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Research

Susceptibility of Larvae and Pupae of the Aphid Parasitoid *Aphelinus abdominalis* (Hymenoptera: Aphelinidae) to the Entomopathogenic Fungus *Beauveria bassiana*

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Abstract

The lettuce aphid, *Nasonovia ribisnigri* (Mosley), is an economically important pest of lettuce worldwide. Recently, the entomopathogenic fungus, *Beauveria bassiana* strain GHA, and the aphelinid parasitoid, *Aphelinus abdominalis* Dalman, have been reported to be potential biological control candidates for use against *N. ribisnigri*. However, no information is available on the interaction between *B. bassiana* and *A. abdominalis* when both are applied. This study therefore examined the compatibility of *B. bassiana* and *A. abdominalis* in laboratory experiments. Specifically, we assessed the susceptibility of two *A. abdominalis* developmental stages (larvae and pupae) to two spore concentrations of *B. bassiana* (high: 1×10^9 and low: 1×10^4 conidia/ml) and a control of 0.01% Tween 80. We found parasitoid larvae to be highly susceptible to infection at the high spore concentration of *B. bassiana*, as measured by rates of mummy formation (mean ± SE: 14% ± 2.23) and adult emergence (mean ± SE: 10% ± 5.56) compared with the control treatment (mummification: mean ± SE: 79% ± 3.22; adult emergence: mean ± SE: 87% ± 4.40). In contrast, *B. bassiana* had no effects on parasitoid development when parasitoid larvae were treated with the lower spore concentration or parasitoid pupae were treated with either high or low spore concentrations. This study suggests that it might be possible to combine *B. bassiana* and *A. abdominalis* for integrated pest management of *N. ribisnigri*. As such, the application of *B. bassiana* should be timed to coincide with the presence of advanced developmental stages of *A. abdominalis* to protect the parasitoid. Another option would be to delay the release of *A. abdominalis* after *B. bassiana* application, when *A. abdominalis* is no longer susceptible to fungal infection.

Key words: lettuce, interaction, biocontrol, insect pathogenic fungi, mortality

The lettuce aphid, *Nasonovia ribisnigri* (Mosley) (Hemiptera: Aphididae), is a major pest of lettuce worldwide (Blackman and Eastop 2000). *Nasonovia ribisnigri* is difficult to control with contact insecticides because it prefers to live and feed deep inside the lettuce head (Mackenzie et al. 1988). This aphid species reduces lettuce yield by causing leaf distortion, reducing seedling vigor and deforming the heads (Stufkens and Teulon 2003). In Europe, different species of aphids, of which the *N. ribisnigri* is the most serious pest, have been reported to cause yield losses up to 70% in field-grown lettuce (ten Broeke et al. 2013).

Insecticides are still widely used for controlling *N. ribisnigri* in major lettuce-producing countries (e.g., United States, Europe, and New Zealand; Palumbo 2000, Kift et al. 2004, Fagan et al. 2010). However, the ability of *N. ribisnigri* populations to develop insecticide resistance (e.g., to organophosphates, carbamates, and pyrethroids; Barber et al. 1999, Kift et al. 2004) and the increasing awareness of environmental concerns related to the use of insecticides (Desneux et al. 2007, Koureas et al. 2012) among the public and farmers have promoted research on biological control methods as an alternative or supplement to chemical control.

The entomopathogenic fungus, *Beauveria bassiana* (Bals.) Vuill (Ascomycota: Hypocreales), is pathogenic to many pest species, including aphids (Ugine et al. 2007, Hesketh et al. 2008, Castrillo et al. 2010), and the strain GHA, commercially available as BotaniGard, has recently been reported as a promising biocontrol candidate for use against the lettuce aphid under field conditions in Europe (Yearbook 2009, Shrestha et al. 2015a). Another biological control agent, *Aphelinus abdominalis* Dalman (Hymenoptera: Aphelinidae), a solitary polyphagous aphid parasitoid believed to be native to Europe, is found over large areas of Europe, Asia, and

North America (Van Lenteren 2012). This commercially available species is an important biological control agent for a broad range of aphid species (Haardt and Höller 1992, Blumel and Hausdorf 1996) including economically important pests such as the potato aphid, *Macrosiphum euphorbiae* (Thomas), and the glasshouse potato aphid, *Aulacorthum solani* (Kaltenbach) (Eilenberg et al. 2000). Moreover, this parasitoid has recently been found to be an effective biological control candidate for use against *N. ribisnigri* (Shrestha et al. 2015b). Although the information regarding greenhouse or field releases of *A. abdominalis* for control of *N. ribisnigri* in Europe or other parts of the world is limited, it has been successfully used for control of *M. euphorbiae* and *A. solani* in European greenhouses (Eilenberg et al. 2000).

In the recent years, growers are increasingly interested in combining different beneficial agents such as parasitoids and insect pathogenic fungi for the control of aphids in glasshouses or field-grown crops (Eilenberg et al. 2000). These beneficial agents are often introduced simultaneously or over a short time interval (Kim et al. 2005). Because aphids are r-selected insects with a high reproductive capacity, parthenogenesis, and short generation time (Diaz and Fereres 2005), it can be difficult to achieve control using only a single beneficial agent. However, entomopathogenic fungi, especially Hypocreales fungi with a generalist nature, can infect nontarget insects, including parasitoids, which can result in direct deleterious effects in the parasitoids (Roy and Pell 2000).

In some cases, however, the additive interactions between pathogens and parasitoids can raise control efficacy (Brodeur and Rosenheim 2000, Roy and Pell 2000). Recent studies have found that *A. abdominalis* causes highest mortality in young and intermediate stages of lettuce aphids (Shrestha et al. 2015b), while *B. bassiana* primarily kills the older aphids (Shrestha et al. 2015a), suggesting that the combined application of these agents may enhance control.

One important way to obtain an additive mortality effect from these biological control agents is to apply the entomopathogenic fungus when the parasitoid is less susceptible to it (van Lenteren and Fransen 1994, Kim et al. 2005). To determine the optimal use which will minimize competition between the beneficials and enhance their additive interactions in controlling aphids, the interaction between beneficials should be examined prior to their application in wide-scale pest control program (Kim et al. 2005).

There is currently no information available on the compatibility of *B. bassiana* and *A. abdominalis* for use in future integrated pest management of *N. ribisnigri* in field or glasshouse grown lettuce in Europe, where both biocontrol agents are commercially available. This laboratory study therefore aimed at investigating the susceptibility of *A. abdominalis* larvae and pupae to *B. bassiana* infection.

Materials and Methods

Insect Cultures

Lettuce aphids, *N. ribisnigri*, were reared on iceberg lettuce (*Lactuca sativa* L. var. "Mirette") plants in nylon net cages (68 by 75 by 82 cm) maintained in a controlled environmental glasshouse (22 ± 1°C, 55–70% relative humidity [RH], and a photoperiod of 16:8 [L:D] h) at Flakkebjerg Research Centre, Aarhus University, Denmark. Fifteen to twenty mummies of *A. abdominalis* (EWH Bio Production, Tappernøje, Denmark) were placed in Petri dishes (diameter: 15 cm) with vented lids, and 30–35 of such Petri dishes were maintained in a controlled-climate cabinet at 22°C, 70% RH, and a photoperiod of 16:8 (L:D) h. Cohorts of adults emerging on the same day (50–60% emergence) were transferred to new Petri dishes (15–20 adults per Petri dish) with a piece of cotton pad soaked in a solution of 10% honey water. These adults were kept for two days under the same laboratory conditions. On the third day, adults were sexed under a stereo microscope and a single female and male subsequently transferred by aspirator to a new Petri dish (diameter: 5 cm) and left for mating for 24 h prior to use in experiments on the following day. All experiments were performed in July–August, 2014.

Fungus Material

Beauveria bassiana strain GHA (BotaniGard), formulated as a wettable-powder (22WP), was provided by Borregaard BioPlant (Aarhus, Denmark). The conidial concentration of the product was determined by counting conidia suspended in a solution of 0.01% Tween 80 under a compound microscope using a hemocytometer (0.0625 mm^2 Fuchs-Rosenthal Merck Eurolab) to calibrate a suspension of 1×10^9 conidia/ml. The lower concentration of fungus (1×10^4 conidia/ml) was prepared by serial dilution and the controls were treated by 0.01% Tween. The viability of conidia was determined by spreading 2 ml of 1×10^6 conidia/ml spore suspension on a plate with 2% Sabouraud dextrose agar and estimating the percent germination with a compound microscope after 18-h incubation at room temperature. This viability check was performed immediately before the start of each experiment. Conidia germination always exceeded 95%.

Production of Parasitized Aphids for Fungus Treatment

A synchronized cohort of second-instar aphids, a stage suitable for parasitization by *A. abdominalis* (Shrestha et al. 2015b), was produced by placing 20–25 adult aphids (10–12 d old) on uninfested lettuce leaves with a fine camel hair brush. The petiole of each leaf was wrapped with a piece of moist cotton pad and inserted into a 1.5-ml Eppendorf tube with demineralized water. One or two such leaves were placed at the bottom of a Plexiglass box with a mesh screened lid (17 by 11 by 3 cm) lined with moist filter paper. These Plexiglass boxes were afterwards moved to a controlled-climate cabinet and held at 22°C, 70% RH, and a photoperiod of 16:8 (L:D) h. Adult aphids were allowed to produce nymphs for 24 h, after which, the adults were removed. Leaves containing newly produced nymphs were also incubated, in boxes, in the controlled-climate cabinet, (as previously described) to allow them to reach the second instar (Diaz and Fereres 2005). The resulting cohort of second-instar *N. ribisnigri* was used to obtain the parasitized aphids.

A group of 50 second-instar individuals of *N. ribisnigri* was transferred onto a clean leaf disc (diameter: 5 cm) and placed at the bottom of a Petri dish (diameter: 9 cm) lined with moist filter paper. Subsequently, five mated adult female parasitoids of same age (4 d old) were released into this arena and allowed to oviposit for 24 h. The ratio of 50 aphid individuals to 5 parasitoids was chosen based on prior experiments yielding parasitism rates of ~96%. After the parasitization period ceased, the leaf disc with parasitized nymphs was gently transferred to a Plexiglass box with uninfested leaves (Eppendorf setup, see above) to allow aphids to translocate themselves to the new leaves. Parasitized aphids were incubated until immature parasitoids in hosts were either larvae (4 d after oviposition) or pupae (8–9 d after oviposition). Parasitized aphids (4 d after oviposition) were identified under a stereo microscope based on external symptoms such as 1) no wing bud development at the thorax of parasitized alates vs. wing bud development at the thorax of unparasitized alates; 2) dorsal banding pattern not visible in parasitized apterae vs. dorsal banding visible in unparasitized apterae (G.S. et al. unpublished data).

Treatment of Parasitized Aphids With Fungus

The experiment was performed to assess the susceptibility of *A. abdominalis* developmental stages (larval and pupal) to two spore concentrations of *B. bassiana* (high: 1×10^9 and low: 1×10^4 conidia/ml) and a control of 0.01% Tween 80. The "high" and "low" spore concentrations of *B. bassiana* relate to the general LC_{50} levels for aphids. Parasitized aphids of the same stage were placed in groups of 10 individuals in a transparent 30-ml medicinal cup capped with a mesh screen lid. Each group was swirled for 5 s in 5 ml spore suspension and the liquid drained off in a Büchner funnel (Hall 1976). The parasitized aphids from each treated group were subsequently transferred with a fine camel hair brush to a lettuce leaf disc (diameter: 5 cm) placed at the bottom of a Petri dish (diameter: 9 cm) lined with moist filter paper. The Petri dish was then sealed with parafilm to maintain saturated humidity in order to facilitate conidial germination. After 24 h, the leaf disc with parasitized aphids (larval stage of parasitoid) was transferred to Plexiglass boxes with a fresh lettuce leaf (Eppendorf setup, see above) or the parasitized aphids (pupal stage of parasitoid, i.e., in the form of mummified aphids) were transferred individually into medicinal cups with screened lids. These Plexiglass boxes and plastic cups were checked at 2–3-d interval for a total of 10 and 15 d, respectively, for larval stage and pupal stage, and the numbers of mummified aphids and emerged adults were recorded. When mummified aphids had been observed in Plexiglass boxes, they were transferred individually into medicinal cups with screened lid and the emergence of adults was observed. Seven replicates (one replicate equals a cup with 10 individuals that had been treated with fungus or Tween 80) were undertaken per treatment (larval or pupal stages). The entire experiment was run twice (providing a total of 14 replicates per spore concentration treatment), each time on a different date.

Treatment of Unparasitized Aphids With Fungus

An additional control experiment was performed to study the course of fungus infection in unparasitized aphids over time; this will provide information on when the host starts to succumb to fungus infection. Additionally, the objective of this experiment was to see whether the unparasitized aphids subjected to different fungal spore concentration and a control of 0.01% Tween 80 work differently for parasitized and unparasitized aphids. Groups of 10 unparasitized aphids (second instar) were treated with two spore concentrations of *B. bassiana* (1×10^9 and 1×10^4 conidia/ml) and a control of 0.01% Tween 80. The treatment of aphids with fungus and their subsequent incubation was similar to the previous experiment. Aphid mortalities were recorded at 1–3-d intervals for 7 d and dead aphids were placed on moist filter paper in Petri dishes to check for fungus sporulation. Treatments were replicated seven times in each run of the experiment, and the whole experiment was done twice, on two separate dates.

Statistical Analysis

One-way analysis of variance (ANOVA) was used to determine the effect of *B. bassiana* on rates of mummification or adult parasitoid emergence when treated as parasitoid larvae or pupae. A test with normal quantile-quantile plot was used to check the normality of residuals and the equality of residual variances. Mortality data from unparasitized aphids were not corrected for control mortality, as this study was to see the mortality effects of the control (0.01% Tween 80) to the mortality effects of the high and low dose treatments. Tukey's post hoc test was used for multiple comparisons among the treatment means. Data were analyzed using the free statistical software package R 2.15.1 (R Development Core Team 2012).

Results

Effect of *B. bassiana* on Unparasitized Aphids

The average cumulative mortality (± SE) of unparasitized aphids treated with two spore concentrations of *B. bassiana*: 1×10^9 and 1×10^4 conidia/ml and a control of 0.01% Tween 80 were: 95.71 ± 1.73, 24.29 ± 2.91, and 10.16 ± 1.82%, respectively, 7 d postinoculation (Fig. 1). Mortality data were supported by data on sporulation; no fungus-infected specimens were found in the control treatments, while 98.47 and 17.64% of dead aphids supporting

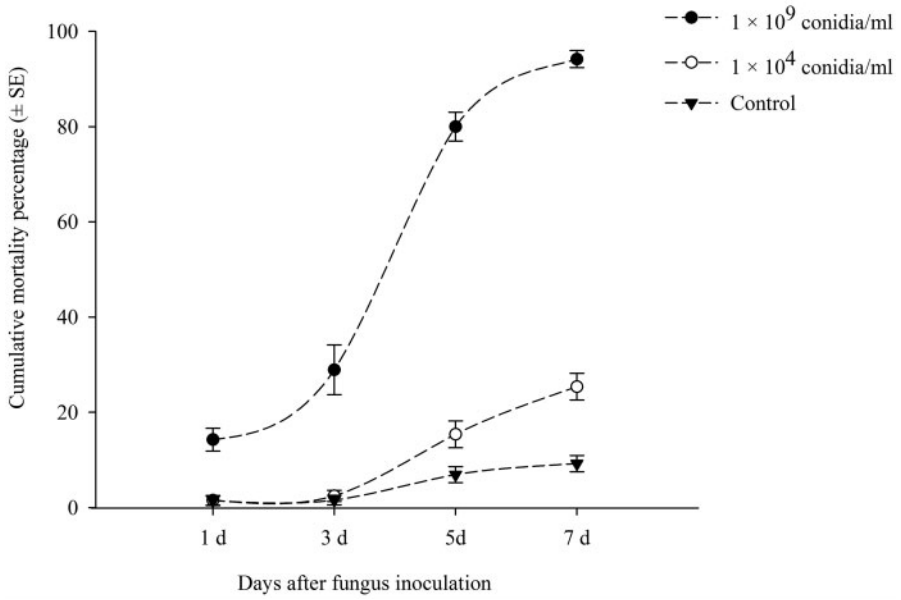

Fig. 1. Cumulative mortality (mean ± SE) over time of unparasitized second-instar *N. ribisnigri* treated with different spore concentrations of *B. bassiana* or a Tween control.

fungus outgrowth were found in the high and low fungal spore treatments, respectively.

Effect of *B. bassiana* on Parasitoid Larvae

This study showed that *B. bassiana* had a significant influence on the rate of mummification and adult parasitoid emergence when applied to parasitized aphids containing parasitoid larvae (4 d after oviposition) with different spore concentrations of fungus (mummification: df = 2, 39; $F = 158.70$; $P < 0.0001$; adult emergence: df = 2, 39; $F = 79.51$; $P < 0.0001$). The lowest rate of mummies or adult parasitoid emergence occurred when parasitoid larvae were treated with the high spore concentration of the fungus, whereas larvae developed most successfully into the highest rate of mummies or adult parasitoids when treated with the low dose of fungus or the control treatment (Figs 2 and 3). Furthermore, there was no significant difference, for either parameter, between the low fungal conidial level and the control (Figs 2 and 3).

Effect of *B. bassiana* on Parasitoid Pupae

We found no significant difference in rates of adult parasitoid emergence among treatments when mummified aphids (containing parasitoid pupae) were treated with different fungal spore concentrations or Tween (the control; df = 2, 39; $F = 1.23$; $P = 0.30$; Fig. 4).

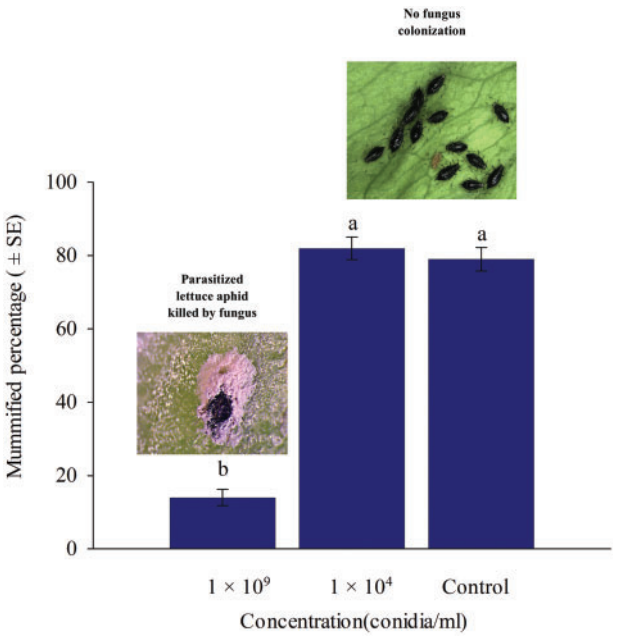

Fig. 2. Rates of mummification of *N. ribisnigri* parasitized by *A. abdominalis* when treated at the parasitoid larval stage (4 d after oviposition) with different spore concentrations of *B. bassiana* or a Tween control. Bars bearing the same letters are not significantly different (Tukey's test, $P > 0.05$).

Discussion

The cumulative mortality of *N. ribisnigri* seven days after application of *B. bassiana* spores confirms the results previously presented by Shrestha et al. (2015a) on the virulence of this fungus for *N. ribisnigri*. This study showed that only *A. abdominalis* larvae were susceptible to the fungus, and only at the high spore concentration as measured by both mummification and adult emergence. In contrast, *B. bassiana* showed no effect on *A. abdominalis* development when the parasitoid larvae were treated with the low spore concentration of fungus nor when parasitoid pupae were treated with either high and low spore concentrations of fungus.

These results support previous studies showing a higher susceptibility to *Lecanicillium* sp. (syn. *Verticillium lecanii*) infection in larvae of *Aphidius colemani* Viereck (Hymenoptera: Aphidiinae) (Kim et al. 2005) and of *Aphidius nigripes* Ashmead (Brodeur and

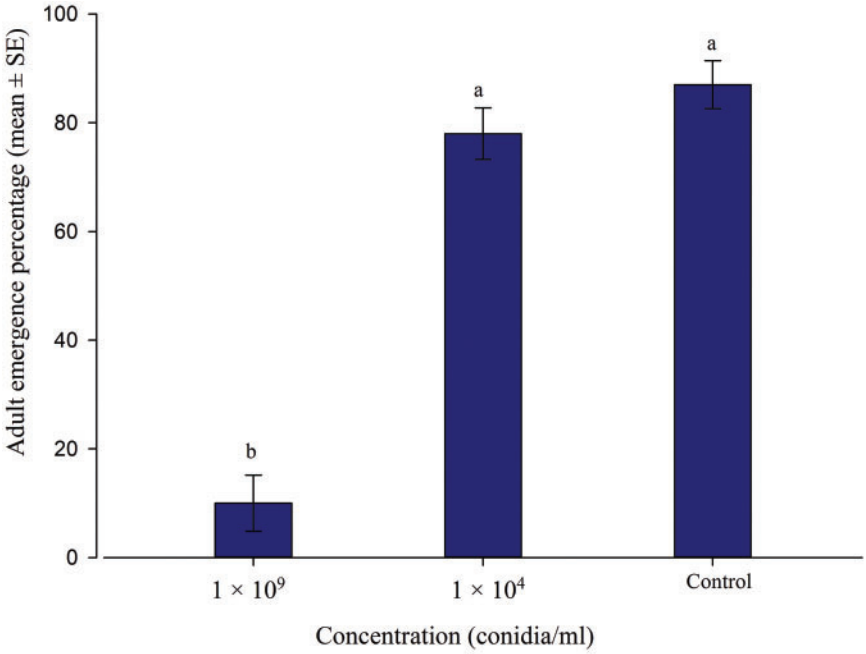

Fig. 3. Rates of adult parasitoid emergence (mean % ± SE) of *N. ribisnigri* parasitized by *A. abdominalis* when treated at the parasitoid larval stage (4 d after oviposition) with different spore concentrations of *B. bassiana* or a Tween control. Bars bearing the same letters are not significantly different (Tukey's test, $P > 0.05$).

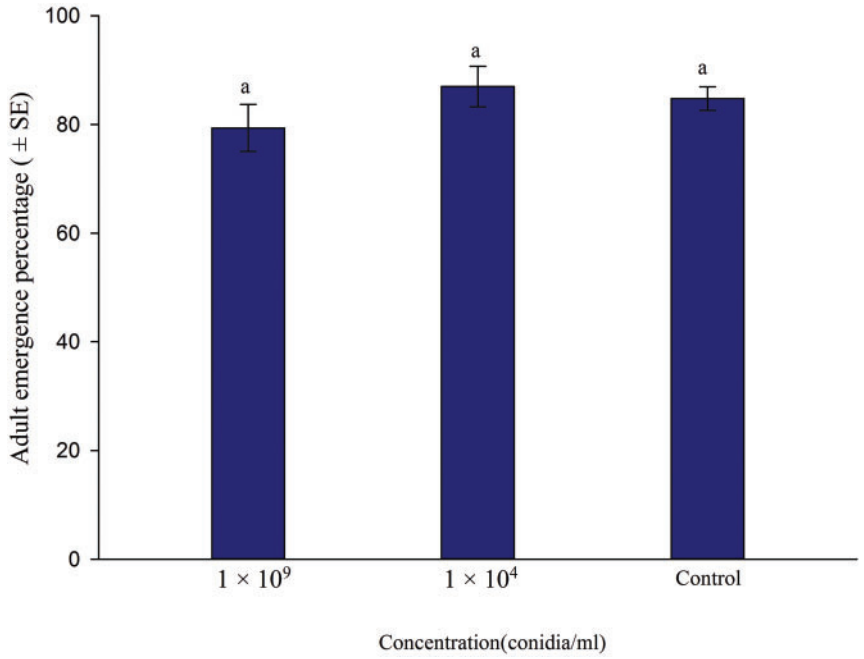

Fig. 4. Rates of adult parasitoid emergence (mean % ± SE) of *N. ribisnigri* parasitized by *A. abdominalis* when treated at the parasitoid pupal stage (8–9 d after oviposition) with different spore concentrations of *B. bassiana* or a Tween control. Bars bearing the same letters are not significantly different (Tukey's test, $P > 0.05$).

Rosenheim 2000), parasitoids of cotton aphid, *Aphis gossypii* Glover (Hemiptera: Aphididae), and potato aphid, *M. euphorbiae*, respectively, as compared with the pupal stages of these species. Furthermore, van Lenteren and Fransen (1994) reported higher susceptibility of *Encarsia formosa* Gahan (Hymenoptera: Aphelinidae) larvae to *Aschersonia aleyrodes* Webber (Deuteromycotina: Coelomycetes) compared to the susceptibility of the pupal stage.

Differences in susceptibility between developmental stages of parasitoids to fungal infection may be influenced by number of factors such as: 1) the timing of fungal spore application, with increased time intervals between parasitoid introduction and fungus application possibly reducing mortality (van Lenteren and Fransen 1994, Kim et al. 2005, Rashki et al. 2009); 2) the conidial load on the body of the target insects, with a low pathogen dose leading to lower rates of mortality (Lacey et al. 1997, Askary and Brodeur 1999); 3) the change in cuticle composition during parasitoid development, with greater cuticle thickness in older developmental stages possibly preventing penetration by fungal hyphae (Brodeur and Rosenheim 2000).

Our data show that the fungus has an effect on *A. abdominalis* larvae and a lack of effect on pupae (at a high dose of conidia), and this differential response might be due to the fungus penetrating the aphid cuticle and parasitoid larval tissues relatively easily. Higher parasitoid larval mortality was also observed for *Aphelinus asychis* Walker (Hymenoptera: Aphelinidae) and *A. nigripes* by Lacey et al. (1997) and Askary and Brodeur (1999), respectively, and only when the aphid populations were treated with high concentration of conidia. Similarly, Mesquita and Lacey (2001), reported that larvae of *A. asychis* (4 d after parasitization) were not susceptible to a low dose of the fungus *Isaria fumosorosea* (=*Paecilomyces fumosoroseus*) Wise (Hypocreales: Cordycipitaceae).

Over the past decade, research interest in the biocontrol of *N. ribisnigri* in glasshouse or field-grown lettuce has gradually progressed in several countries, particularly in Europe (Department for Environment Food and Rural Affairs [DEFRA] 2007, Yearbook 2009, Shrestha et al. 2015a), New Zealand (Fagan et al. 2010), Canada (Fournier and Brodeur 2000), and United States (Hopper et al. 2011). Potential biocontrol candidates of *N. ribisnigri* in field-grown lettuce are syrphid species in California (Hopper et al. 2011), and ladybeetles in New Zealand (Fagan et al. 2010). In Europe, lacewings and the parasitoid *A. abdominalis* have been reported as major biocontrol agents in glasshouse and field-grown lettuce (Shrestha and Enkegaard 2013, Shrestha et al 2015a). Another potential biocontrol candidate for controlling *N. ribisnigri* in Europe is the insect pathogenic fungus, *B. bassiana*, strain GHA (DEFRA 2007, Yearbook 2009, Shrestha et al. 2015b). However, currently, there are no scientific reports that outline methods for combining different biocontrols in integrated pest management strategies of *N. ribisnigri* in Europe or the other parts of world. This is the first study to provide evidence that suggests that *B. bassiana* strain GHA and *A. abdominalis* can be used in conjunction for control of *N. ribisnigri* in Europe.

Beauveria bassiana, strain GHA has been used successfully as a biocontrol of several pests including the *N. ribisnigri* and is registered for use against arthropod pests in glasshouse and field crops in Europe (DEFRA 2007, Yearbook 2009). However, in some European countries such as in Denmark, it has not been registered yet for outdoor use. *B. bassiana* persists longer in phylloplane at lower temperatures and low solar radiation compared to high temperatures and high solar radiation (Ignoffo 1992, Inglis et al.1997, Jaronski 2010). Therefore, in temperate and mediterranean regions, this entomopathogenic fungus may provide better control of *N. ribisnigri* in field-grown lettuce during spring or autumn as compared to the summer season. This is supported by lettuce field trials during the spring season in Spain (Yearbook 2009) and the autumn season in United Kingdom (DEFRA 2007). At the same time, the use of other biocontrols of *N. ribisnigri* such as lacewings (Shrestha and Enkegaard 2013) or parasitoids (Shrestha et al. 2015b) are often

limited during spring and autumn, especially in the northern temperate regions, due to suboptimal climate conditions. For glasshouse lettuce production, *B. bassiana*, strain GHA may be applied regardless of season because proper environmental conditions can be maintained throughout the production cycle.

This preliminary study indicates that *B. bassiana* and *A. abdominalis* should be used simultaneously with caution, as *A. abdominalis* larvae were found to be susceptible to the high spore concentration of *B. bassiana*, a concentration likely to be 10× times higher than the manufacturer's recommended dose rate. In practice, it would be difficult to time *B. bassiana* applications so that the larvae of *A. abdominalis* are not also infected. This is because *B. bassiana* is typically applied very frequently to obtain efficient control of pests, as suggested by biocontrol companies (e.g., BioWorks 2016). Our results suggest that the timing and frequency of *B. bassiana* applications need to be adjusted in order to integrate *B. bassiana* and *A. abdominalis* for managing *N. ribisnigri*. The best strategy for integrating these two biocontrol agents would be to use each biocontrol agent at different times during the lettuce cropping cycle.

Beauveria bassiana could be used first to control the *N. ribisnigri* during the early growth stages of the lettuce plants, as *N. ribisnigri* usually colonize the heart leaves first, followed by other leaves in later stages (Liu 2004). We have recently reported that *B. bassiana* has the ability to cause mortality of *N. ribisnigri* in all leaves of lettuce plants before the heads formed (Shrestha et al. 2015a). In contrast, *A. abdominalis* is likely to be less efficient at controlling *N. ribisnigri* during early lettuce growth stages since the aphid prefers to feed inside heart leaves, which makes it difficult for *A. abdominalis* to locate the hosts. Hence, *A. abdominalis* could be released 5–6 d after application of *B. bassiana*, when the number of viable spores on the lettuce leaves will be reduced (Shrestha et al. 2015a); therefore, the fungus's impact on the parasitoid will be minimal. Another option would be to delay applications of *B. bassiana* with respect to release of the parasitoids, to coincide with the occurrence of parasitoid pupae rather than larvae. This would likely reduce parasitoid mortality and better conserve parasitoids in the cropping system.

In summary, these preliminary results suggest that combining *B. bassiana* and *A. abdominalis* may be feasible for integrated pest management of *N. ribisnigri* in field and glasshouse grown lettuce in Europe. Another aspect which could influence the integration of *B. bassiana* and *A. abdominalis* for controlling *N. ribisnigri* is, whether or not *A. abdominalis* will able to discriminate between aphids infected with fungus vs. noninfected aphids, or to avoid the fungus-treated lettuce leaves. Studies have shown that this plays an important role in hosts–parasitoids–fungus interactions (Brobyn et al. 1988, Rännbäck et al. 2015, Oreste et al. 2016). For example, Brobyn et al. (1988) and Rännbäck et al. (2015) showed that the parasitoids *Aphidius rhopalosiphis* De Stef (Hymenoptera: Aphidiinae) and *Trybliographa rapae* Westwood (Hymenoptera: Figitidae) were able to choose healthy rose grain aphids *Metopolophium dirhodum* (Wlk) and cabbage root flies, *Delia radicum* L. (Diptera: Anthomyiidae), for oviposition as compared to the hosts infected with *Erynia neoaphidis* Remaudiere & Hennebert (Entomophthorales: Entomophthoraceae) and *Metarhizium brunneum* Metsch (Ascomycota: Hypocreales), respectively. Therefore, more studies on this aspect of the *N. ribisnigri–A. abdominalis–B. bassiana* system are necessary to improve overall control efficacy of *N. ribisnigri*, and to improve methods for combing these two biocontrol agents.

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