

Short Communication

Transmission of *Pandora neoaphidis* in the presence of co-occurring arthropods

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Abstract

Transmission of the entomopathogenic fungus *Pandora neoaphidis* to the nettle aphid *Microlophium carnosum* was assessed in the presence of arthropods that co-exist with the fungus within the habitat but do not compete for aphid hosts. The presence of a parasitoid significantly enhanced transmission, and transmission rates were similar for both enemy and non-enemy parasitoids. Although herbivory of nettle leaves by Peacock butterfly (*Inchis io*) caterpillars indirectly reduced the number of *M. carnosum* by >30% due to a reduction in leaf area for feeding, the addition of *I. io* significantly increased transmission of *P. neoaphidis* in the remaining aphids. It is likely that enhanced transmission in the presence of *A. rhopalosiphii* and *I. io* is due to disturbance and subsequent movement of the aphid, resulting in contact with conidia deposited on the leaf surface. The presence and impact of co-occurring arthropods should be taken into consideration when assessing the transmission of fungal entomopathogens.

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The aphid-specific entomopathogenic fungus *Pandora neoaphidis* (Remaudière & Hennebert) Humber (Entomophthorales: Entomophthoraceae) is a naturally occurring pathogen in temperate regions where it infects both pest and non-pest aphid species (Pell et al., 2001; Shah et al., 2004). *P. neoaphidis* disperses through the release of infective conidia from sporulating cadavers. Conidia are forcibly discharged either into air currents or are deposited on the substrate surrounding the cadaver. Presence of other natural enemy guild members, i.e. species which utilise the same aphid resource as the fungus, such as the seven-spot ladybird *Coccinella septempunctata* (L.) and the hymenopteran parasitoid *Aphidius ervi* (Haliday) (on pea aphids *Acyrtosiphon pisum* (Harris)) can enhance transmission of *P. neoaphidis* to other host aphids (Baverstock et al., 2007; Pell et al., 1997; Roy et al., 1998; Roy and Pell, 2000). Although *C. septempunctata* is able to vector the

fungus directly to host aphids, enhanced transmission is predominately thought to be as a result of aphid escape movements in response to attack resulting in greater contact with *P. neoaphidis* conidia (e.g. Baverstock et al., 2007; Roy and Pell, 2000). This is supported by the fact that transmission of *P. neoaphidis* to mobile pea aphids colonising a plant is approximately double that to immobile aphids feeding on a plant (Baverstock et al., 2005). Potentially, arthropods that co-occur with aphids but are not their natural enemies may also indirectly affect aphid behaviour and, therefore, the fungus-aphid interaction.

Over 100 species of insects can be found on the perennial stinging nettle *Urtica dioica* (L.) and these may affect the interactions that occur between *P. neoaphidis* and the nettle aphid *Microlophium carnosum* (Buckton) (Hemiptera: Aphididae) (Davis, 1991). Here we assess whether the presence of a hymenopteran parasitoid that does not attack *M. carnosum*, or a butterfly caterpillar that feeds directly on nettles affect the transmission of *P. neoaphidis* to

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M. carnosum compared to transmission in the presence of the hymenopteran parasitoid *Aphidius microlophii* (Pennacchio & Tremblay) (Hymenoptera: Aphidiidae), that does attack *M. carnosum*.

Aphidius rhopalosiphi (De Stefani Perez) (Hymenoptera: Aphidiidae) is a cereal aphid natural enemy, and is found nettle patches around cereal fields. Unlike *A. microlophii*, which is a natural enemy of *M. carnosum*, *A. rhopalosiphi* does not attack *M. carnosum*, but it may utilise nettle patches as refuges or for supplementary food sources (aphid honeydew). The transmission of *P. neoaphidis* to *M. carnosum* was assessed in the presence of either *A. rhopalosiphi* or *A. microlophii*. Nettle plants that were 28 days old were infested with 50 mixed-instar *M. carnosum* and maintained in Perspex cages (1 m × 0.5 m²) at 18 °C (16L:8D) within a controlled environment room for 11 days prior to the start of the experiment. Three cages (experimental unit) each containing four plants (pseudoreplicates) were prepared. Five batches of two *P. neoaphidis* cadavers that had been placed on discs of 1.5% tap-water agar (10 mm diameter, 3 mm depth) for 16 h and were sporulating profusely were placed on the adaxial surface of random leaves on each plant. One cage was supplemented with five female *A. rhopalosiphi*, one with five female *A. microlophii* and one cage was not supplemented with either parasitoid. After 7 days the number of *P. neoaphidis*-sporulating cadavers on each plant was assessed. The experiment was repeated four times, with treatments randomised to cages such that each treatment occurred in each cage at least once. All data analyses were done using GenStat 9th Edition (Payne et al., 2006). The data were transformed ($\log_{10}(n + 1)$) and analysis of variance (ANOVA), with the treatment df partitioned into two single df contrasts, used to assess whether parasitoid foraging

affected transmission of *P. neoaphidis* and whether transmission differed in the presence of enemy and non-enemy parasitoid species. Transmission of *P. neoaphidis* to *M. carnosum* occurred in all three treatments, with means ($n = 16$ plants) of 0.710 (back-transformed = 4.1), 1.137 (12.7) and 1.013 (9.3) sporulating cadavers being found in the fungus only, *A. rhopalosiphi* and *A. microlophii* treatments, respectively. The number of sporulating cadavers was greater in the presence of parasitoids than in their absence ($F_{1,4} = 7.91$, $p < 0.05$; Fig. 1). However, there was no effect of parasitoid species on the number of *P. neoaphidis*-sporulating cadavers recovered ($F_{1,4} = 0.73$, $p > 0.05$; Fig. 1).

The Peacock butterfly, *Inachis io* (L.) (Lepidoptera: Nymphalidae), utilises nettles in spring as a host plant on which to lay eggs and subsequently as a food source for the developing caterpillars. It is during this period that epizootics of *P. neoaphidis* in populations of *M. carnosum* are most commonly observed (Hemmati, 1998; Shah and Pell, 2003) and, therefore, it is likely that *P. neoaphidis* and *I. io* caterpillars co-occur. The transmission of *P. neoaphidis* to *M. carnosum* was assessed in the presence and absence of laboratory reared *I. io* caterpillars. A large nettle leaf (length ~12 cm) was placed abaxial side uppermost in a seed tray (15 cm × 21 cm) containing damp tissue paper and infested with approximately 75 mixed-instar *M. carnosum*. After 2 h an agar disc containing three sporulating *P. neoaphidis* cadavers (as described above) was placed on the centre of the leaf. Twenty replicate seed trays were prepared. A single final instar *I. io* caterpillar that had been starved for 24 h prior to the start of the experiment was placed in ten randomly selected seed trays. Each tray was covered using a polythene bag to maintain a high humidity (>95%) and maintained at 18 °C (16L:8D) within a controlled environment room. After 24 h the caterpillars were

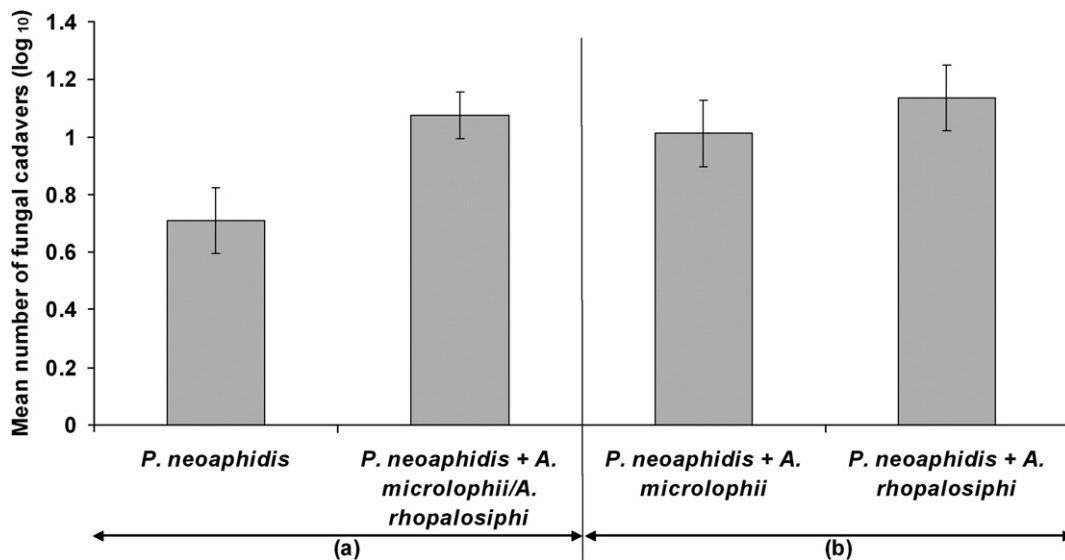


Fig. 1. Mean number (on \log_{10} scale) of *M. carnosum* recovered from nettle plants infested with *P. neoaphidis* in (a) the absence ($n = 16$ plants) and presence ($n = 32$ plants) of parasitoids and (b) the presence of an enemy parasitoid, *A. microlophii*, or a co-occurring non-enemy parasitoid, *A. rhopalosiphi* (both $n = 16$ plants). Error bars show standard error of difference of combined means.

removed and the number of aphids feeding on each of the twenty nettle leaves assessed. Finally, the trays were covered with a perforated bag and maintained at 18 °C for a further 5 days after which time the number of *P. neoaphidis*-sporulating cadavers was assessed. The experiment was done on two occasions. ANOVA was used to assess whether the presence of *I. io* affected the number of aphids (untransformed) remaining on the nettle leaves after 24 h. The proportions of aphids that became infected with the fungus were analysed using logistic regression (generalized linear model with binomial error and logit link) to determine whether *I. io* caterpillars affected transmission of *P. neoaphidis*. There was a significant effect of *I. io* on the number of living aphids remaining, with means of 50.6 and 74.9 aphids remaining in the presence and absence of the caterpillar, respectively, (SED = 10.15; $n = 20$; $F_{1,36} = 5.69$, $p < 0.05$). Transmission occurred in both the presence and absence of *I. io*. However, when *I. io* was present a larger proportion of aphids (back-transformed proportion = 0.25) became infected with *P. neoaphidis* than when *I. io* was absent (0.03) ($F_{1,36} = 35.90$, $p < 0.001$ after allowing for over-dispersion; Fig. 2).

Pandora neoaphidis co-exists within a diverse community of arthropods. Whilst a small percentage of these arthropods are guild members which compete with the fungus directly for aphids, the majority of these arthropods co-occur as they utilise the same host plant as the fungus-infected aphid. Previous studies have shown that both negative and positive intraguild interactions occur between *P. neoaphidis* and arthropods. For example, the presence of *Aphidius ervi* (Haliday) increases the transmission of *P. neoaphidis* at a cost to its own reproductive potential whereas intraguild predation of entire *P. neoaphidis* sporulating cadavers by the coccinellid *Harmonia axyridis* (Pallas) may prevent transmission of the fungus (Baverstock et al., 2007; Roy et al., 2008). However, unlike guild mem-

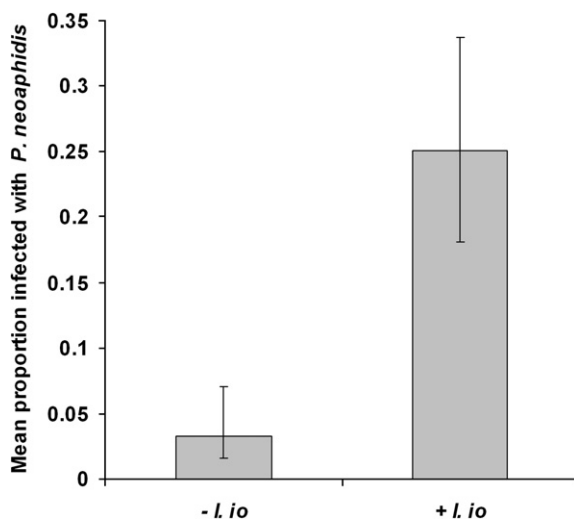


Fig. 2. Mean proportion of *M. carnosum* infected with *P. neoaphidis* in the absence ($n = 20$) and presence ($n = 19$) of *I. io*. Means and 95% confidence intervals back-transformed from the logit scale.

bers that compete with *P. neoaphidis* for host aphids, non-guild members do not utilise the same resource as the fungus and will not suffer a direct fitness cost through their interactions with the fungus. Indeed, the results presented here indicate that the presence of two species of co-occurring arthropods, *A. rhopalosiphi* and *I. io*, benefit *P. neoaphidis* through significantly enhancing its transmission to susceptible host aphids. Although competition for resources frequently occurs among distantly related herbivorous insects, with exploitative competition for plant tissue occurring between chewers and sap feeders (Kapan and Denno, 2007), the additional impact of the dominant competitor (*I. io*) on the susceptibility of the inferior competitor (*M. carnosum*) to an entomopathogen has not been previously considered.

The presence of parasitoids significantly increased the transmission of *P. neoaphidis* to *M. carnosum* and this supports previous studies done on a crop system which indicated that foraging parasitoids enhance fungal transmission (Baverstock et al., 2007; Fuentes-Contreras et al., 2007). Although the enhanced transmission of *P. neoaphidis* in the presence of *A. microlophii* may have been due to aphid escape responses to avoid parasitisation, it is unlikely that this was the case for *A. rhopalosiphi*. Parasitoids use visual and chemical cues released from both the host aphid and the plant on which the aphid is feeding to accurately determine whether an aphid is a suitable host for oviposition (Battaglia et al., 2000a,b). It is therefore unlikely that *A. rhopalosiphi*, which is cereal aphid specialist, would attempt to oviposit in *M. carnosum*. However, it is possible that aphids do not differentiate between enemy and non-enemy parasitoids and, therefore, the response of the aphid to *A. microlophii* attempting to oviposit and *A. rhopalosiphi* feeding on honeydew may be similar. Indeed, *A. rhopalosiphi* has been observed feeding on aphid honeydew and spends longer feeding on honeydew secreted from *M. carnosum* than that secreted from a host aphid, *S. avenae* (W. Powell, personal communication). This association between *A. rhopalosiphi* and *M. carnosum* may explain why the enhanced transmission of *P. neoaphidis* in the presence of enemy and non-enemy parasitoids is similar.

Transmission of *P. neoaphidis* in the presence of *I. io* caterpillars is approximately eight times greater than in the absence of the caterpillar. This is likely to be a result of *M. carnosum* making contact with conidia that had been deposited on the substrate whilst moving to an alternative feeding site following disturbance by *I. io*. However, the increase in transmission will be dependent on the extent of herbivory by *I. io*. In this experiment the caterpillar was allowed to forage on the nettle leaf for 24 h, in which time the surface area of the leaf was greatly reduced, although in no replicate was the leaf consumed entirely. In the presence of *I. io* the number of aphids feeding on the nettle leaf decreased by approximately 32%. This may be due to aphids being unable to find a suitable feeding site on the diminished nettle leaf and seeking an alternative

food source. If herbivory of the nettle leaf by *I. io* was to continue and entire leaves were consumed the aphid population would collapse and this would prevent the occurrence and transmission of *P. neoaphidis*. The effect of herbivores on the transmission of *P. neoaphidis* is therefore likely to be dependent on the extent of herbivory.

It is important when assessing the role of *P. neoaphidis* as a conservation biological control agent to understand the habitats in which the fungus naturally occurs, how the fungus propagates within this habitat, and the transmission of the fungus both within this habitat and between the habitat and cropped areas. These results highlight the importance of co-occurring arthropods on the transmission of *P. neoaphidis* and confirm previous studies which describe enhanced transmission of *P. neoaphidis* in the presence of guild members. Future research is now required to assess whether the presence of *P. neoaphidis* in naturally occurring nettle patches is affected by the diversity within the community of co-occurring arthropods.

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