specific fungal pathogen *Erynia (= Pandora*) *neoaphidis* has potential as a control agent of cereal aphids. However, epidemics often occur too late in the season to retain aphid populations below the economic threshold. We hypothesise that managed field margins could provide a constant reservoir for this fungus from where it could spread into adjacent crops earlier than normal thereby retaining crop aphids below damaging levels.

- An experimental field margin has been planted at Rothamsted. Field sampling strategies have been devised and used to determine the spatial and temporal distribution of aphid, fungus and parasitoid populations in this margin, in nettle plants around the field perimeter and in the adjacent cereal crop for 3 years. These data are being analysed using SADIE (Spatial Analyses by Distance Indices), a software package which identifies the spatial distributions of organisms from count data. SADIE is also being used to detect whether there are any associations between infection and parasitism in the field margin and wheat crop. Aphid abundances in the field margin and wheat crop were low or very low in 2000 and 2002. Moderate numbers were found in 2001 allowing preliminary analysis.

- Plants identified as potential aphid hosts in the margin include the grasses Yorkshire fog and crested dogstail, the legumes Bird's foot trefoil, clover and bean species. The large nettle aphid (*Microlophium carnosum*) is a common early-season source of *E. neoaphidis*.

- Molecular techniques have been developed to discriminate between *E. neoaphidis* and other species of fungus infecting aphids and also between groups of isolates within the species. Phylogenetic analysis showed that the different isolates of *E. neoaphidis* screened fell into three main groups which were not dependent on aphid host or geographical area. The lack of relationship between isolate and aphid host species suggests that isolates can move between different aphid species in the environment.

- *Erynia neoaphidis* and *E. kondoiensis* infection can be identified within aphids collected in the field using species specific PCR primers based on the ITS region of the genome. These techniques in combination with the field studies described above, will allow us to identify particular groups of isolates in the field and track them between the margin and the crop.

- Laboratory studies demonstrated that aphid species varied in their susceptibility to *E. neoaphidis*. *Acyrthosiphon pisum* (pea aphid) and *Metopolophium dirhodum* (rose-grain aphid) were highly susceptible to most isolates, whereas *Myzus persicae* (peach-potato aphid) and *Sitobion avenae* (English grain aphid) were moderately susceptible, and *Brevicoryne brassicae* (cabbage aphid) and *Rhopalosiphum padi* (bird cherry-oat aphid) were the least susceptible.

- Susceptibility of *A. pisum*, *M. dirhodum* and *M. persicae* was affected by the host plant on which the aphids were maintained, but not the cultivar.

Summary of results from reporting year:

Results are described against milestones:

1.3 Experimental field margins monitored for spatial and temporal dynamics throughout the year. Spread of *P. neoaphidis* in crop aphid species determined in laboratory and caged field experiments.

Field margin and wheat crop: Aphid densities during monitoring at Rothamsted (Stubbings field) were the lowest of the three years so far. Peak numbers of living aphids occurred at the end of July, and there were very few infections in *Sitobion avenae* which was the only aphid species to occur. This species also infested grasses in the margin. (Figs. 1, 2). In addition, the botanical composition of the field margin was very poor this year due to a combination of a low height for plant cutting in September 2001 and a very dry April 2002.

Nettle aphids: Nettle aphids were of moderate to high densities and useful information was collected on their abundance and distribution in two sites; the perimeter of Stubbings field and under an oak tree at the entrance to the field. Both studies showed that peak nettle aphid populations occurred in early June, while peak fungal infection was during mid - end June (Figs. 3,4). This indicates that in normal pest aphid years, *E. neoaphidis* would be established in nettle aphids before aphid populations in the adjacent crop began increasing. Therefore, infection has the potential to move from this source in nettles to other areas (margin and crop) limiting aphid population development. Samples for subsequent laboratory molecular characterisation were also collected and preserved.

Dispersal studies: Attempts were made to determine the dispersal and spread of *E. neoaphidis* spores from artificial sources placed in winter bean and winter wheat fields. At some of the sample distances, laboratory-reared aphids (*A. pisum* or *M. dirhodum*) were also placed in containers (sentinel aphids) for 24 hour exposure periods. Initial information was obtained on within-crop spread of the spores (Figure 5), as well as limited data on aphid infection (Figure 6). There were no natural infestations of aphids in either crop.

Cage infection studies: Infection levels in sentinel aphids (*A. pisum* or *M. dirhodum*) placed in 3.4 m^3 cages in bean and wheat crops were assessed. Two cages were positioned in each crop and fungal mycelium was positioned in the centre of one of each pair of cages. Very low infection levels were observed (1-2% in wheat cage only) and it was concluded that large cages were inappropriate settings for such experiments and that, in the first instance, future tests should be made either in caged pot plants or in muslin sleeves enclosing field crops.

2.3 Relative susceptibility of 3 crop aphid species on selected varieties including transgenics.

Three crop aphid species were tested for differences in *E. neoaphidis* infection while on standard and alternate varieties of host plant. For *A. pisum*, the standard broad bean variety was The Sutton and the alternate variety was Victoria; for *M. dirhodum*, the standard barley variety was Gleam and the alternate was Regina; for *M. persicae*, the standard Chinese cabbage variety was Wong Bok and the alternate was Kasumi.

Alternate varieties had been recommended because of their commercial use (Victoria, Kasumi) or presence on recommended seed lists (Regina). No statistical differences in time to infection or amount of infection were found between the two varieties for either of the three aphid species. Data on nymphal production between cultivars are being analysed.

In a separate study, a wheat cultivar (Bob White) was modified to include the GNA snowdrop lectin, which is active against a range of sucking pests. Genetically unaltered and modified seeds of Bob White were supplied by Dr A. Gatehouse (Univ. Newcastle) for testing. There appeared to be no differences in *E. neoaphidis* infection, numbers of nymphs produced, or time to infection between *M. dirhodum* adults kept on the GM or non-GM types of Bob White wheat. Data are currently being analysed.

3.3 Molecular 'fingerprint' of field collected isolates produced and used to identify spatial and temporal occurrence of isolates in the field.

- Primers specific for the species *E. neoaphidis* have been developed and used to identify the fungus within field collected infected aphids. DNA was extracted from infected and uninfected aphids but bands indicating the presence of the fungus were only found in the infected aphids. Further development of this technique will allow us to identify infected aphids in the field before symptoms are visible.

- Molecular screening using ERIC, RAM and RAPD primers has demonstrated differences in banding patterns amongst isolates of *E. neoaphidis*. Some isolates have bands that are either unique or shared by only a few other isolates (Figure 7; red arrows indicate these unique/ shared bands). Phylogenetic analysis showed that the different isolates of *E. neoaphidis* screened fell into three main groups which were not dependent on aphid host or geographical area. The UK isolates fell into two of these groups. The lack of relationship between isolate and aphid host species suggests that isolates can move between different aphid species in the environment.

- The development of group or isolate specific primers is still underway. Bands which appear to be unique or shared by only a few isolates are being cloned and sequenced to develop primers which will identify particular isolates. These primers can then be used to identify groups of isolates within infected nettle aphids collected from the field this year. Due to the low numbers of aphids in the crop, it will not be possible to identify spatial and temporal occurrence of particular isolates in the field but this may be possible in future years.

Supplementary Work

HGCA student bursary awarded to Ms Elcie Chan (Bath University) to develop an enzyme assay for aphid resistance to *P. neoaphidis*. Project completed and report submitted to HGCA.

Key issues to be addressed in the next year:

Milestones for the next year are
1.4 Experimental field margins monitored for spatial and temporal dynamics throughout the year.
2.4 Spread of *E. neoaphidis* in margin aphid species determined in laboratory and caged field experiments. Relative susceptibility of insecticide-resistant and susceptible *Myzus persicae* determined on a single host plant
3.4 Molecular 'fingerprint' used to identify spatial and temporal occurrence of isolates in the field