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Intraguild interactions between the entomopathogenic fungus *Pandora neoaphidis* and an aphid predator and parasitoid at the population scale

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ABSTRACT

The interactions that occur between the entomopathogenic fungus *Pandora neoaphidis* and a predator (*Coccinella septempunctata*) and a parasitoid (*Aphidius ervi*) were assessed in microcosm and polytunnel experiments. Transmission of *P. neoaphidis* to the pea aphid, *Acyrthosiphon pisum*, was enhanced in the presence of both *C. septempunctata* and *A. ervi* in microcosm experiments done under fixed abiotic conditions. In contrast, the reproductive success of *A. ervi* was reduced in the presence of *P. neoaphidis*. Despite the increased fungal transmission in the presence of *C. septempunctata*, there was no additional decrease in the aphid population indicating that *P. neoaphidis* is functionally redundant in the presence of the coccinellid. In polytunnel experiments the reproductive success of *A. ervi* was not affected by *P. neoaphidis*. These results do not support those of the microcosm and may be due to the more natural abiotic conditions in the polytunnel reducing the competitive advantage of the fungus. Microcosms therefore provide an arena in which the interactions between fungal pathogens and other aphid-natural enemies can be assessed however, further assessments at increased spatial scales under more natural abiotic conditions are also required to accurately determine the outcome of these interactions.

1. Introduction

The regulation of insect pests by their natural enemies (ecosystem service providers) is valued at $4.5 billion annually (Losey and Vaughan, 2006). The efficiency of this regulation may be influenced by the intraguild interactions that occur between the service providers, with positive intraguild interactions decreasing the equilibrium density of the pest and aiding overall control. In contrast, negative intraguild interactions may disrupt pest control. There is conflicting evidence concerning the effect of natural enemy species richness on the control of pest species with different authors reporting positive, neutral and negative effects of enhanced diversity on pest control (Pell, 2007). Straub et al. (2008) suggest that the effect of diversity on pest control is dependent on niche complimentarity (positive effect), functional redundancy (neutral effect) and intraguild predation (negative effect). Although complimentarity between natural enemies of exopterygote insects such as aphids should not be as great as that between endopterygotes that undergo extensive morphological changes (Wilby and Thomas, 2002), temporal separation between specialist groups within the aphidophagous guild occurs. For example, fungal entomopathogens are most effective in cool humid conditions (usually found at the start and end of the growing season) which are in contrast to the activity requirements of arthropod natural enemies (Pell, 2007). However, generalist predators that occur throughout the growing season such as coccinellids are coincidental intraguild predators (the intraguild prey is consumed whilst developing within its herbivore host) of both fungus-infected and parasitised aphids and may disrupt pest control (Meyhofer and Klug, 2002; Pell et al., 2008; Roy and Pell, 2000; Roy et al., 2008; Wells et al., 2001; Wheeler et al., 1968).

Although insects have been observed avoiding patches containing intraguild predators (Meyling and Pell, 2006; Nakashima et al., 2004), laboratory studies have shown that avoidance does not occur between all members of the aphidophagous guild. For example, the coccinellid *Coccinella septempunctata* (L.) and the hymenopteran parasitoid *Aphidius ervi* (Haliday) will both enter aphid colonies containing the aphid-specific entomopathogenic fungus *Pandora neoaphidis* (Remaudière and Hennebert) Humber where they subsequently predate fungal cadavers or oviposit in aphids infected with the fungus (Baverstock, 2004; Baverstock et al., 2005a). If these species are to be encouraged to co-exist as part of conservation biological control programmes, the outcomes of intraguild interactions and their effect on the population size of both the pest and their natural enemies needs to be assessed. The impact of intraguild predation on biological control has been assessed for a number of systems and, in a recent meta-analysis by Rosenheim

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and Harmon (2006), coincidental intraguild predation was found to enhance the overall strength of biological control whereas omnivorous intraguild predation (the prey plus its natural enemy are targeted by a second predator) had only a negligible effect.

Numerous factors affect the outcome of intraguild interactions, including: foraging mode, prey preference, response to prey density and microhabitat use (Straub et al., 2008). These factors are rarely incorporated into experiments designed to assess intraguild interactions, indeed, the interactions between *C. septempunctata*, *A. ervi* and *P. neoaphidis* described above were all done at the laboratory scale in artificial arenas such as Petri dishes or on single plants. The outcome of laboratory scale experiments may, therefore, not accurately reflect the intraguild interactions that occur at larger spatial scales. Microcosms provide an arena in which intraguild interactions and their effect on the prey population can be assessed within closed populations and communities can be assessed in an environment more similar to that of field conditions. Unlike field studies, the biotic and abiotic conditions within microcosms can be manipulated to test specific hypotheses regarding the outcome of intraguild interactions. The objective of the experiments described in this manuscript is to determine whether the intraguild interactions that occur at small spatial scales between *P. neoaphidis* and either *C. septempunctata* or *A. ervi* also occur when assessed at a larger spatial scale. The effect of *C. septempunctata* on transmission of *P. neoaphidis* and the subsequent effect on the aphid population are assessed followed by the intraguild interactions that occur between *A. ervi* and *P. neoaphidis* and their effect on the reproductive success of the fungus and parasitoid. In addition to this, the effect of increased spatial scale and semi-natural abiotic conditions on the two-way intraguild interactions that occur between *A. ervi* and *P. neoaphidis* are assessed in a polytunnel.

2. Methods and materials

2.1. Plant, fungus and insect cultures

The dwarf broad bean plant, *Vicia faba* (L.) (cultivar The Sutton), was used in this study. Plants were approximately 15 days old when used for experiments. An *in vivo* culture of *P. neoaphidis* (isolate X4, from Rothamsted Research collection, original host = *A. pisum*) was used in these experiments and was maintained by regular passage through *A. pisum* as described by Wilding (1970). Dried *P. neoaphidis* cadavers were stored at 20% relative humidity within a 4 °C incubator in darkness until required. To rehydrate the fungus, batches of five dried fungal cadavers were placed on solidified 1.5% tap-water agar poured to a depth of 3 mm and maintained at >95% relative humidity for 16 h. A size 5 cork borer (10 mm diameter) was then used to cut around the batches of sporulating fungal cadavers. Henceforth these will be referred to as '*P. neoaphidis* discs'. A mixed-sex culture of laboratory reared *C. septempunctata* that were 2–12 weeks post-ecolsion and had only been fed on *A. pisum* was used in the experiments. The coccinellids were transferred in batches of six to a Petri dish (90 mm diameter) containing a small piece of wet filter paper and maintained at 18 °C (16L:8D) for 24 h prior to the start of the experiment. This ensured that the coccinellids were starved and would forage during the experiment. The hymenopteran parasitoid *A. ervi* was used in these experiments and was cultured on *A. pisum* feeding on *V. faba* plants and maintained at 18 °C (16L:8D). Parasitoids were taken from a mixed-sex culture and were not more than 5 days old when used for experiments.

2.2. Cage microcosm experiments

Cages were made from Perspex (0.5 m × 0.5 m, 1 m tall) and contained a Perspex plant-pot frame (0.45 m × 0.45 m, 0.85 m tall) which had space for nine pots (85 mm diameter) each containing a single *V. faba* plant (four cages in total). The frame ensured that plants were equidistant from each other and allowed movement of insects between plants. The matting on the floor of the cage was kept wet at all times to provide water for the plants and to ensure a relative humidity higher than 90%. The cages were maintained within a controlled environment room (18 °C, 16L:8D).

Interactions between *C. septempunctata* and *P. neoaphidis*: Thirty, 8-day-old *A. pisum* (fourth instar) were placed at the base of each of the eight peripheral plants in each of the four treatment cages. The central plant remained uninfested. After 3 h the aphid-natural enemies were added to the cages to provide either: no enemies (control), *P. neoaphidis*, *C. septempunctata* or *P. neoaphidis* + *C. septempunctata*. Where required, six *P. neoaphidis* discs were placed in random positions on the leaves of the central plant and/or six coccinellids were released onto the central plant. After 8 days, the number of living aphids (adults and nymphs) and *P. neoaphidis*-sporulating cadavers on the eight peripheral plants were counted. The central release plant was not assessed.

Interactions between *A. ervi* and *P. neoaphidis*: The method described for transmission in the presence of *C. septempunctata* was repeated with the following modifications. The *V. faba* plants were infested with only ten 8-day-old *A. pisum* (to prevent the plant from becoming over-infested during the course of the experiment) and six *A. ervi* (3♂ + 3♀) were released at the base of the central plant in place of *C. septempunctata*. This provided the following four treatment cages: no enemies (control), *P. neoaphidis*, *A. ervi* or *P. neoaphidis* + *A. ervi*. After 8 days the number of *P. neoaphidis*-sporulating cadavers on each of the eight peripheral plants was assessed. Aphids were not assessed as destructive sampling would have been required. The plants were then placed within a large Perspex 'holding cage' and maintained at a low humidity (18 °C, 16L:8D) for a further 8 days. This ensured that the development of both *P. neoaphidis* and *A. ervi* within aphids would continue but subsequent sporulation and transmission of the fungus would be prevented. The number of *P. neoaphidis*-sporulating cadavers and *A. ervi* mummies was then assessed. Aphids were not assessed after 16 days as nymphs would have been produced whilst the plants were being maintained in the holding cage.

Each of the experiments described above was repeated on four occasions (four true replicates per treatment) with each treatment tested in a different cage on each occasion according to a Latin square design. Data were transformed to logarithms (base 10 after adding an offset of one to allow for zeros) so the data satisfied the assumptions underlying the analysis. Analysis of variance (ANOVA), with blocking structure for occasion and cage, was used to assess whether there was an effect of treatment on the number of aphids and/or natural enemies remaining in the cages. Values given in the text are back-transformed from the geometric mean following analysis.

2.3. Polytunnel experiment

The effect of *P. neoaphidis* on the reproductive success of *A. ervi* was assessed at a larger spatial scale under semi-natural abiotic conditions within a polytunnel. The polytunnel was made from a steel tube semi-circular frame (5.5 mm wide × 3 m high) covered in clear polythene. The light cycle and temperature within the polytunnel were dependent on external abiotic conditions. A bank of four fans drew air in through the south-facing end of the polytunnel and out through the north-facing end (flow rate of approximately 54 cm/s). Two patches, each comprised of nine pots (125 mm diameter, each containing three *V. faba* plants), placed in three rows of three pots (300 mm apart) were placed 6 m upwind from a parasitoid release point. Patches were adjacent to each other and separated by 1 m. Thirty eight-day-old *A. pisum* were