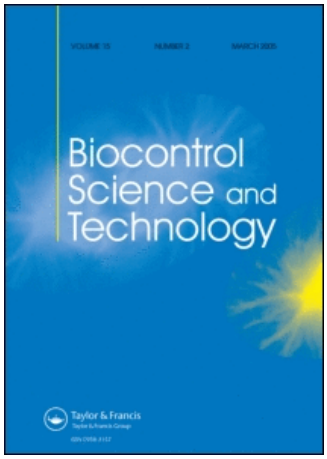

Biocontrol Science and Technology
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713409232

Field Evaluation of Beauveria bassiana: Its Persistence and Effects on the Pea Aphid and a Non-target Coccinellid in Alfalfa

Rosalind R. James; Brenda T. Shaffer; Brian Croft; Bruce Lighthart

Online Publication Date: 01 December 1995
To cite this Article: James, Rosalind R., Shaffer, Brenda T., Croft, Brian and Lighthart, Bruce (1995) 'Field Evaluation of Beauveria bassiana: Its Persistence and Effects on the Pea Aphid and a Non-target Coccinellid in Alfalfa', Biocontrol Science and Technology, 5:4, 425 - 438
To link to this article: DOI: 10.1080/09583159550039620
URL: http://dx.doi.org/10.1080/09583159550039620

Biocontrol Science and Technology (1995) 5, 425–437

Field Evaluation of *Beauveria bassiana*: Its Persistence and Effects on the Pea Aphid and a Non-target Coccinellid in Alfalfa

ROSALIND R. JAMES,[1] BRENDA T. SHAFFER,[1] BRIAN CROFT[2] AND BRUCE LIGHTHART[3]

[1]*ManTech Environmental Technology, Inc., 200 SW 35th Street, Corvallis, OR 97333, USA;* [2]*Department of Entomology, Oregon State University, Corvallis, OR 97339, USA;* [3]*US Environmental Protection Agency, Environmental Research Laboratory, 200 SW 35th Street, Corvallis, OR 97333, USA*

(*Received for publication 28 October 1994; revised manuscript accepted 29 July 1995*)

Several strains of the entomopathogenic fungus Beauveria bassiana *have been considered for use as microbial insecticides. Experimental sprays were conducted in an alfalfa field with an aphid-derived strain of* B. bassiana *to determine its persistence and its effects on pea aphids,* Acyrthosiphon pisum *(Homoptera: Aphididae) and a non-target aphid predator,* Hippodamia convergens *(Coleoptera: Coccinellidae).* B. bassiana *conidia persisted in the field for at least 28 days, when approximately 10% of the original inoculum was still present. In the lower canopy, more conidia were present than on other plant parts and they persisted longer on the leaves in this location. However, conidia were still abundant in the upper canopy, where 97.9% of the aphids and 95.5% of* H. convergens *larvae were found. Thus, both insect species were exposed to the fungus for at least 1 month. However, pea aphid populations were not affected by the fungus. The predator's incidence was reduced by 75–93% (depending on application rate) early in the season, but was not affected later in the season. Insect life history patterns and weather conditions are likely causes for the differences seen in field effects.*

Keywords: *microbial pesticide, insect pathogen,* Acyrthosiphon pisum, Hippodamia convergens, Beauveria bassiana

INTRODUCTION

Many entomopathogenic fungi have broad host ranges (Goettel *et al*., 1990), and the use of such microorganisms to control insect pests may present risks to predatory insects that are important biological control agents themselves. The US Environmental Protection Agency requires that the potential effects of microbial pest control agents on non-target organisms be assessed before

Correspondence to R. R. James, Department of Forest Science, Oregon State University, Corvallis, OR 97331–7501, USA.

0958–3157/95/040425–12

registration. In the past, assessing risks to non-target arthropods usually involved laboratory bioassays, and only a few studies have been done in the field (i.e. see Flexner *et al.*, 1986).

In the field, the risk of an insect population to a particular microbial pesticide depends both on the innate susceptibility of the insect, the degree of its exposure and environmental conditions. Laboratory bioassays can give some indication of innate susceptibilities and the effects of different environmental conditions. However, the degree of exposure depends on the likelihood that the insect will come into contact with the microbe, which, in turn, is dependent on factors such as persistence of the microbial agent, its distribution in the canopy and the behavior and morphology of the insect.

Beauveria bassiana is an entomopathogenic fungus (class Hyphomycetes) that has a very broad host range, infecting all the major insect orders (Goettel *et al.*, 1990). It has been considered as a pest control agent for a variety of pests, principally from, but not restricted to, the orders Coleoptera, Lepidoptera and Hemiptera (Tanada & Kaya, 1993). The suggestion that it be used for aphid control (Pavliushin, 1983; Feng *et al.*, 1990; Dorschner *et al.*, 1991) raises concerns for coccinellids. In the laboratory, Magalhães *et al.* (1988) found that *B. bassiana* was pathogenic towards two non-target aphid predators (both coccinellids), and *Hippodamia convergens* (Coleoptera: Coccinellidae) was later found to be susceptible to two different strains of the fungus (James & Lighthart, 1994). One of these strains, ARSEF 2883, was of particular interest for further study because it was originally isolated from an aphid, *Schizaphis gramminum* (Homoptera: Aphididae), and has shown pathogenicity towards several other Aphididae (Feng *et al.*, 1990; Dorschner *et al.*, 1991).

Using strain ARSEF 2883, field experiments were conducted in alfalfa to determine: (1) the persistence and distribution of *B. bassiana* within the crop canopy after spray application; and (2) its effects on populations of a pest insect, the pea aphid *Acyrthosiphon pisum* (Homoptera: Aphididae) and a predator of this pest, *H. convergens*. The behavior of the insects in the greenhouse was also observed in order to determine their distribution in the alfalfa canopy. This information was used to assess the relative risks to exposure for the two insects. Alfalfa is an ideal crop for testing fungal agents because humidity levels within the canopy are very high due to the dense growth of the foliage, and activities of many entomopathogenic fungi are enhanced under conditions of high humidity.

MATERIALS AND METHODS

Field Experiments

Field site. The experimental site, a 2-year-old alfalfa field on the Oregon State University Entomology Farm, less than 2 miles east of Corvallis, OR, was maintained as a hay crop by a local farmer. The 105×82 m field was divided into a grid, with 7.6-m spacing between intersects, and each intersect was a potential plot location, giving 108 possible plot locations. Each treatment and replicate was randomly assigned to one of these locations. The whole experiment was repeated twice during the season, but the same plot location was not used more than once. Each 'plot' consisted of a $1 \times 1 \times 1$-m screen cage (mesh size 0.125×0.10 cm), with the screening buried approx. 25 cm into the ground. The top of the cage opened to allow access for spraying plots and sampling plants, and was fastened closed with Velcro.

Experimental design. For the first experiment, 0.5 g of live aphids were added to each cage at the end of March to allow them to become established before treatments were applied. On 25 April, 20 neonate *H. convergens* larvae were added to each cage. Two days later, *B. bassiana* treatments were applied. Three concentrations of *B. bassiana* ARSEF 2883 were tested: 10^4 (low), 10^6 (medium) and 10^8 (high) conidia ml^{-1}. A water control was also included. The fungus was cultured on Sabaroud maltose agar with yeast extract (SMAY, Difco Laboratories, Detroit, MI, USA) at 25°C. Conidia were removed from the agar plates after 1 month and stored at -80°C until needed (after 25 days for the first experiment and 3 months for the second one). The evening before a spray application took place, conidia were mixed in water using a tissue

grinder. The conidia–water mixture was adjusted to the desired concentrations using a hemocytometer to enumerate the conidia.

Conidia suspensions were applied to the plots with a gas (N_2) pressure backpack sprayer at a rate of 137 ml/plot for coverages of 0, 1.4×10^{10}, 1.4×10^{12} and 1.4×10^{14} conidia ha^{-1}. Plants were sprayed to just before run-off at this rate. There were eight cages/treatment: four of these cages were used for a single destructive sample for insects and four were used for sampling plants for *B. bassiana* persistence. Thus, there were four replicates for each spray treatment.

The experiment was repeated on 1 July after the first hay crop was taken off the field. The second experiment was set up like the first, except aphids were placed in the cages 2 days before the spray treatment and second instar *H. convergens* (3 days old) were used. Slightly older larvae were used because *H. convergens* recovery rates were low in the first experiment. All insects released in the field experiments were laboratory reared as described by James and Lighthart (1992).

Sampling for B. bassiana *persistence—stomacher samples.* To estimate the persistence of fungal conidia in the alfalfa field, plots were sampled just after they were sprayed and on the following days after spray: 1, 2, 4, 10, 16 and 28 for the first experiment and 1, 2, 4, 7, 14 and 21 for the second experiment. To determine if the initial spray was distributed evenly in the canopy, and to see if conidial persistence was dependent on the location of the sample in the canopy, four different types of samples were taken. Four plants were randomly sampled from each plot. Alfalfa grows as a clump of several stems from one plant. Each of these stems is referred to here as a 'plant', even though it may only be a ramet. Use of the term 'stem' is reserved for the structural stem of the plant, as opposed to the leaves. The plant samples were divided into stems and leaves, and the stems and leaves were divided into those that came from the top half of the plant and those that came from the bottom half of the plant. These plant parts were grouped by plot and put into sterile plastic bags. In the laboratory, 20 ml of sterile deionized water were added and the sample was processed for 1 min in a stomacher blender (Tekmar, Cincinnati, OH, USA). This macerated the plants and dislodged the conidia. A standard plate count technique was then used to determine the number of conidia/sample. A culture medium selective for *B. bassiana* described by Sneh (1991) was used, except that SMAY made up the nutrient base. Sneh (1991) used a wheat germ extract, but the authors found that a more uniform medium could be obtained using SMAY, and it was easier to prepare.

After the liquid extract in the stomacher samples had been plated, the plant material was filtered, dried at 60°C and weighed to determine dry biomass. The samples could then be standardized based on dry plant biomass.

Stomacher samples were used to determine the initial distribution and density of *B. bassiana* conidia after spray, and the persistence of conidia on different plant parts and in canopy locations. To determine whether or not conidia were initially distributed evenly with respect to plant parts and height within the canopy, a multivariate analysis of variance was used to determine if treatment concentration, plant part and/or canopy location had an effect on conidial density on day 0. Each experiment was analyzed separately. If significant interactions were found between treatment concentrations and any other main effect, the analysis was conducted for each concentration separately. If there were interactions between the other main effects, then a Tukey's LSD (SAS, 1990) was used to compare the means of each sample type.

The persistence of conidia was defined as the rate of decline in density over time, as determined by the slope of the linear regression of log conidial density (the number of conidia g^{-1} of plant sample (dry weight) over time (days post-spray)). A separate regression analysis was carried out for each replicate. The slopes were then compared using multivariate analysis with dose, plant part and canopy sample height as the main effects (SAS, 1990).

Sampling for B. bassiana *persistence—leaf blot samples.* To determine the number of conidia on the top and bottom surfaces of a leaf, the leaf was sandwiched between two contact surface plates filled with the selective medium. For each plot, five leaves were randomly sampled from

the top halves of plants and five leaves from the bottom halves of plants. The sample days were the same as for the stomacher samples.

Statistical analysis of these samples was the same as for stomacher samples, except that differences in spore counts between leaf sides and between leaf locations within the canopy were compared. The five samples from each cage were pooled, and each cage was considered a replicate. The mean number of conidia recovered per sample was the dependent variable used in regressions for determining the rate of decline in conidia.

Weather. Daily weather conditions were recorded at the site for both field experiments using a Campbell Scientific (Logan, UT, USA) data logger. Hourly rainfall, mean hourly temperature, humidity and incident solar radiation were recorded. Measures for temperature, humidity and incident solar radiation were taken within the plant canopy inside a cage, and in the air above a cage. This was to give a measure of the effects of the cages.

Sampling for insect survival. Previous tests done in the authors' laboratory (James & Lighthart, 1994) found that all *H. convergens* mortality due to *B. bassiana* occurs within the first 10 days after exposure, with the majority occurring within the first 6 days, at 25°C. To make laboratory and field data comparable, insects were sampled 11 days after treatment (rain on day 10 precluded sampling). A Shuh shaker (Gray & Shuh, 1941) was used to sample for pea aphids and *H. convergens* larvae. Whole plants were cut and placed in the sampler with methylethyl ketone, which anesthetized the insects, allowing them to be shaken off the plants into a collection jar. The plots were also inspected after they were cut to collect any larvae that had fallen when the plants were cut. The total dry biomass was determined for each insect species, as well as the number of individuals for *H. convergens* larvae. An *F*-test (SAS, 1990) was used to test for a significant effect of treatment on insect survival. If a significant effect was seen, a Tukey's LSD (SAS, 1990) was used to compare treatments.

Greenhouse Experiments

Greenhouse experiments were conducted to determine where in the canopy, and on which plant parts, pea aphids and *H. convergens* larvae occurred and the relative proportion of time spent in each area. Twenty-four 0.6×0.6-m alfalfa plots were established in a 6.6×4.2-m array in the ground in a greenhouse. Pea aphid populations were established in the plots, but not *H. convergens*.

To determine the pea aphid distribution, 25 plants were randomly sampled from three different plots, and the number and location of all aphids on these plants were recorded. Plant parts were categorized as follows: the terminal bud; the upper and lower halves of the stem; and the upper and lower leaf surfaces in the upper and lower halves of the plant. A total of 1262 aphids were sampled and the proportion of aphids found in each location was determined.

To sample *H. convergens* larvae, 20 neonate larvae were released into each plot. Larvae were then sampled 2, 5, 7 and 9 days after their release, to allow for sampling over the time period of the field samples. On each day, three plots were randomly selected (although the same plot was never used twice). All plants in a plot were cut and inspected for larvae, and the number and location of all larvae was noted. The plant part locations were categorized as above. A total of 84 larvae were recovered.

RESULTS

Field Experiments

Weather conditions during experiments. The temperature fluctuated between 6 and 20°C during the first half of the first experiment and there was considerable rainfall (54.36 cm^3; it rained on 12 of the 28 days of the experiment). As a result, the foliage was constantly wet, and the relative humidity (RH) was always high. Daily means ranged from 82 to 96%, and the daily highs were $\geq 96\%$ RH. Temperatures were fairly cold for *H. convergens*, which has a developmental

threshold of 13°C (Miller, 1992), but were somewhat warmer in the second experiment, fluctuating between 10 and 25°C. The total number of degree-days (above 0°C) for the first experiment was 410, but there were only 69 degree-days above 13°C, whereas for the second experiment there were 372 total degree-days, with 93 degree-days above 13°C. Thus, there were more thermal units available to the insects in the second experiment, even though it was a week shorter in duration. The daily means in RH in the second experiment ranged between 72 and 96%, and the daily highs were always $\geq 96\%$. Thus, night time highs were similar to those in the first experiment but the RH dropped to lower levels during the day. This effect was, in part, due to the lower levels of rainfall (17.78 cm^3).

Cages reduced light levels by 32%, somewhat dampened the fluctuations in RH and had little effect on temperature. The weather conditions reported above are for within the canopy in the cages.

B. bassiana *persistence—stomacher samples.* In both experiments, location within the canopy had no significant effect on the initial spray deposition (day 0) at any concentration (Figures 1 and 2). In the first experiment, a slight increase was seen in the number of conidia sampled on the second day over the first day. This increase must have been due to a sampling error that underestimated the number of viable conidia just after spray, and occurred consistently over all sample treatments.

In both experiments, conidia persisted for longer than the sampling period (21–28 days) in both the high and medium spray rates. The conidial density declined to about 10% of initial densities after 21 days, although the rate of decline did depend on the treatment and sample type. In the first experiment, a significant difference was seen between doses in the rate of decline ($F = 11.22$, $P \leq 0.001$), with the middle concentration treatment (mean regression slope $= -0.23$) showing the most rapid decline in spore density (Figure 1). There was also a significantly faster decline in the upper canopy (mean regression slope $= -0.20$) than in the lower canopy (mean regression slope $= -0.12$) ($F = 10.90$, $P \leq 0.003$). There was no difference between leaves and stems in the persistence of the fungus, and there were no interactions between the main effects (dose, plant part and location in the canopy).

The persistence of conidia in the second experiment was similar to that seen in the first experiment (Figure 2). The effect of dose on the rate of decline in conidia was significant ($F = 5.16$, $P \leq 0.01$), but the lowest dose had the greatest rate of decline and the highest dose had the slowest rate of decline. There was also a faster decline in conidia in the upper canopy (mean regression slope $= -0.28$) than in the lower canopy (mean regression slope $= -0.17$) ($F = 88.24$, $P \leq 0.0001$). Unlike in the first experiment, there was also a difference between leaves and stems ($F = 7.15$, $P \leq 0.002$). The conidia on the stems declined at a faster rate (mean regression slope $= -0.25$) than those on the leaves (mean regression slope $= -0.20$). The interaction between dose and location within the canopy was significant ($F = 7.15$, $P \leq 0.003$). Visual inspection of Figure 2 reveals the low dose as the probable cause of this statistical interaction. When the low dose was removed from the analysis, dose, location within the canopy and plant part were all significant effects, and there were no interactions. When the low dose was analyzed separately, similar responses were seen, except the differences in the rate of conidial decline between the upper and lower canopy were greater (Figure 2), thus explaining the interaction seen.

B. bassiana *persistence—leaf blot samples.* In the first experiment, there was no difference between leaf sides nor locations in the canopy on the initial deposition of conidia (Figure 3). This is consistent with the results from the stomacher samples, where there was also no effect of location in the canopy. In the second experiment, there were significantly fewer conidia in the lower canopy than in the upper canopy ($F = 10.80$, $P \leq 0.007$), but the effect was small (Figure 4). For the high concentration treatment, this effect was only seen on the leaf bottoms ($F = 6.53$, $P \leq 0.007$).

For both experiments, there was a greater rate of decline in conidia in the upper canopy than

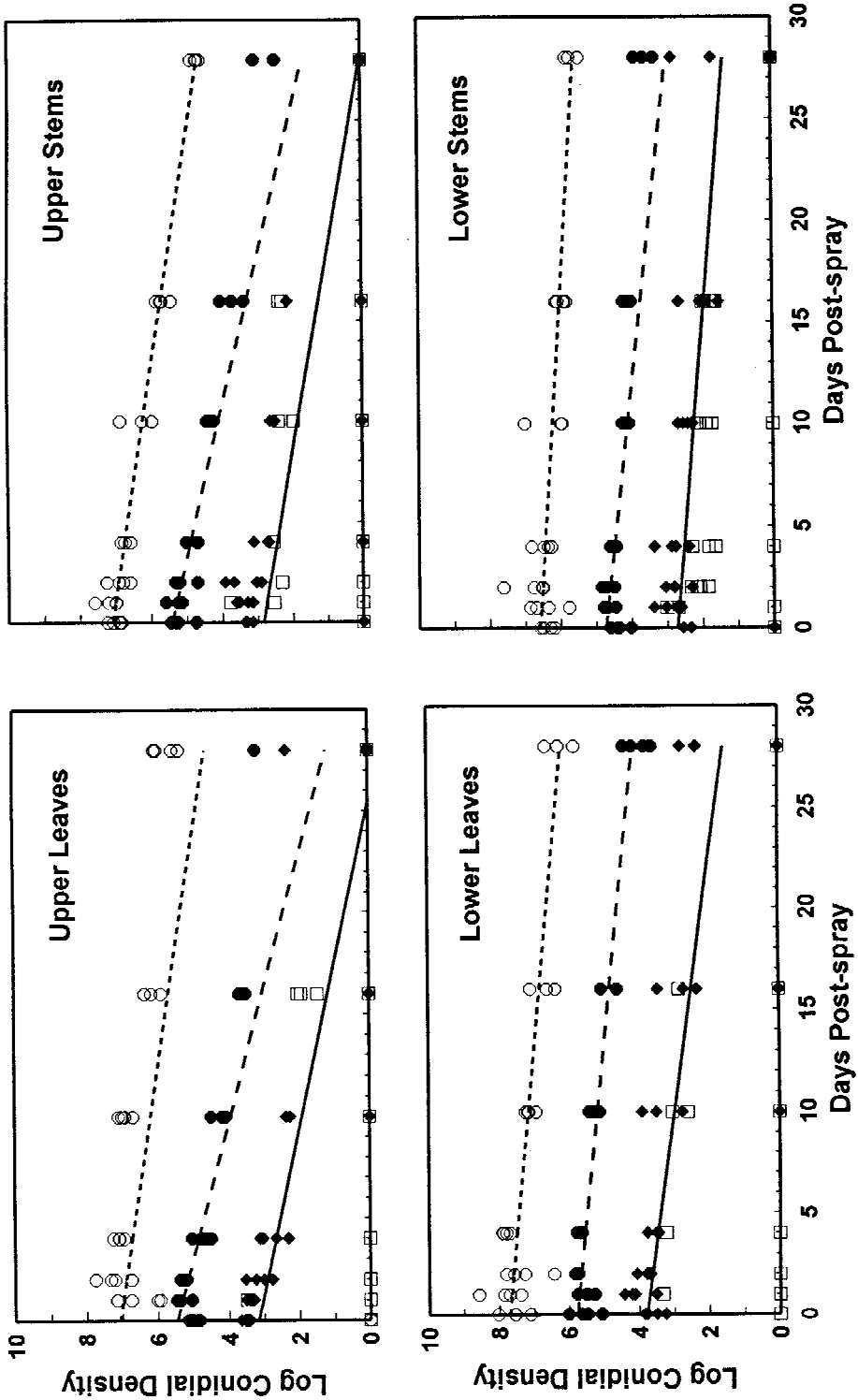

FIGURE 1. *B. bassiana* persistence after spray application to alfalfa, as determined from stomacher samples in the first experiment. The conidial density is the number of conidia sampled g^{-1} of plant dry weight. Symbols correspond to different spray concentrations: □, controls; ◆, low; ●, medium; and ○, high. The lines represent fitted regression models for each sample type, all of which had highly significant fits ($P \leq 0.0003$, except for the low dose on lower stems, where $P \leq 0.004$).

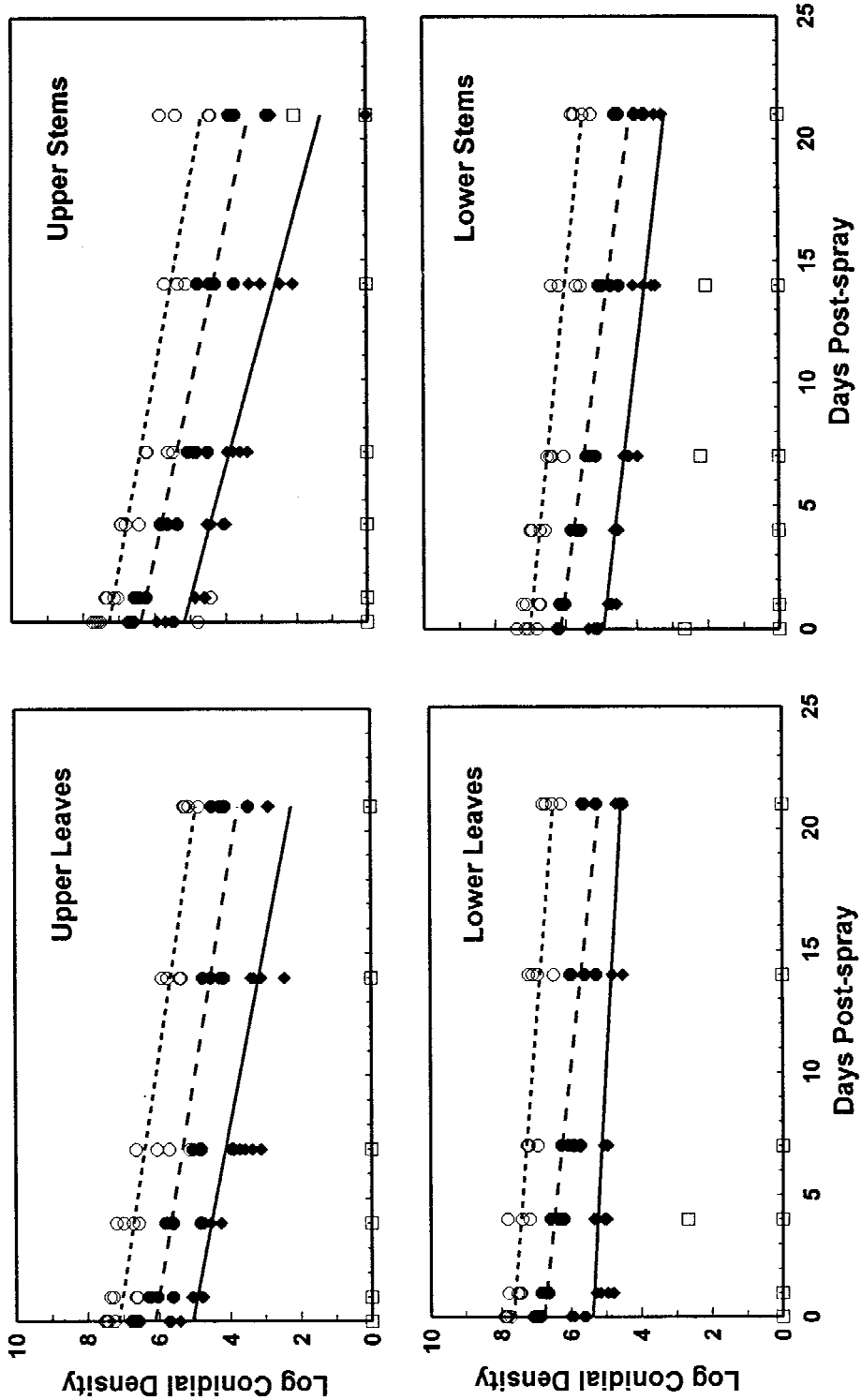

FIGURE 2. *B. bassiana* persistence after spray application to alfalfa, as determined from stomacher samples in the second experiment. The conidial density is the number of conidia sampled g^{-1} of plant dry weight. Symbols correspond to the different spray concentrations; □, controls; ◆, low; ●, medium; and ○, high. The lines represent fitted regression models for each sample type, all of which had highly significant fits ($P \leq 0.0001$).

in the lower canopy (experiment 1, $F = 8.92$, $P \leq 0.007$; experiment 2, $F = 222$, $P \leq 0.0001$) (Figures 3 and 4). Although there was not a statistically significant difference in conidial persistence between leaf sides in the first experiment, the rate of decline was always somewhat greater on the leaf top. This effect was significant in the second experiment ($F = 5.33$, $P \leq 0.03$).

Insect samples. In the first experiment, pea aphid populations were not affected by any of the *B. bassiana* treatments, but *H. convergens* populations were (Table 1). Control plots had significantly more *H. convergens* larvae than treated plots ($F = 8.92$, $P \leq 0.006$), but there was no significant difference between plots treated with different concentrations of *B. bassiana*. In the second experiment, there was no significant effect of *B. bassiana* on either insect (Table 1).

Greenhouse Experiments

Insects were not distributed evenly over the entire plant. Nearly all the aphids were found in the upper part of the canopy (98.5%) (Figure 5), with more than half on the terminal buds. Aphids on the terminal buds were 93.9% nymphs, which was a considerably higher proportion than that found elsewhere. For example, only 66.6% of the aphids on the leaf undersides (the second most common location at which to find aphids) were nymphs.

Likewise, 95.6% of *H. convergens* larvae were located in the upper canopy, with about half found on the stems (Figure 5). Larvae were also abundant on the undersides of leaves in the upper canopy, with the proportion found there (26.5%) being similar to that for aphids.

DISCUSSION

The initial distribution of conidia was similar throughout the crop canopy, but conidia persisted for longer in the lower canopy. The survival of conidia in the field is likely to be affected by UV light, moisture and temperature (McCoy *et al.*, 1988). The lower canopy generally has temperatures that are lower during the day and higher at night than the upper canopy, and direct sunlight is reduced due to shading. The lower persistence of conidia in the upper canopy may have been due to plant growth, rain washing the conidia into the lower canopy, poorer survival in the upper canopy due to UV exposure or some combination of these factors. The pattern of persistence between the two sides of leaves was consistent with either rainwater or UV effects. If plant growth was the only factor involved, patterns of persistence would be expected to be the same for both sides of leaves, whereas rain may wash conidia from the upper surfaces, and solar exposure is generally greater on this surface. Moisture may also have had a differential impact on survival if conidia were wetted, dried and rewetted. Conditions producing such an effect were more likely to occur in these experiments in the upper canopy on leaf surfaces exposed to sunlight.

The total rate of decline observed in *B. bassiana* in this study was similar to that seen by Inglis *et al.* (1993) in alfalfa. However, measurable levels of viable conidia were found in the field as late as 28 days after application, and the levels of conidia found at this time in the high concentration treatment were sometimes greater than the initial levels found in the middle concentration treatment. This suggests a high persistence of *B. bassiana* conidia under fairly mild, wet field conditions.

Despite its high persistence, *B. bassiana* had no effect on pea aphid populations in the field cages in this study. These results are consistent with those of Dorschner *et al.* (1991), who saw up to 100% mortality in hop aphids (*Phorodon humuli*) exposed to *B. bassiana* in the laboratory, but the fungus did not control aphids in the field. This result may be due to either the aphids not receiving a sufficient dose in the field or to weather conditions not being sufficiently suitable for infection to occur. Germination of *B. bassiana* is greatly reduced at RH levels less than 95% (Hart & MacLeod, 1955; Walstad *et al.*, 1970; Doberski, 1981). In the field experiment, RH daily highs were always adequate for high germination, but occurred at night and in the early morning during the lowest daily temperatures.

Poor recovery of *H. convergens* larvae hampered measurements of the effects of *B. bassiana*

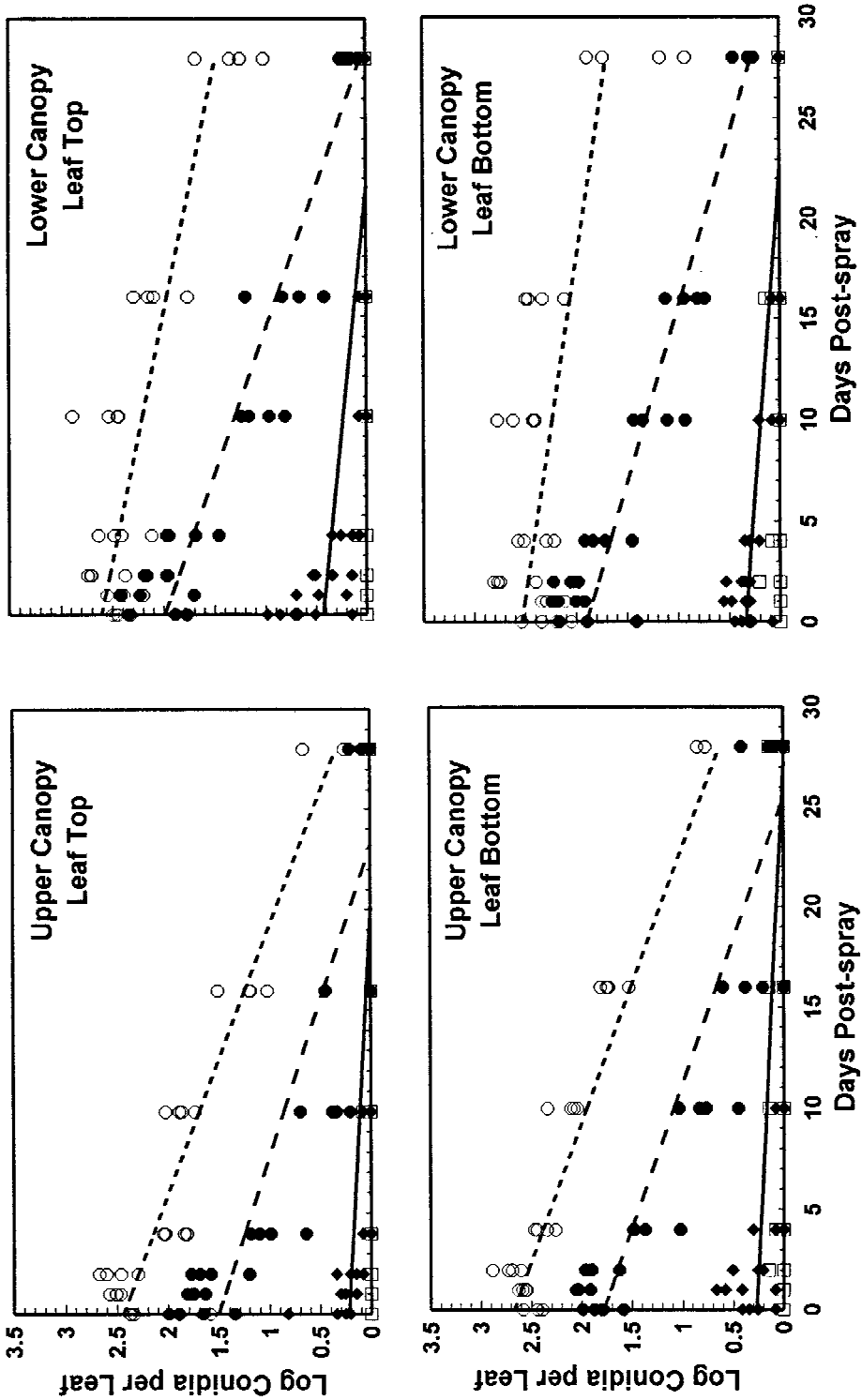

FIGURE 3. The persistence of *B. bassiana* on leaf surfaces after spray application to alfalfa for the first experiment, from leaf blot samples. The top and bottom sides of the leaves were measured separately. Symbols correspond to the different spray concentrations: □, controls; ◆, low; ●, medium; and ○, high. The lines represent fitted regression models for each sample type, all of which had highly significant fits ($P \leq 0.0003$, except for the low dose on lower stems, where $P \leq 0.004$).

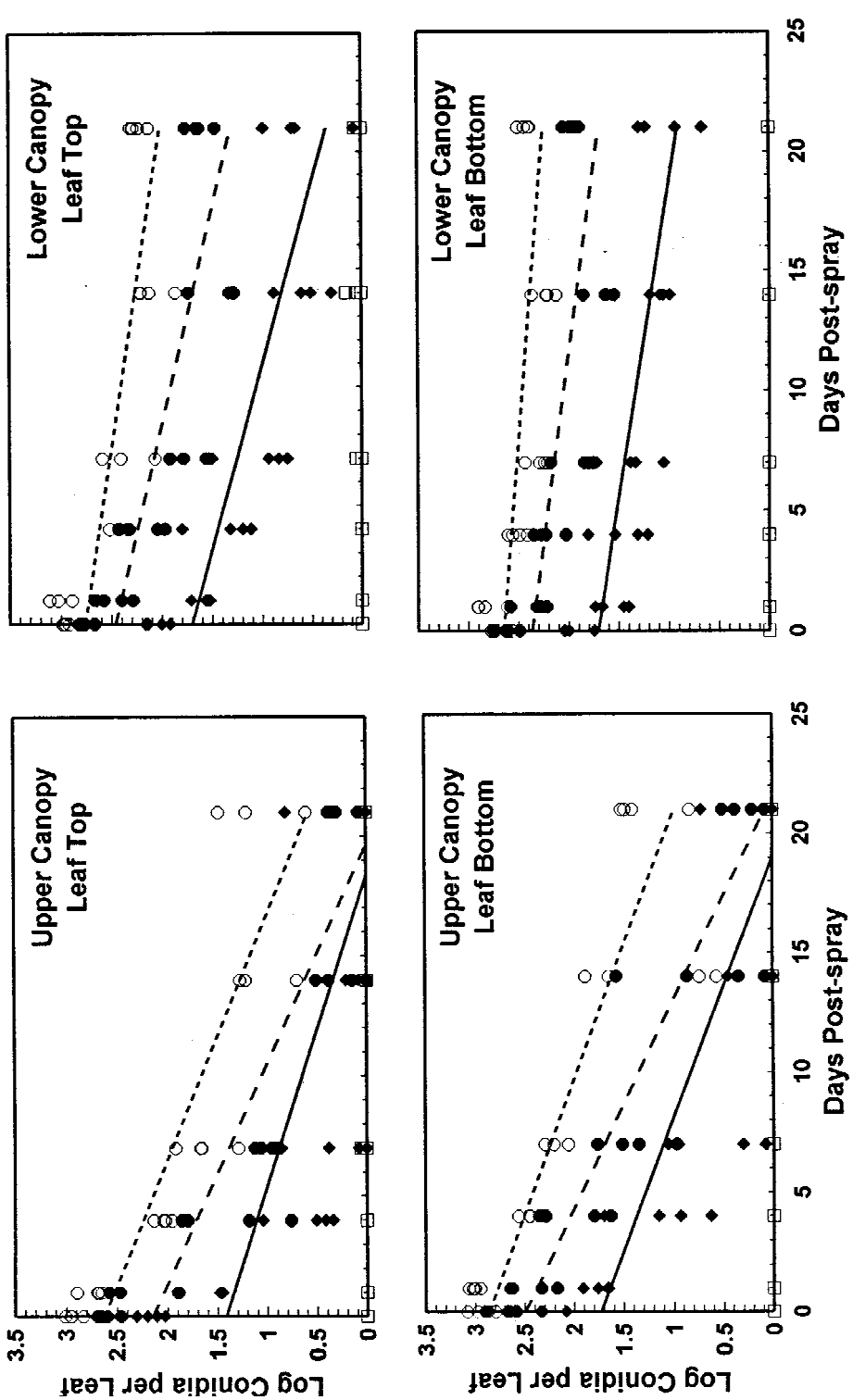

FIGURE 4. The persistence of *B. bassiana* on leaf surfaces after spray application to alfalfa for the second experiment, from leaf blot samples. The top and bottom sides of the leaves were measured separately. Symbols correspond to the different spray concentrations: □, controls; ◆, low; ●, medium; and ○, high. The lines represent fitted regression models for each sample type, all of which had highly significant fits ($P \leq 0.0001$).

TABLE 1. Mean (±SE) recovery of *H. convergens* larvae and *A. pisum* (pea aphids) from alfalfa field plots 11 days after the plots were sprayed with *B. bassiana* conidia at three different concentrations: 10^4 (low), 10^6 (medium) and 10^8 (high) conidia ml^{-1}

Treatment concentration	Mean no. of *H. convergens*/plot (±SE)[a]	Mean pea aphid biomass/plot (g)
April–May (experiment 1)		
Control	7.00 (±2.83)a	3.98 (±2.52)a
Low	1.75 (±0.05)b	2.31 (±1.57)a
Medium	1.00 (±1.73)b	3.15 (±2.30)a
High	0.04 (±1.15)b	1.44 (±0.45)a
July (experiment 2)		
Control	7.00 (±1.63)a	0.16 (±0.07)a
Low	8.00 (±1.41)a	0.22 (±0.12)a
Medium	9.00 (±0.82)a	0.12 (±0.02)a
High	7.25 (±1.25)a	0.19 (±0.09)a

[a]Numbers in a column followed by different letters are significantly different ($P \leq 0.05$), as determined by Tukey's LSD.

on its survival. However, it is clear that larvae were greatly affected early in the season, even by the low concentration treatment. The effect was greater at the low concentration treatment than would be expected from laboratory studies, because the low concentration in the field was 1/100th of the laboratory LC_{50} of 3.24×10^6 colony forming units ml^{-1} of conidia (James & Lighthart, 1994). Yet in the second experiment, later in the season, the survival of larvae did not appear to be affected by *B. bassiana* at all, despite the fact that initial conidial densities in the field were somewhat higher than in the first experiment. This result suggests that *H. convergens* may be more susceptible to *B. bassiana* in the field than the pea aphid is, but that its susceptibility is greatly affected by weather conditions. Environmental stress has been shown to affect insects' ability to resist infection (Donegan & Lighthart 1989).

Both insect species occurred almost exclusively in the upper canopy. This behavior would lead to higher initial exposures, but less risk to residues. *H. convergens* larvae were found more frequently on stems and in the lower canopy than were aphids. This distribution may be a result of *H. convergens* having to traverse these areas to move from one leaf to the next and from one plant to the next. Conversely, *H. convergens* larvae were found at a much lower frequency on the terminal buds than were the pea aphids. This area may act as an aphid refuge, particularly for nymphs.

The difference between predator and prey distributions on the plants does not explain the differences in survival seen between the aphid and the beetle in the first experiment. Both insects spent a similar amount of time on the upper part of the plant, and there was no significant difference in spore densities between leaves and stems in the first experiment. Other aspects of behavior may be more important. For example, *H. convergens* larvae spend more time moving around on the foliage than aphids. This behavior may lead to greater rates of conidial pick-up, or *H. convergens* larvae may increase their risk to infection when they consume infected aphids because *B. bassiana* can infect through the gut (Gabriel, 1959). Aphids, on the other hand, have sucking mouthparts, and internal ingestion of conidia would be low. Non-behavioral characteristic differences may also play a role. For example, coccinellid larvae have more hairs than aphids, which may facilitate conidial pick-up.

An important difference between laboratory bioassays and many field experiments is that laboratory bioassays assess individuals and many field trials assess populations. Pea aphids have a very short generation time, approximately 6 days at 22°C (Hochberg *et al.*, 1986), and a very high fecundity rate. It takes 3–6 days for the fungus to kill an adult aphid, and that same adult

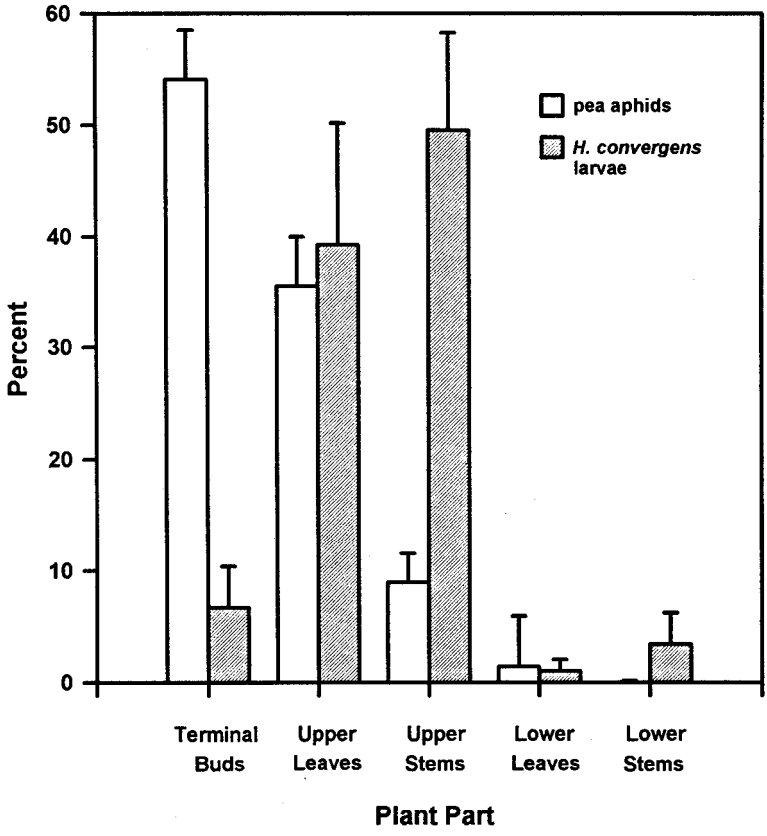

FIGURE 5. Distribution of pea aphids *A. pisum* and larvae of the convergent lady beetle *H. convergens* (all instars) on alfalfa foliage in the greenhouse. Distribution is represented by the percentage of individuals found on each plant part for each species.

may continue to reproduce during the incubation period. This time delay in mycosis development may impede the short-term efficacy of *B. bassiana* for aphids.

To improve control with *B. bassiana*, more needs to be known about the environmental conditions necessary for the pathogen to be effective. The optimum conditions for infection are still unknown. Improving formulations to increase infection rates may help to reduce dependence on favorable weather conditions. Several frequent applications may be required for pests such as aphids, that have a short generation time and a high fecundity, particularly if the pest is not highly mobile and has little contact with the substrate, thus reducing its exposure to residues. The time duration between sprays should be less than the generation time of the pest. Unfortunately, such a tactic may pose an even greater risk to non-target species such as *H. convergens*.

ACKNOWLEDGEMENTS

Toni Hoyman and Lynn Bucao helped considerably in sample collection and processing. Toni also provided the insects used in the experiments. The authors are grateful to Dr L. Ganio for her advice on statistical analyses and to the following people for their comments on preliminary drafts of this manuscript: Dr P. Rossignol, Dr J. Wolff, Dr J. Miller, Dr J. DeAngelis and Dr M. Whalon. This research was funded by the US Environmental Protection Agency through contract #68-C4–0019 to ManTech Environmental Technology, Inc.

REFERENCES

DOBERSKI, J.W. (1981) Comparative laboratory studies on three fungal pathogens of the elm bark beetle *Scolytus scolytus*: effect of temperature and humidity on infection by *Beauveria bassiana, Metarhizium anisopliae*, and *Paecilomyces farinosus. Journal of Invertebrate Pathology* **37**, 195–200.

DONEGAN, K. & LIGHTHART, B. (1989) Effect of several stress factors on the susceptibility of the predatory insect, *Chrysoperla carnea* (Neuroptera: Chrysopidae), to the fungal pathogen *Beauveria bassiana. Journal of Invertebrate Pathology* **54**, 79–84.

DORSCHNER, K.W., FENG, M.G. & BAIRD, C.R. (1991) Virulence of an aphid-derived isolate of *Beauveria bassiana* (Fungi: Hyphomycetes) to the hop aphid, *Phorodon humuli* (Homoptera: Aphididae). *Environmental Entomology* **20**, 690–693.

FENG, M.G., JOHNSON, J.B. & KISH, L.P. (1990) Virulence of *Verticillium lecanii* and an aphid-derived isolate of *Beauveria bassiana* (Fungi: Hyphomycetes) for six species of cereal-infesting aphids (Homoptera: Aphididae). *Environmental Entomology* **19**, 815–820.

FLEXNER, J.L., LIGHTHART, B. & CROFT, B.A. (1986) The effects of microbial pesticides on non-target, beneficial arthropods. *Agriculture, Ecosystems, and Environment* **16**, 203–254.

GABRIEL, B.P. (1959) Fungus infection of insects via the alimentary tract. *Journal of Insect Pathology* **1**, 319–330.

GOETTEL, M.S., POPRAWSKI, T.J., VANDENBERG, J.D., LI, Z. & ROBERTS, D.W. (1990) Safety to nontarget invertebrates of fungal biocontrol agents, in *Safety of Microbial Insecticides* (LAIRD, M., LACEY, L.A. & DAVIDSON, E.W., Eds) CRC Press, Boca Raton, FL, USA, pp. 209–231.

GRAY, K.W. & SHUH, J. (1941) A method and contrivance for sampling pea aphid populations. *Journal of Economic Entomology* **34**, 411–415.

HART, M.P. & MACLEOD, D.M. (1955) An apparatus for determining the effects of temperature and humidity on germination of fungus spores. *Canadian Journal of Botany* **33**, 289–292.

HOCHBERG, M.E., PICKERING, J. & GETZ, W.M. (1986) Evaluation of phenology models using field data: case study for the pea aphid, *Acyrthosiphon pisum*, and the blue alfalfa aphid, *Acyrthosiphon kondoi* (Homoptera: Aphididae). *Environmental Entomology* **15**, 227–231.

INGLIS, G.D., GOETTEL, M.S. & JOHNSON, D.L. (1993) Persistence of the entomolphagous fungus, *Beauveria bassiana*, on phylloplanes of crested wheatgrass and alfalfa. *Biological Control* **3**, 258–270.

JAMES, R.R. & LIGHTHART, B. (1992) *Protocol for Testing the Effects of Fungal Pesticides on Nontarget Beetles using* Hippodamia convergens (*Coleoptera: Coccinellidae).* National Technical Information Service Publication No. PB92–271–488, Springfield, VA, USA.

JAMES, R.R. & LIGHTHART, B. (1994) Susceptibility of the convergent lady beetle (Coleoptera: Coccinellidae) to four entomogenous fungi. *Environmental Entomology* **23**, 190–192.

MAGALHÃES, B.P., LORD, J.C., WRAIGHT, S.P., DAOUST, R.A. & ROBERTS, D.W. (1988) Pathogenicity of *Beauveria bassiana* and *Zoophthora radicans* to the coccinellid predators *Coleomegilla maculata* and *Eriopis connexa. Journal of Invertebrate Pathology* **52**, 471–473.

MCCOY, C.W., SAMSON, R.A. & BOUCIAS, D.G. (1988) Entomogenous fungi, in *Handbook of Natural Pesticides, Vol. 5, Microbial Insecticides, Part A, Entomogenous Protozoa and Fungi* (IGNOFFO, C.M. & MANDAVA, N.B., Eds) CRC Press, Inc., Boca Raton, FL, USA, pp. 151–236.

MILLER, J.C. (1992) Temperature dependent development of the convergent lady beetle (Coleoptera: Coccinellidae). *Environmental Entomology* **21**, 197–201.

PAVLIUSHIN, V.A. (1983) White muscardine disease of the pea aphid, in *Sistematika i Eklogiia Tlei—Vreditelei Rastenii: Tezisy Dokladov I Mezhreshpublikanskogo Afidologicheskogo Simpoziuma* (RUPAID, A.A., Ed.) Zinatne, Riga, pp. 52–53 (in Russian).

SAS (1990) *SAS/STAT User's Guide*, 4th edn. SAS Institute, Inc., Cary, NC, USA.

SNEH, B. (1991) Isolation of *Metarhizium anisopliae* from insects on an improved selective medium based on wheat germ. *Journal of Invertebrate Pathology* **58**, 269–273.

TANADA, Y. & KAYA, H. (1993) *Insect Pathology*. Academic Press, San Diego, CA, USA.

WALSTAD, J.D., ANDERSON, R.F. & STAMBAUGH, W.J. (1970) Effects of environmental conditions on two species of muscardine fungi (*Beauveria bassiana* and *Metarrhizium anisopliae*). *Journal of Invertebrate Pathology* **16**, 221–226.