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Entomopathogenic fungi stimulate transgenerational wing induction in pea aphids, *Acyrthosiphon pisum* (Hemiptera: Aphididae)

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Abstract. 1. Aphid natural enemies include not only predators and parasitoids but also pathogens, of which fungi are the most studied for biological control. While wing formation in aphids is induced by abiotic conditions, it is also affected by biotic interactions with their arthropod natural enemies. Wing induction via interactions with arthropod natural enemies is mediated by the increase in their physical contact when alarmed (pseudo-crowding). Pathogenic fungi do not trigger this alarm behaviour in aphids and, therefore, no pseudo-crowding occurs.

2. We hypothesise that, while pathogenic fungi will stimulate maternally induced wing formation, the mechanism is different and is influenced by pathogen specificity. We tested this hypothesis using two entomopathogenic fungi, *Pandora neoaphidis* and *Beauveria bassiana*, an aphid specialist and a generalist respectively, on the pea aphid, *Acyrthosiphon pisum* Harris.

3. We first demonstrate that pea aphids infected with either pathogen and maintained in groups on broad bean plants produced a higher proportion of winged morphs than uninfected control aphids. We then show that, when maintained in isolation, aphids infected with either pathogen also produced higher proportions of winged offspring than control aphids. There was no difference between *P. neoaphidis* and *B. bassiana* in their effects on wing induction in either experiment.

4. Unlike the effect of predators and parasitoids on pea aphid wing induction, the effect of pathogens is independent of physical contact with other aphids, suggesting that physiological cues induce wing formation in infected aphids. It is possible that aphids benefit from wing induction by escaping infected patches whilst pathogens may benefit through dispersion. Possible mechanisms of wing induction are discussed.

Key words. *Acyrthosiphon pisum*, *Beauveria bassiana*, *Pandora neoaphidis*, parasitism, pathogens, pea aphid, phenotypic plasticity, wing induction.

Introduction

Polyphenism, a special case of phenotypic plasticity, describes the environmentally induced expression of alternative morphological, physiological, or behavioural traits of a genotype. Some examples of alternative phenotypes among insects include elytra colour patterns in *Harmonia axyridis* (Komai, 1956), production of gregarious offspring in *Schistocerca gregaria* (Maeno *et al*., 2011), and differential production of metabolites within members of ant colonies (Law *et al*., 1965). It allows a given genotype to explore multiple habitats and/or at different seasons, and to develop new features whilst preserving the established ones (West-Eberhard, 1989; Nijhout, 1999). In order to better understand the population dynamics of organisms, it is crucial to observe

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the environmental cues that trigger phenotypic plasticity (Storz *et al*., 2011).

Among insects, aphids have conspicuous annual phenotypic plasticity. The annual life cycle of most species consists of sexual morphs during autumn and exclusively parthenogenetically reproducing females during summer (Minks & Harrewijn, 1987; Dixon, 1998; Williams & Dixon, 2007). In genetically identical sexual morphs, none, one, or both sexes will have wings, depending of the species, whereas parthenogenetic females produce winged or unwinged offspring in response to biotic and abiotic stimuli (Minks & Harrewijn, 1987; Dixon, 1998; Williams & Dixon, 2007). The number of winged offspring produced by an aphid varies among species (Mondor *et al*., 2005; Kunert *et al*., 2008) and within clones of a species (Sutherland, 1969; Weisser, 2001).

Aphids may trigger wing formation directly during their nymphal stage or maternally under adverse conditions (Braendle *et al*., 2006). In the pea aphid, *Acyrthosiphon pisum* Harris, wing formation is triggered exclusively by maternal effects, e.g. in response to crowding (Sutherland, 1969), host nutrition (Johnson, 1966; Lees, 1967; Sutherland, 1967), temperature (Johnson, 1966; Lees, 1967; Hodgson *et al*., 2005), and the presence of predators (Dixon & Agarwala, 1999; Weisser *et al*., 1999; Kunert & Weisser, 2003) and parasitoids (Sloggett & Weisser, 2002, 2004). In pea aphids, wing induction triggered by arthropod natural enemies is indirectly mediated by alarm pheromone emitted when aphids are attacked. When aphids perceive their alarm pheromone, they withdraw their stylet and walk or drop from the plant. This movement increases the encounter rate with other aphids and simulates constant physical contact, thus mimicking crowded conditions and triggering wing induction (Kunert *et al*., 2005). Therefore, wing induction in pea aphids is dependent not only on the alarm pheromone but also on the presence of additional aphids to maintain the physical contact. Whilst the effect of arthropod natural enemies on the ecology of aphids, including their biological control, is well studied, pathogens are less frequently investigated (Roy & Cottrell, 2008). Pathogens could cause alterations in the traits of a host, affecting its population biology and biotic interactions, although these effects may not be immediately apparent (Lefèvre *et al*., 2009).

Pandora neoaphidis (Remaudiére and Hennebert) Humber (Zygomycota: Entomophthorales) is a specialist pathogenic fungus of aphids causing epizootics in aphid populations in temperate regions (Hemmati *et al*., 2001). Conidia of this fungus are dispersed on wind currents (Wilding & Perry, 1980; Hemmati *et al*., 2001) and by arthropod natural enemies (Roy *et al*., 1998; Baverstock *et al*., 2006, 2009a). In the latter case, conidia may attach and form secondary conidia on the surface of non-target insects which subsequently vector the fungus to previously uninfected aphid populations (Pell *et al*., 1997; Roy *et al*., 1998, 2001). In addition, natural enemies can indirectly increase infection levels by alarming aphids and enhancing the probability of making contact with conidia deposited on the plant surface (Roy *et al*., 1998; Baverstock *et al*., 2008). Once attached to the aphid, conidia germinate and penetrate the cuticle and initiate the infection process (Völkl *et al*., 2007).

Beauveria bassiana (Balsamo) Vuillemin (Ascomycota: Hypocreales) is a generalist entomopathogenic fungus that infects many insect species from different orders (Vega *et al*., 2009). Like *P. neoaphidis*, conidia of *B. bassiana* can be aerially dispersed (Shimazu *et al*., 2002) or vectored by arthropod natural enemies, however, in the latter the conidia may germinate and infect the vector (Meyling *et al*., 2006).

These two species of entomopathogenic fungi are both highly virulent towards aphids, with previous studies giving LC_{50} values of 15.4 conidia mm^{-2} for *P. neoaphidis* infecting *A. pisum* (Shah *et al*., 2004) and 6.17 $\log_{10}$ conidia ml^{-1} for *B. bassiana* GHA infecting *Aphis fabae* (Hesketh *et al*., 2008). Although these two pathogens reduced the fecundity of adult pea aphids, they do not affect the intrinsic rate of increase of progeny (Baverstock *et al*., 2006). The indirect effect of pathogens on morph formation of offspring remains unexplored.

Here we evaluated the effect of both these pathogens on maternally induced wing formation in aphid offspring. We asked the following questions: (i) Do entomopathogenic fungi induce wing formation in aphid offspring? (ii) Does the co-evolved aphid-specific pathogen, *P. neoaphidis*, have a stronger effect on aphid life-cycle than the generalist *B. bassiana*? (iii) How do the mechanisms of wing induction by entomopathogenic fungi compare to mechanisms of arthropod predators? For this purpose, we determined the effect of pathogen species and number of offspring on the proportion of winged offspring. In order to investigate whether the pseudo-crowding effect is necessary to induce wing formation, aphids were inoculated with pathogens and maintained either in isolation or in groups, and the morphs of their offspring were compared.

Materials and methods

Aphids and pathogens

Pink pea aphids of clone BP (Weisser *et al*., 1999; Sloggett & Weisser, 2002; Kunert *et al*., 2005) were reared on 3-week-old broad bean plants, *Vicia faba* L. (cultivar The Sutton; Nickerson-Zwaan, U.K.) in cages and maintained at 18 °C and LD 16 : 8 h. This was the same clone that was studied for the effect of *Coccinella septempunctata* L. and *Aphidius ervi* (Haliday) on wing induction (Weisser *et al*., 1999; Sloggett & Weisser, 2002) and its mechanism via alarm pheromone (Kunert *et al*., 2005).

Pandora neoaphidis isolate X4, from the Rothamsted Research collection, was maintained as an *in vivo* culture by regular passage through *A. pisum* (Wilding, 1970). Dried *P. neoaphidis*-infected cadavers were stored at 4 °C and 20% RH in darkness until required and for no longer than 6 months. For experiments, three dried cadavers were placed on the surface of water agar (1.5%) in a Petri dish (5 cm i.d.) and incubated at 10 °C and >95% RH for 18 h in darkness to initiate sporulation (Baverstock *et al*., 2005).

Beauveria bassiana (Mycotech strain GHA) was stored at −86 °C in cryovials with 10% glycerol as a cryoprotectant. Prior to experiments, the fungus was defrosted, streaked onto

the surface of Sabouraud Dextrose Agar (SDA) plates and incubated at 25 °C in darkness. After 2 weeks, the fungus had grown sufficiently for conidia to be harvested and used for experiments. For both pathogens, inoculums were applied at sufficiently high doses to achieve 100% infection of the host (Roy *et al*., 2005; Baverstock *et al*., 2006).

Experiment 1: crowded aphids

Aphid lines. Fifteen aphid lines were established for this experiment according to Kunert *et al*. (2005) with few modifications. Each line provided aphids that were used as one replicate per treatment. To start a line, a wingless adult aphid (f_0 generation) was randomly selected and kept on a 3-week-old broad bean plant. Plants were covered with a cellophane bag (30 × 20 × 10 cm, Armin Zeller, Nachf. Schütz & Co, Langenthal, Switzerland) and maintained in a cabinet at 18 °C; LD 16 : 8 h. After 48 h, adult aphids were removed and their progeny (f_1 generation, 10–12 individuals per line) were left on plants for a further 10 days under the same conditions until they became adults. Eight of these adult aphids from a plant were then transferred, individually, to a new 3-week-old broad bean plant using a brush, covered with a cellophane bag and maintained as described above for 48 h. The adults were then removed and the nymphs (f_2 generation, 64–72 aphids per line) maintained until they became adults, at which time they were inoculated with either *P. neoaphidis* or *B. bassiana* or used as controls (Roy *et al*., 2005), as described below.

Pandora neoaphidis. Inoculation arenas consisted of a Petri dish (9 cm i.d.) that was half filled with 1.5% agar and had a broad bean leaf embedded with the abaxial side uppermost in the water agar. For each line, three wingless adult aphids (f_2 generation) were transferred to each of five Petri dish arenas (15 aphids in total). The aphids were then covered with a small Petri dish (5 cm i.d.) containing sporulating *P. neoaphidis* cadavers. These arenas were covered with a tissue paper followed by their lids (9 cm i.d.) and aphids were inoculated with *P. neoaphidis* conidia for 24 h. Seventy-five arenas were prepared to inoculate all 15 lines simultaneously. As a control treatment, another 75 inoculation arenas with the same number of aphids were prepared as described above for each line and covered with a small Petri dish (5 cm i.d.) containing only water agar.

After 24 h, all 15 inoculated aphids (f_2 generation) from each line were transferred to a new 3-week-old broad bean plant using a brush (one plant per line, 15 plants in total), covered with a cellophane bag and maintained at 18 °C, LD 16 : 8 h, and ca 40% RH. Control aphids from the same line were also transferred to new bean plants (15 control plants in total).

All aphids (f_2 generation) were observed daily for 3 days and then transferred to a new Petri dish containing a bean leaf to ensure that they succumbed to fungal infection without infecting their offspring. The offspring (f_3 generation) were left on the plants until they became fourth-instar nymphs or early adults, at which point the morph type was determined.

Beauveria bassiana. Beauveria bassiana (Mycotech strain GHA) was cultivated on 10 SDA plates using the same conditions as described previously. Conidia were scraped from the agar surface and mixed with 20 ml of 0.03% Tween 80 in a 50 ml Falcon tube. The suspension of conidia and mycelium was then vortexed for 5 min and filtered through four layers of muslin. The concentration of conidia was determined using a Neubauer haemocytometer at 400× magnification (6.1×10^8 conidia ml^{-1}). A bean leaf was then placed in a Petri dish (9 cm i.d.) and dipped in 0.5 ml of the conidia suspension until coated. Once dried, the leaf was transferred to another Petri dish that contained a filter paper (Whatman No. 1) that had been soaked in a further 0.5 ml of the conidia suspension. As for *P. neoaphidis*, 15 wingless aphids (f_2 generation) from each line were distributed equally among five Petri dishes containing the *B. bassiana*-treated leaf and filter paper and covered with a tissue paper and their lids. Petri dishes were enclosed in plastic bags for 24 h with a wet filter paper and kept under controlled conditions (18 °C; LD 16 : 8 h). This procedure was repeated for all 15 lines. As a control treatment, 15 aphids (f_2 generation) from each line were maintained on five bean leaves treated with just 0.03% Tween 80 solution. After 24 h, these aphids were transferred to bean plants as described previously. The adults were observed daily for 3 days and transferred to Petri dishes containing bean leaves and observed to ensure that they succumbed to fungal infection. The offspring were maintained and assessed as described previously. The *P. neoaphidis* and *B. bassiana* experiments were done simultaneously.

Experiment 2: uncrowded aphids

Aphid lines. Seventeen aphid lines (f_0 generation) were established on bean leaves in Petri dishes as described above and were allowed to reproduce for 24 h. Adults were then removed and their progeny (f_1 generation) maintained on leaves for 10 days to become adults. The aphids (f_1 generation) were then inoculated with either *P. neoaphidis* or *B. bassiana* as described below.

Pandora neoaphidis. Prior to infection, *P. neoaphidis* was prepared to sporulate from infected aphid cadavers as described previously. One wingless adult aphid (f_1 generation) was transferred to an inoculation arena using a brush and covered with a Petri dish containing the sporulating cadavers. The infection procedure and abiotic conditions were the same as used for crowded aphids. This procedure was repeated for all 17 lines. As a control treatment, 17 aphids (f_1 generation; one per line) were transferred individually to Petri dish inoculation arenas and covered with a Petri dish containing only water agar.

Aphids showered with *P. neoaphidis* conidia were individually transferred to new bean leaves in Petri dishes and covered with a tissue paper and their lids. Aphids were kept under controlled conditions (18 °C; LD 16 : 8 h) and observed daily. The adults were removed after 3 days to avoid infecting their offspring (f_2 generation) and transferred to new dishes and

maintained individually to ensure that they succumbed to fungal infection. The offspring (f_2 generation) were left on leaves until they became fourth-instar nymphs or early adults, at which point the number of nymphs was counted and the morph type determined.

Beauveria bassiana. Conidia from 10 SDA plates were harvested, the conidia concentration determined (7.5×10^8 conidia ml^{-1}), and the broad bean leaves and filter paper soaked in the conidia suspension as described previously. As for *P. neoaphidis*, one wingless aphid (f_1 generation) from each of the 17 lines was placed individually on a treated leaf and filter paper in a Petri dish and covered with a tissue paper followed by the lid. Petri dishes were enclosed in plastic bags with a wet filter paper to create a high RH to allow infection and maintained under controlled conditions (18 °C; LD 16 : 8 h) for 24 h. As a control, 17 aphids (f_1 generation; one per line) were also kept individually on leaves and filter papers treated with only Tween 80 solution. Aphids treated with *B. bassiana* were transferred to new bean leaves in Petri dishes, maintained and their offspring (f_2 generation) assessed as described previously.

Statistical analysis

Crowded and uncrowded aphid experiments were analysed separately but in the same way. The effects of pathogen species (*P. neoaphidis* or *B. bassiana*), infection (infected or uninfected), and number of offspring on the proportion of winged morphs were examined in one analysis by a generalised linear model (GLM) with a quasibinomial error distribution. Aphid lines were used as random effects. Numbers of offspring produced were analysed by a GLM with a quasipoisson error distribution using aphid lines as random effects and pathogen species and infection as independent explanatory variables. Models were simplified to the minimal adequate model by removing non-significant interactions followed by independent variables if these were not included in any significant interaction (Crawley, 2007b). Among non-significant independent variables or interactions with the same number of variables, the one with the highest P-value was removed first, followed by the others in a descending order. After removing a parameter, the new model was only accepted if the removal did not significantly increase deviance compared with the previous model after a F-test ($P > 0.05$) (Crawley, 2007a,b). Otherwise, the previous model was retained and the simplification continued with the next non-significant interaction or variable. Data were analysed using R software 2.9.0 (www.r-project.org).

Results

Experiment 1: crowded aphids

Most treated adult aphids (f_2 generation) succumbed to infection by the end of this experiment: 12.8 ± 0.42 adults ($85.33 \pm 2.66\%$) succumbed to *P. neoaphidis* and 12.13 ± 0.61 adults ($80.87 \pm 4.07\%$) succumbed to *B. bassiana* on each plant.

The number of offspring produced during this experiment was not significantly affected by the pathogen species ($F_{1,57} = 1.475$, $P = 0.23$), infection ($F_{1,57} = 2.161$, $P = 0.147$), or the interaction between both factors ($F_{1,55} = 3.847$, $P = 0.055$; Fig. 1). There was also no significant difference among aphid lines ($F_{14,42} = 0.942$, $P = 0.525$).

There was a significant positive relationship between the total number of nymphs and the proportion that were winged ($F_{1,56} = 4.723$, $P = 0.03$, Fig. 2a). Neither pathogen species ($F_{1,67} = 1.527$, $P = 0.221$), aphid line ($F_{14,43} = 1.23$, $P = 0.292$) or any interaction between factors were significant. Among aphids that were crowded, infected aphids produced a significantly greater proportion of winged offspring than uninfected aphids ($t_{58} = 2.116$, $P = 0.038$, Fig. 2b).

Experiment 2: uncrowded aphids

In this experiment all inoculated aphids (f_1 generation) succumbed to infection after being removed from leaves. Aphids that were infected with *P. neoaphidis* survived 4.94 ± 0.26 days post-inoculation (p.i.), whereas aphids infected with *B. bassiana* survived 5.5 ± 0.27 days p.i. There was no significant difference between the number of offspring produced by infected or uninfected aphids ($F_{1,67} = 1.963$, $P = 0.054$), or between pathogen species ($t_{66} = 1.981$, $P = 0.487$). Aphid line ($F_{16,48} = 1.023$, $P = 0.453$) and the interaction between pathogen species and infection ($F_{1,47} = 3.127$, $P = 0.084$) were not significant (Fig. 3).

The proportion of winged offspring was significantly affected by infection ($F_{1,65} = 10.301$, $P < 0.01$, Fig. 4), with infected aphids producing a greater proportion of winged offspring than uninfected control aphids. Pathogen species, aphid

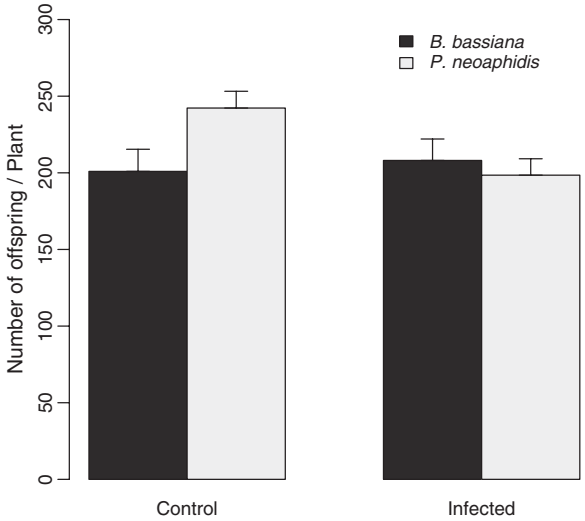

Fig. 1. Number of offspring from groups of 15 aphids inoculated with either uninfected control and *Pandora neoaphidis* or *Beauveria bassiana* ($n = 15$). Bars show mean values + SE.

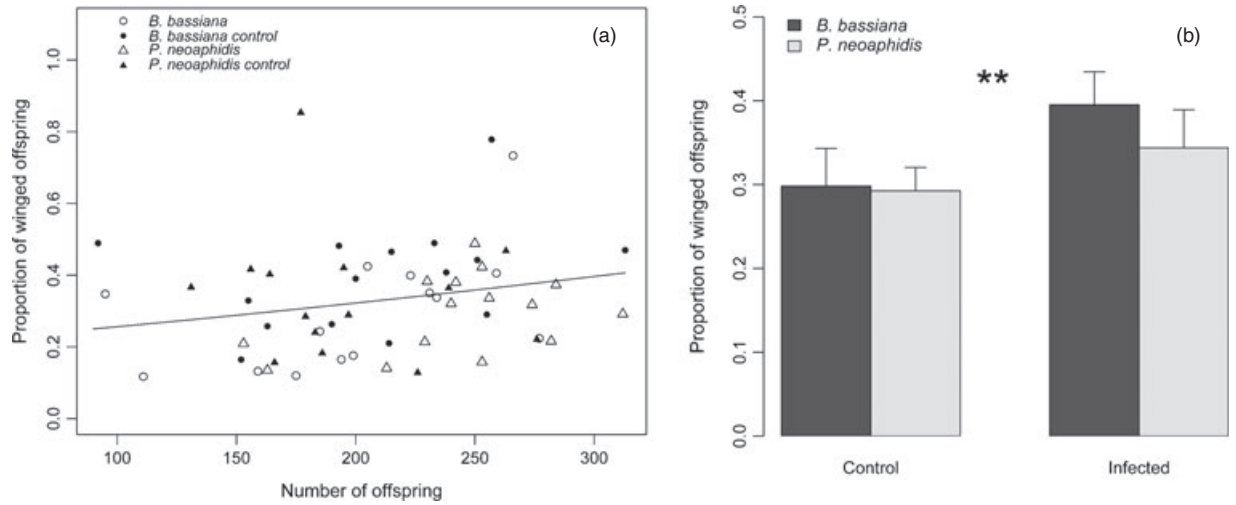

Fig. 2. (a) The relationship between the proportion of winged aphids and the number of offspring from groups of aphids treated with either *Pandora neoaphidis* or *Beauveria bassiana* or the respective controls, $n = 15$. Open circles represent aphids treated with *B. bassiana* and black circles its control; open triangles represent aphids treated with *P. neoaphidis* and black triangles represent its control. The linear regression over all 60 points showed a significant correlation between the number of offspring and the proportion of winged offspring independent of treatment ($r^2 = 0.0065$, $F_{1,56} = 4.723$, $P = 0.03$). (b) The proportion of winged offspring from crowded aphids treated with either uninfected control and with *P. neoaphidis* or *B. bassiana* differed significantly ($P = 0.038$), $n = 15$. Bars show mean values + SE.

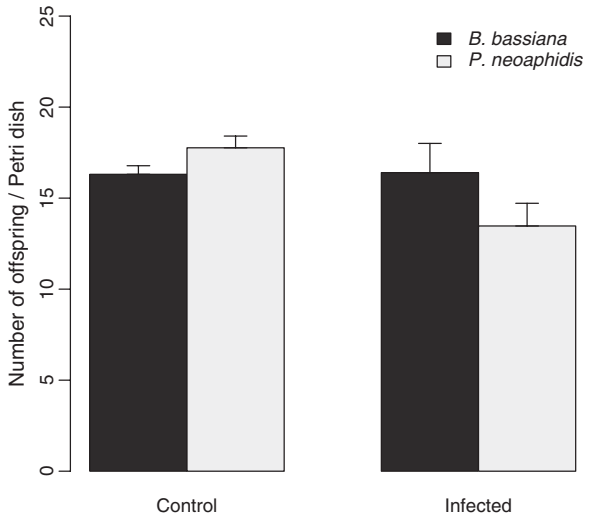

Fig. 3. Number of offspring from isolated aphids treated with uninfected controls and *Pandora neoaphidis* or *Beauveria bassiana* ($n = 17$). Bars show mean values + SE.

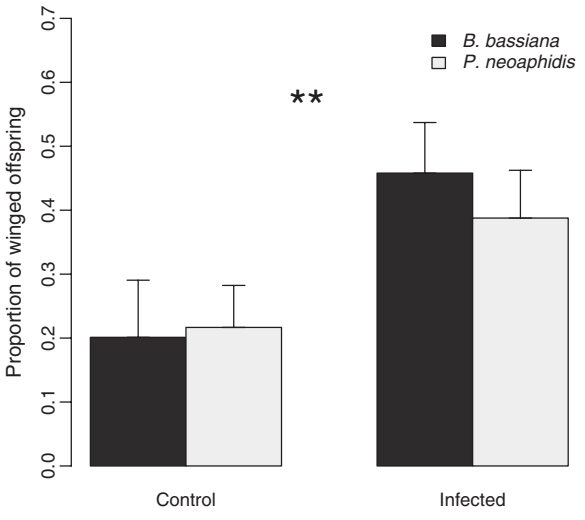

Fig. 4. The proportion of winged offspring from uncrowded aphids infected with *Pandora neoaphidis* or *Beauveria bassiana* and respective controls differed significantly ($P < 0.01$, $n = 17$). Bars show mean values + SE.

line, and number of offspring did not significantly affect wing induction ($F_{1,65} = 0.047$, $P = 0.829$; $F_{16,48} = 0.748$, $P = 0.638$ and $F_{1,64} = 0.843$, $P = 0.859$, respectively). Furthermore, none of the interactions between factors were significant.

Discussion

We demonstrated that the entomopathogenic fungi *P. neoaphidis* and *B. bassiana* affected aphid phenotypic plasticity by the induction of wing formation in offspring. Unlike wing induction triggered by predators and parasitoids, the effect of entomopathogenic fungi did not require physical contact with conspecifics and, consequently, is not mediated by the pseudocrowding effect. Interestingly, wing induction was not different between pathogen species, making it plausible that the same mechanism triggers wing formation for both pathogens.

Wings facilitate dispersal to explore new patches and to propagate the genotype whilst minimising competition and predation levels. Although there is a potential fitness cost of inducing wings through a lower reproduction rate and

longer development time (Dixon, 1998), the response shown here allows aphid genotypes to leave patches containing entomopathogenic fungi. Poethke *et al*. (2010) showed for predator–prey interactions that such behaviour, termed delayed predator-induced dispersal (PID, cf. Weisser, 2001), can evolve if there is substantial temporal correlation in predation risk and weak competition among prey. As most aphid colonies in the field do not grow to a size where intraspecific competition becomes important (Weisser, 2000; Weisser & Härri, 2005), the conditions of weak competitive interactions appears to be fulfilled in aphids. Because pathogen infection of aphids on a plant increases greatly the risk for the next (offspring) generation of aphids also to be infected by the fungus, pathogen infection is also likely to fulfil the condition of high temporal correlation in predation risk (Poethke *et al*., 2010).

It is, however, also possible that pathogens benefit from aphid wing induction: aphids can move to a new host plant to avoid infection sites, but they may also vector conidia, facilitating pathogen dispersal to new patches and contaminating a new or an existing colony, as has been observed for other species (Roy *et al*., 1998, 2001; Meyling *et al*., 2006; Baverstock *et al*., 2009b). Winged morphs of *Myzus persicae* Sulzer, *Brevicoryne brassicae* L., and *Lypaphis erysimi* Kaltenbach captured from the field were already infected by entomopathogenic fungi including *P. neoaphidis*, which subsequently established in young aphid colonies (Feng *et al*., 2007; Huang *et al*., 2008).

Entomopathogens may also modify emission dynamics of aphid alarm pheromone in pea aphids. Roy *et al*. (2005) showed that *P. neoaphidis*-infected aphids produce more alarm pheromone whilst *B. bassiana*-infected aphids decrease emission compared with uninfected controls. However, in contrast to predator attack, pathogen infection on its own does not lead to alarm pheromone emission. Infection only modifies the strength of alarm pheromone release upon attack by a natural enemy. Thus, it is unlikely that wing induction by pathogens in our grouped aphid experiment was mechanistically triggered by the pseudo-crowding effect.

When predators attack pea aphids, wing induction is strongly dependent of colony density, with a greater proportion of winged offspring produced when colony density is high (Kunert & Weisser, 2003). In addition, isolated pea aphids that were exposed to alarm pheromone to simulate the attack of a predator did not differ in wing induction from isolated control aphids (Kunert *et al*., 2005). However, in our experiment with single aphids, the proportion of winged offspring from infected aphids differed from control aphids, showing that the mechanism used by entomopathogens is not density-dependent. Thus, fungal infection is a cue to induce wing formation in *A. pisum*. Interestingly, single pea aphids that were parasitised by the braconid wasp *A. ervi* did not directly promote wing induction (Sloggett & Weisser, 2002), emphasising the difference in mechanisms used by insect parasitoids and fungal parasites. However, direct physiological manipulation for wing induction by a parasite was observed when the rosy apple aphid, *Disaphis plantaginea* Passerini, was infected with the *Disaphis plantaginea* densovirus (DpIDNV) (Ryabov *et al*., 2009). This virus was essential to induce wings since virus-free aphids did not produce any winged offspring, even in crowded conditions or poor plant quality (Ryabov *et al*., 2009). As this virus increases the proportion of winged offspring and mobility of aphids, it is likely that it also benefits viral transmission (Ryabov *et al*., 2009).

Crowding is important to induce wing formation as described not only for *A. pisum* (Sutherland, 1969), but for many other aphid species, including *Megoura viciae* Buckton (Lees, 1967), *M. persicae* (Sutherland & Mittler, 1971), and *A. fabae* (Shaw, 1970). In our group experiment, we observed a positive effect of number of offspring on the proportion of winged morphs. Possibly the offspring also contributed to the crowding effect and, therefore, induced the f_2 generation to produce winged morphs. Crowding was a significant factor only in our group experiment but worked independent of infection to induce wing formation. In conclusion, although *P. neoaphidis* and *B. bassiana* are under different selection pressures, both trigger wing induction equally in *A. pisum*, and independently of the pseudo-crowding effect. The costs and biochemical pathways for these fungi to trigger wing induction remain to be elucidated.

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