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Original article

Impact of a treatment of *Beauveria bassiana* (Deuteromycota: Hyphomycetes) on honeybee (*Apis mellifera*) colony health and on *Varroa destructor* mites (Acari: Varroidae)*

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Abstract – In 2 experiments bee colonies in southern France were treated with conidia of a *Beauveria bassiana* isolate collected from *Varroa* mites in the region. Objectives were to evaluate treatment effect on colony weight, adult bee mass, and capped brood and honey, and on *Varroa* fall onto sticky boards. Treatments included conidia formulated with either wax powder or wheat flour, flour alone, or control. Treatment did not affect colony health. Colonies treated with conidia and wax powder had higher mite fall compared to controls while those treated with conidia and wheat flour did not. The proportion fallen, infected mites in both conidia treatments was higher than controls for up to a week. Higher mite fall and infection rates were observed in treated hives in the 2nd, smaller experiment. The relationships between dosage and proportion fallen, infected mites, and between ambient temperature and infection duration, were examined. Future experiments will explore *Varroa* control using conidia.

***Apis mellifera* / *Varroa destructor* / *Beauveria bassiana* / biopesticide / formulation**

1. INTRODUCTION

Varroa destructor Anderson and Trueman is one of the most serious pests of honeybees (*Apis mellifera* L.) (Hymenoptera: Apidae) worldwide (Martin, 1998; Chandler et al., 2001). Mites weaken larvae and adults by feeding on haemolymph, transmitting diseases, and inducing deformities (Chandler et al., 2001; Martin, 2001). The impact of *Varroa* mites on domesticated and feral colonies of honeybees in the U.S. has been high; feral populations of *A. mellifera*, once common, have been almost completely eliminated by the mites (Rinderer et al., 2001). Biological control, particularly the use of entomopathogenic fungi, is seen as promising (Chandler et al., 2001), and fungal isolates have been identified and tested in the lab and the field (Shaw et al., 2002; Kanga et al., 2003, 2006; James et al., 2006; Meikle et al., 2006).

Entomopathogenic fungi are useful for controlling arthropod pests, and isolates are often collected from the target pests themselves to maximize the probability of finding an isolate adapted to the pest and to its ecology.

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For example, an isolate of *Metarhizium anisopliae* (formerly *M. flavoviride*) (Metsch.) Sorokin (Deuteromycota: Hyphomycetes), obtained from cadavers of the bird locust *Ornithacris cavroisi* (Finot) (Orthoptera: Acrididae) during an epizootic in Niger, was found to be highly effective against the desert locust *Schistocerca gregaria* Forskål (Orthoptera: Acrididae), in part because the fungus was well adapted to the high temperatures and low humidities of the region (Lomer et al., 1997). That isolate formed the basis for a biopesticide program against desert locusts in Africa (Cherry et al., 1999). Honeybee colonies are special environments from a pathogen's point of view because of high temperatures, high humidity, the properties of wax and propolis, and other factors. Meikle et al. (2006) reported the collection and evaluation of isolates of *Beauveria bassiana* (Balsamo) Vuillemin (Deuteromycota: Hyphomycetes) found infecting *Varroa* mites in honeybee colonies. One of these isolates significantly increased *Varroa* fall and infection in subsequent field experiments (Meikle et al., 2007).

Two key characteristics of a desirable biopesticide are high virulence against the target pest, and little or no virulence against nontarget organisms. This is particularly important in the case of *V. destructor* mite control, since any pathogen that harms the honeybee colony would not be acceptable, regardless of its virulence against *Varroa* mites. *Beauveria bassiana* has one of the broadest host ranges among entomopathogenic fungi (Tanada and Kaya, 1993), so potential risks to nontarget organisms are not negligible. In laboratory bioassays Meikle et al. (2006) observed *B. bassiana* infections of bee pupae that had been exposed to treated *Varroa* mites. Whether susceptibility of bee pupae to hyphomycete fungi is a problem in hives is unclear. Kanga et al. (2003) reported that 4 of 24 colonies treated with conidia of *Metarhizium anisopliae* (Metschnikoff) "swarmed (or left the bee hive)" within days after treatment, compared to 1 of 12 untreated colonies. Meikle et al. (2007) reported the death of one colony out of a total of six colonies treated with *B. bassiana* in two experiments and considered further work specifically targeting colony health after treatment with entomopathogenic fungal conidia to be essential.

The main objective of the 1st experiment was to evaluate the impact of an application of entomopathogenic fungal conidia on colony health and on *Varroa* mite fall. Mite "control" in the form of a long-term or permanent reduction in *Varroa* density was not an objective; in general, bee colonies are not treated against *Varroa* in the spring in France. This experiment was conducted at the start of a nectar flow, when brood densities and foraging activity are high and the sensitivity of hives to a perturbation likewise high. For these reasons, spring was deemed a good time to test impact on colony health. Widespread infection within a colony would be expected to measurably affect adult bee and brood populations, and possibly colony food stores and weight gain. Here we measured colony growth rates per week, total adult bee weight and the amounts of sealed brood and honey. The use of growth rates, which are independent of colony size, was intended to facilitate comparison of these results with other studies. *V. destructor* mite fall and the proportion fallen mites that were infected were measured as in Meikle et al. (2007). The main objective of the 2nd experiment was to observe the changes in mite fall and proportion fallen mites that were infected at a different time of year.

2. MATERIALS AND METHODS

2.1. Preparation of formulation

Cultures of *Beauveria bassiana* (Balsamo) Vuillemin (Deuteromycota: Hyphomycetes) isolate Bb05002, isolated from *Varroa destructor* mites in southern France (Meikle et al., 2006) were grown on Sabouraud dextrose agar with yeast (SDAY) (Goettel and Inglis, 1997) for a minimum of 15 days. Conidia were harvested by scraping the surface of the cultures onto glass petri dishes with a metal spatula, and placing the petri dishes in a crystallizing dish containing silica gel for 20–24 hours at room temperature for drying. Conidium viability was assessed by plating a suspension sample onto SDAY, incubating the plates at 22 °C for 24 h, and examining >200 conidia for germ tubes under a light microscope. Formulated and unformulated

conidia were stored in a refrigerator at 4 °C. A second batch of conidia of the same isolate was prepared, using the same technique, to measure germination at 22, 32 and 34 °C.

2.2. First field experiment

Three formulations were prepared prior to treatment: wax powder and conidia ("powder + conidia"); flour and conidia ("flour + conidia"); and flour alone. The per colony dose of powder + conidia consisted of 1.0 g Bb05002 conidia mixed with 9.0 g Entostat® powder, a refined, electrostatically-chargeable carnauba wax powder (Exosect, Winchester, UK) and 0.05 g hydrated silica (Hi-Sil-233, Pittsburgh Plate Glass, Pittsburgh, PA, USA) perpared on 24 May. The per colony dose of flour + conidia consisted of 1.0 g Bb05002 conidia mixed with 9.0 g commercially-prepared wheat flour (type 55, Générale des Farines France, Paris, France) and 0.05 g silica prepared on 18 May. The dose per colony of flour alone was 10.0 g wheat flour mixed with 0.05 g silica prepared on 24 May. All formulations were mixed using a food processor (Valentin Mini Chopper, SEB, Dijon, France). Flour moisture content was estimated by weighing six 1.5 g samples of flour, placing the samples in an oven at 100 °C for 7 h, and weighing the samples again. The density of colony-forming units (cfu) per g formulation was determined at the time of colony treatment by plating three sub-samples of the formulation diluted in distilled water and Tween 80 (Merck, Munich, Germany) onto potato-dextrose agar, and counting the number of colonies 96 h after plating.

In May 2006, 22 honeybee colonies were selected for the field experiment. The colonies were part of an apiary of 52 colonies near Lattes, in southern France. The bee colonies were kept in painted, 10-frame, wooden Dadant brood boxes (56 L capacity) with telescoping lids and with screens underneath the frames. On 15 May one sticky board (31 × 42 cm, Mann Lake Ltd, Hackensack, MN, USA) was placed under each colony. The boards were replaced on 23 May with fresh boards. All mites adhering to the used boards were counted, and 40-mite samples were taken from each board and plated on water agar (6.0 g/L) with chloramphenicol (0.4 g/L). If a board had 40 or fewer mites, all mites were plated. Plated mite samples were incubated at 23 °C and examined for sporulation after 15 days. Sticky boards were replaced, and mites counted and plated, on 30 May and 31 May, and twice a week thereafter until 27 June.

On 23 May each hive was weighed using a portable electronic balance with a precision of 50 g (OHaus Corporation model Champ CQ100L, Pine Brook, NJ, USA). After weighing, each hive was opened, and each hive part (i.e. brood box, lids, colony base, and frames after shaking them free of bees) was weighed using a smaller portable electronic balance with a precision of 1g (Kern & Sohn model 12K 1N, Balingen, Germany). Digital photographs were taken of each side of each frame using a 3.3 megapixel camera (Nikon Coolpix 990, Tokyo, Japan), and the area of sealed brood and sealed honey per photograph was estimated using ArcView 3.0 (Environmental Systems Research Institute, Redlands, CA, USA). Brood areas were inspected closely for any signs fungal infection. The hive was then reassembled, and one super containing 9 frames with wax foundation was weighed and placed on top of each colony. Hives were then weighed in their entirety once per week thereafter until 27 June for a total of 6 sampling occasions. On 27 June, the brood box frames were again weighed individually, following the same procedure, and the super was also weighed.

Colonies were treated on 30 May. Five colonies were selected for treatment with powder + conidia, five colonies for treatment with flour + conidia, four colonies for treatment with flour alone and seven colonies were kept as controls. Colonies were randomly assigned treatments, but treatments were occasionally re-assigned so that at least one untreated colony was placed between all treated colonies. For each colony treatment, a plastic laboratory wash bottle (Nalge Nunc International, Rochester, NY) was filled with a single dose of preparation, the hive lid removed, the formulation blown between all the frames in the brood box by squeezing the wash bottle, and the lid replaced.

To calculate colony and adult bee weight, hive weight was divided into a "non-colony" part, consisting of the hive pieces, e.g., brood box, lids, super, hive base, and 10 empty frames with foundation comb, and the "colony" part, consisting of the adult bees, brood, honey, pollen and wax (other than foundation comb). Adult bee weight was calculated as the difference between the sum of the weights of all the hive parts and the observed hive weight. The non-colony weight was calculated as the total weight of all the hive parts except brood box frames, plus the weight of 10 empty frames. The average weight of an empty frame was estimated

by weighing 22 frames with only foundation comb using an electronic balance (Sauter model 4021, Albstadt Ebingen, Switzerland). Colony weight was calculated by subtracting the non-colony component from the total hive weight. Colony entrances were inspected for unusually large numbers of dead bees.

A weather station (HOBO micro station, Onset Computer Corporation, Bourne, MA, USA) was used to monitor ambient temperature. Temperature loggers (Thermachron iButton, Dallas Semiconductor, Sunnyvale, CA, USA) were placed in 4 hives at the top a frame in the center of the brood box to record internal temperature hourly starting the day of treatment. In one of those hives, a second logger was also placed in the center at the base of the hive, underneath the frames.

2.3. Second field experiment

In December 2006, 7 honeybee colonies, near Prades le Lez in southern France, were selected for an experiment to examine the change in mite fall and infection over time after treatment with formulated *B. bassiana* conidia. The bee colonies were maintained in painted, 10-frame, wooden Dadant brood boxes (56 L capacity) (Ickowicz, Bollène, France). The colonies were all queen right and several colonies had supers. The colonies were covered with telescoping lids. The *B. bassiana* conidia had been produced the previous May and stored at 4 ^{o}C. The day before application, hive doses consisting of 1.0 g conidia mixed with 9.0 g carnauba wax powder (Strahl & Pitsch Inc., West Babylon, NY, USA) and 0.05 g hydrated silica, were prepared as described above.

Sticky boards were placed under all the colonies on 4 December, removed on 12 December immediately prior to treatment, and replaced with fresh boards. All the mites adhering to the boards were counted, and 40-mite samples were plated on water agar with chloramphenicol, incubated at 23 °C and examined for sporulation after 15 days. Four bee colonies were treated with formulation, and cfu density per g formulation was determined, in the same manner as the 1st experiment. The remaining 3 hives were kept as controls. Sticky boards were replaced on 13 December and twice per week thereafter until 14 January.

2.4. Statistical analysis

Data for both experiments were analyzed using SAS (SAS Institute, Inc., Cary NC, USA) software. Multiple regression analyses (α = 0.05) were conducted for a linear mixed model using PROC MIXED of SAS (Littell et al., 1996) with either mite fall (log transformed), or the proportion mites infected by fungi (arcsine square-root transformed) as the response variable and with 3 fixed effects: treatment, date and their interaction. The covariance matrix of both response variables was inspected for patterns and residual plots were assessed visually for variance homogeneity. Colony number was incorporated as a random effect. The degrees of freedom were calculated using the Satterthwaite method. Analyses were designed to maximize the degrees of freedom for detection of differences among treatments. Insignificant main effects were excluded from the model but if the interaction was significant both main factors were retained. Post hoc contrasts of the least squares means differences were conducted for all significant factors, using Bonferroni adjustment for the t-value probability. Because excess formulation on sticky boards immediately after treatment may cause spurious infection data, the 1st sample after treatment was excluded from analyses for both experiments. For adult bee weights and surface areas of sealed brood and honey in the 1st experiment, the daily intrinsic natural rate of increase, r, was calculated by dividing the post treatment value (27 June) by the pretreatment value (23 May), and then dividing the logarithm of that ratio by the number of days between these two dates (35).

3. RESULTS

3.1. First field experiment

Percentage germination of conidia used in the field experiment was 96% at 22 °C. Percentage germination of conidia from the second batch was 94% at 22 °C, 88% at 32 °C, and 88% at 34 °C, although growth was slower at 34 °C than 32 °C. Cfu density at time of treatment was 1.96×10^{10} cfu/g for the powder + conidia formulation, and 6.33×10^{9} cfu/g for the flour + conidia formulation. Flour moisture content was 9.8% (s.d. = 0.5). Average weight of an empty frame was 287 g (s.d. = 24).

In the analysis of colony weight *r* values, treatment ($F_{3,97}$ = 7.10, P = 0.0002), and date ($F_{4,97}$ = 25.53, P < 0.0001) were significant factors, but their interaction was not (P = 0.840) (Fig. 1). The *r* values for the powder + conidia (t_{97} = 2.94, P = 0.0041), flour + conidia (t_{97} = 2.49, P = 0.0146), and flour alone (t_{97} = 4.40, P < 0.0001) groups were all significantly higher than the control. During the experiment, colonies treated with powder + conidia gained an average of 10.3 kg (s.e. 3.0), colonies treated with flour + conidia gained an average of 7.0 kg (s.e. 2.5), colonies treated with flour alone gained an average of 14.5 kg (s.e. 2.6) and the control colonies gained an average of 2.9 kg (s.e. 1.7). One colony in the flour alone group gained 22.0 kg during the experiment, exceeding by 5.1 kg the next highest colony weight gain. The only colony to lose weight, 1.7 kg, was in the control, as were the two colonies with the lowest weight gains (0.8 kg and 0.3 kg). Treatment with conidia did not significantly affect total adult weight change (P = 0.380), or change in the surface areas of sealed brood (P = 0.754) or honey (P = 0.728) (Tab. I). One control colony had no queen at the end of the experiment, so data on mite fall and hive health for that colony were removed from the analyses. No infected brood were observed in any photographs.

Average temperature at the top of the brood boxes was 34.3 °C (average minimum = 32.5; average maximum = 36.0 °C). In the hive with dataloggers at both the top of the brood box and the base, temperature at the base was on average 2.2 °C (s.e. = 0.1) lower than that at the top of the brood box; the difference was larger at the time of treatment and decreased as the external temperature rose (Fig. 2).

In the analysis of mite fall, treatment ($F_{3,206}$ = 7.33, P < 0.0001) was significant but neither date (P = 0.37) nor interaction (P = 0.999) were. Compared to the control group mite fall was significantly higher in the powder + conidia group (t_{227} = 4.33, adj. P < 0.0001) and the flour alone group (t_{227} = 3.07, adj. P = 0.0143) but not in the flour + conidia group (adj. P = 0.99) (Fig. 3). Likewise, mite fall in the powder + conidia group was significantly higher than in the flour + conidia group (t_{227} = 2.80, adj. P = 0.0334) but not the flour alone group (P = 0.99). The flour + conidia group and the flour alone group were not significantly different (adj. P = 0.51).

Regarding the proportion fallen mites that were infected, treatment ($F_{3,37}$ = 15.07, P < 0.0001), Date ($F_{9,55}$ = 20.82, P < 0.0001) and treatment × date interaction ($F_{27,55}$ = 4.07, P < 0.0001) were all significant. The proportions of infected mites in both the powder + conidia (t_{37} = 4.91, adj. P < 0.0001) and the flour + conidia (t_{37} = 5.64, adj. P < 0.0001) groups were significantly higher than the control whereas the flour alone group was not (adj. P = 0.785). At least one infected mite was found in 14 of the 22 hives in the two weeks before treatment. Elevated densities of infected mites, probably due to bee drift or robbing, were observed in non treated colonies after treatment application, as was reported in Meikle et al. (2007).

3.2. Second field experiment

Cfu density at the time of application was 1.76×10^9 cfu/g for the powder + conidia formulation. One treated colony died during the experiment. That colony had a mite fall more than twice as high as the next highest colony, and likely collapsed due to varroosis. Data for that colony were removed from the mite fall analysis. Mite fall in treated colonies was significantly higher than in the control ($F_{1,64}$ = 59.06, P < 0.0001) but neither date (P = 0.15) nor the interaction term (P = 0.81) were significant. In the analysis of proportion fallen mites that were infected, treatment × date was significant ($F_{9,13}$ = 3.98, P < 0.046), but neither treatment (P = 0.120) nor date (P = 0.060) were (Fig. 4), and post hoc contrasts showed significant differences 5 and 12 days after treatment. Infected mites were found in untreated colonies.

4. DISCUSSION

The goals of this study were to determine the effects of one application of *Beauveria bassiana* conidia, formulated in two ways,

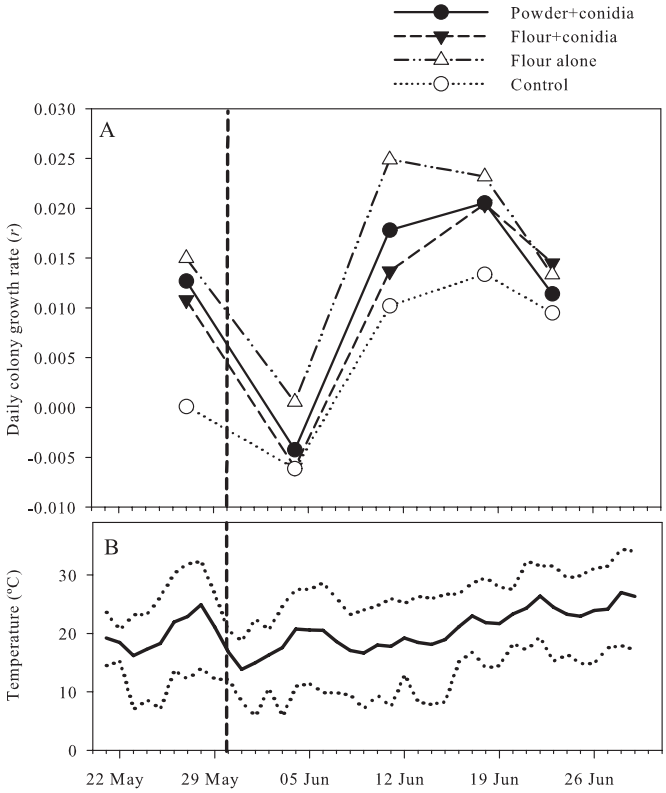

Figure 1. Bee colony growth for hives treated with *B. bassiana* conidia plus wax powder, conidia plus wheat flour, flour alone, or nothing (control), and ambient temperature, in May 2006 near Lattes in southern France. (A) Average intrinsic rates of increase, *r*, of bee colonies; (B) Daily minimum, maximum and average temperature. Vertical dashed line shows treatment date.

on bee health and on *V. destructor* mite fall. Long-term control of mites was not a goal; this work was intended to add to our understanding of the effects of entomopathogenic fungi in *V. destructor*-infested bee colonies and thus to the development of a biopesticide against *V. destructor*. In the 1st experiment we found no negative effect of application of entomopathogenic fungi on colony health, measured as the colony growth rate, total adult bee weight, surface areas of capped brood and honey, and colony survivorship. Colony growth among all groups was lowest immediately after application, but this was likely due to food consumption prior to a nectar flow. Colony growth increased among all groups thereafter. No treatment differences were observed in either total adult weight change or changes in the amounts of sealed brood or honey. No colonies were lost in the 1st experiment. One treated colony was lost in the 2nd experiment, but this was likely due to a heavy initial mite infestation rather than treatment. A lack of a negative impact by *B. bassiana* on honeybees has been observed in previous work which involved different response variables (Jaronski et al., 2004).

Amounts of adult bees, sealed brood and honey are valuable indicators of hive health but weighing and photographing each frame was labor intensive. These data are usually estimated visually (e.g. Kanga et al., 2003; Savary, 2006), which is fast and causes little disruption, but which requires training for consistent results. In contrast, the use of electronic balances, digital cameras and photo analysis

Table I. Total adult weight, and total surface areas of sealed brood and honey, for bee colonies before and after treatment with a formulation of *B. bassiana* conidia.

			23 May		27 June		
Variable	Treatment	N	avg[1]	s.d.	avg[1]	s.d.	Daily *r*
Total adult	Powder + conidia	5	3.301 ab	0.899	4.773 a	0.954	0.0105
weight	Flour + conidia	5	2.805 b	0.575	4.245 ab	0.535	0.0118
	Flour alone	4	4.305 a	0.513	4.711 ab	0.866	0.0026
	Control	7	2.161 b	0.865	3.242 b	1.083	0.0116
Sealed brood	Powder + conidia	5	4532 a	551	3712 a	732	–0.0057
surface area	Flour + conidia	5	3721 ab	982	3665 a	596	–0.0004
	Flour alone	4	4447 a	548	3790 a	587	–0.0046
	Control	7	2867 b	1105	2812 a	1018	–0.0006
Sealed honey	Powder + conidia	5	4846 a	1800	5489 a	1099	0.0036
surface area	Flour + conidia	5	2836 a	1750	3593 a	1870	0.0068
	Flour alone	4	4535 a	849	6009 a	1545	0.0080
	Control	7	4516 a	1431	5203 a	2524	0.0040

[1] Averages within a variable and within a date followed by different letters are significantly different using Tukey's HSD at $P < 0.05$.

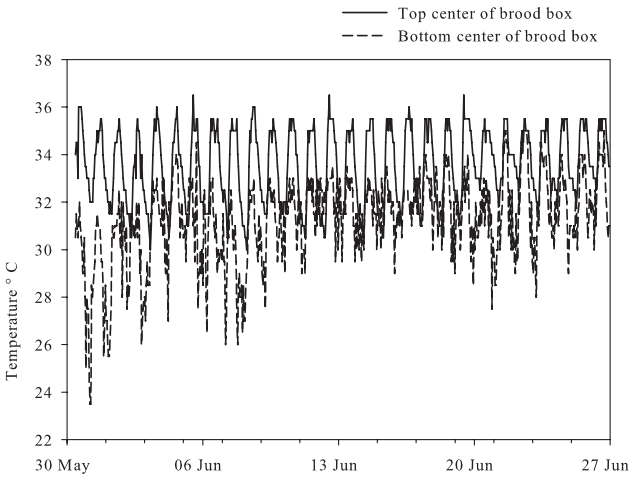

Figure 2. Within-hive hourly temperature for one hive between 31 May and 26 June 2006 near Lattes in southern France. Solid line shows temperature at the top center of the brood box, and dotted line shows the temperature at the bottom center of the brood box.

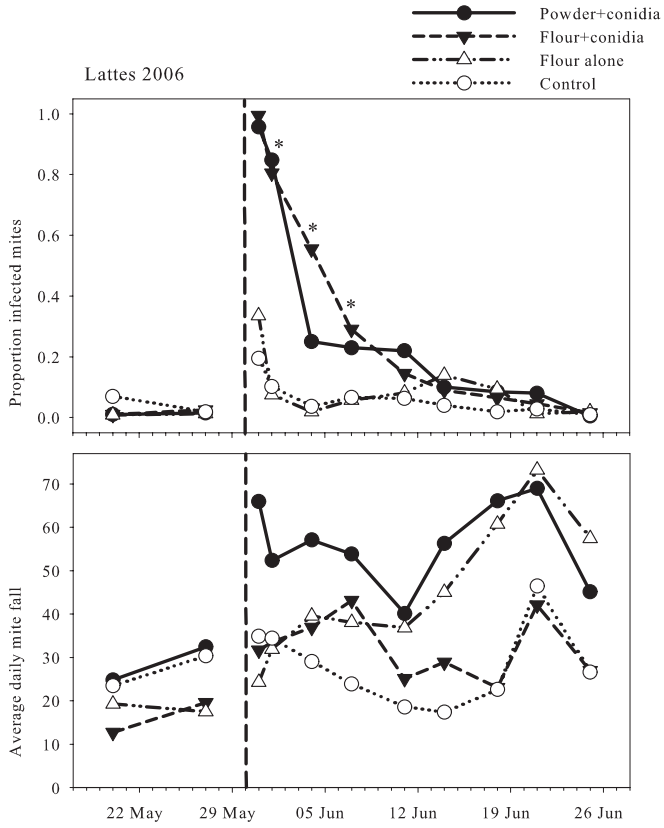

Figure 3. Effects on *V. destructor* mite density by treatment of bee colonies with *B. bassiana* conidia plus wax powder, conidia plus wheat flour, flour alone, or nothing (control) on 30 May 2006 near Lattes in southern France. (A) Average proportion fallen mites that were infected per colony over time; (B) average (geometric) daily mite fall. Points marked with an asterisk are significantly different from the control ($P < 0.05$). Vertical dashed line shows date of treatment.

software requires little training and the photographs form a permanent record, allowing re-analysis. Dead adult bees were not examined for infection because the data would have been difficult to interpret. Meikle et al. (2007) and Kanga (2003) found that bees retained a significant number of cfus on their body for at least a week after treatment. *Beauveria bassiana* conidia grow readily on cadavers (Tanada and Kaya, 1993) and since the amount of time between a bee's death, its ejection from the hive and subsequent collection is unknown, the presence of fungal growth on these cadavers was not seen as a reliable indicator of whether the fungus killed the bee, even if the bee was surface sterilized. Instead, an emphasis was placed on measuring changes in the living adult population. The number of adult bees increased in all treatments and the number in treated hives compared favorably with the number in control hives.

Mite fall in colonies treated with powder + conidia in the 1st experiment was significantly higher than in control colonies, as was observed by Meikle et al. (2007) using the same isolate, and it was signficantly higher than in the flour + conidia group. Mite falls in the powder + conidia group and the flour alone group were not significantly different although Meikle et al. (2007) did observe

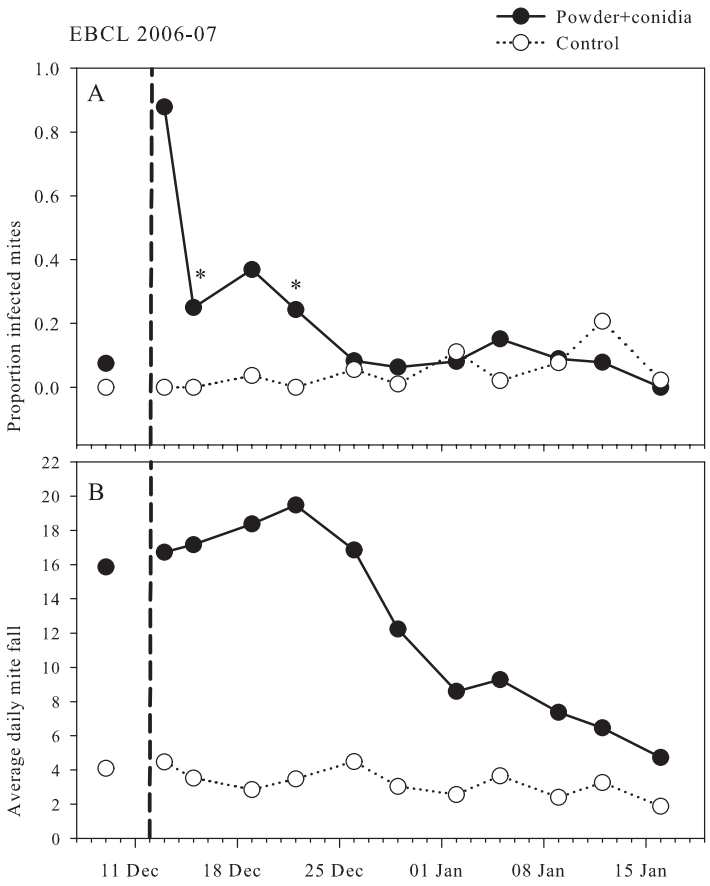

Figure 4. Effects on *V. destructor* mite populations by treatment of bee colonies with *B. bassiana* conidia and wax powder or nothing (control) on 12 December 2006 near Prades le Lez in southern France. (A) Average proportion fallen mites that were infected per colony over time; (B) average (geometric) daily mite fall. Points marked with an asterisk are significantly different from the control ($P < 0.05$). Vertical dashed lines show treatment date.

significant differences between hives treated with powder + conidia and those treated with wax powder alone. Mite fall due to powder alone could be expected (Fakhimzadeh, 2001; Macedo et al., 2002) but 99% of the effect would occur within 18 h (Fakhimzadeh, 2001) and thus would not explain the entire treatment effect. Mite fall was significantly higher in treated hives in the 2nd experiment, although the dose was lower. *V. destructor* mite infestations were not in any sense "controlled" in the treated hives in either experiment, but control was not expected. Any conidia treatment would only have affected exposed mites – those on bees and on frames and comb – with untreated mites emerging from capped brood cells after treatment.

Results of experiments using *M. anisopliae* against *V. destructor* have been variable: Kanga et al. (2003, 2006) reported significantly higher mite fall due to treatment but at a relatively high conidium dose (15–93 g per hive; cfu density per g not provided) while James et al. (2006) found no "control" of mites using a conidium dose per hive (3.5×10^{10} cfu) similar to ours ($1.8–19.6 \times 10^{10}$ cfu)

but applied either in aqueous or oil formulation, as "fungal bands", or unformulated. James et al. (2006) also measured mite density on adult bees, rather than mite fall as was done here, so results are difficult to compare directly. Branco et al. (2006) related mite fall on sticky boards to mite densities on adult bees and pupae, but they found that the relationship became weak for severely infested colonies.

Fungal conidia are often combined with other materials in order to stabilize the conidia during storage, facilitate application, protect the conidia, and enhance conidia activity (Jones and Burges, 1998). Burges (1998) recommended cereal flour for wet or humid environments, the flour acting as a nutrient additive for germinating fungal conidia, and a hydrophobic, lipophilic material such as oil for dry environments. Conidia were formulated for both types of environments in this study, although a hard plant wax powder was used rather than oil. Wax powder is hydrophobic and inert, with no nutritive value for the conidia. Flour is comparatively hygroscopic (Rückhold et al., 2001) and is not inert because it is a potential source of nutrients for germinating conidia. The powder + conidia treatment was significantly different from control whereas the flour + conidia treatment was not, although the two treatments were not significantly different themselves. Inspection of mite fall data over time does suggest a somewhat different dynamic, even if not born out by statistical significance. Further work needs to be conducted on the exact role of the formulation ingredient.

A second implication of these results concerns mite fall, proportion fallen mites that were infected, and application efficacy. While large numbers of fallen mites were infected with *B. bassiana* in the flour + conidia treatment, they were clearly not all dying from infection because the mite fall was the same as that observed in controls, which had little infection. As noted above, *B. bassiana* grows readily on fresh cadavers. A mite with viable conidia on its cuticle may fall for other reasons, and in the 3–4 days between board replacement conidia could germinate on the dead or dying mite, resulting in a false positive because the fungus did not cause the mite to drop. Surface sterilization of the mites would reliably remove only some of those false positives – those less than 1–2 days old (the time needed by the fungus to establish itself within a mite), and might cause false negatives by killing infections in damaged cadavers. Given the false positives and the low probability that an unsterilized mite which does not sporulate died from fungal infection, the proportion of fallen mites that were infected should be considered upper-bound estimates of the true percentage of infection, and is not necessarily related to how well the treatment works against *V. destructor*.

Spore viability was not directly measured in the hives, so the question remains to what extent *B. bassiana* can survive there. This question has two parts: what is meant by environmental conditions of the hive, and whether the fungus can survive and germinate in those conditions. Brood mass temperatures range from 33–36 °C (Winston, 1987; Southwick, 1991), but broodless areas tend to be cooler (Simpson, 1961). The average temperatures of the hive in the 1st experiment were 34.3 °C at the top center but only 32.1 °C at the bottom center (bottom center was only 30.3 °C for the first 10 days after treatment).

Aerial conidia are known to tolerate high temperatures (Burges, 1998), including temperatures encountered in the hive. Hong et al. (2001) tested conidia of 8 *B. bassiana* isolates and found that they all survived several days at 50 °C, provided moisture content was low. Using the relationship between r.h. and conidium moisture content described by Hong et al. (2002), and a simulation model of conidium longevity (Meikle et al., 2003), at 35 °C the half lives of the eight *B. bassiana* isolates described in Hong et al. (2001) were estimated to be 43–135 d at 40% r.h. and 4–13 d at 70% r.h. Some *Beauveria bassiana* isolates can grow at higher temperatures: Davidson et al. (2003) observed growth in all 7 of their *B. bassiana* isolates at 30 °C and in 5 of those isolates at 35 °C, and Fargues et al. (1992) observed growth in all 3 isolates at 32 °C and 1 of those at 35 °C. The isolate used here, found on *V. destructor* mites collected from a beehive (Meikle et al., 2006) germinated at 34 °C, so apparently conditions in much of the hive

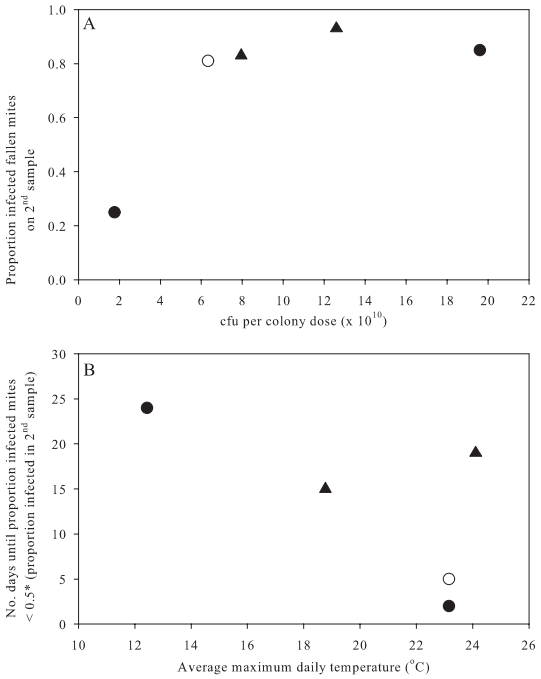

Figure 5. Factors influencing the proportion fallen mites that were infected across 4 field experiments. (A) Proportion fallen mites that were infected on the 2nd sample after treatment with respect to the initial per colony dose in cfu; (B) Number of days until the infection rate dropped below half that observed in the 2nd sample after treatment with respect to the average maximum temperature the 1st week after treatment. Data are from Meikle et al. (2007) (triangles), and from experiments described here (circles). All solid data points pertain to treatments of conidia plus wax powder, while the open circle pertains to the treatment of conidia plus wheat flour.

would not have prevented conidium survivorship or germination.

The proportion fallen mites that were infected may best be used as a measure of conidium duration in the hive environment. Here we defined the initial proportion fallen mites that were infected as the proportion of infection observed in the 2nd sample after application (the 1st sample being contaminated by excess formulation on the sticky boards). Comparing that value with respect to dosage for these experiments and those described in Meikle et al. (2007) showed that the lowest dose had the lowest initial proportion of infection and that proportion of infection increased about threefold when dosage increased about threefold. At higher dosages proportion of infection was above 80% (Fig. 5A). The powder + conidia formulation appears to distribute well in hives; Meikle et al. (2007) found the cfu density per bee using that formulation compared favorably with cfu density reported by Kanga et al. (2003) for unformulated conidia, despite a large dosage difference.

The proportion fallen mites that were infected in the colonies treated with conidia in the 1st experiment was significantly higher than in controls for about a week after application while in the 2nd experiment it was higher up to 12 days after application. Meikle et al. (2007) reported significantly higher infection more than a month after application in 2 previous experiments. Infection half life, defined here as the number of days after application until the proportion of infection fell to less than half that observed in the 2nd sample, would have been affected by colony dynamics, weather conditions, and their interaction (Fig. 5B). The net loss of capped brood and net gain of adults among all groups in the 1st

experiment here indicates that many bees and probably *V. destructor* mites emerged after application; such an emergence would have diluted cfu density among bees and mites and thus shortened infection half life as it was measured here. Comparatively few bees or mites would have likely emerged in the 2nd experiment because colonies in southern France tend to have little brood between November and February (Savary, 2006). As discussed above, the relatively high ambient temperatures and humidity (from nectar drying inside the hive) in the 1st experiment would have also shortened conidium longevity.

In these experiments no impact of *B. bassiana* on colony health was observed. A single application of *B. bassiana* with wax powder increased mite fall relative to the control treatment during the five weeks after treatment, but longer-term effects of a single treatment were doubtful. Future experiments will include more replicates per treatment, to better distinguish any treatment effects, and other measures of mite density, such as number of mites per adult bee, in addition to mite fall onto sticky boards. The results thus far are encouraging, but further work is clearly needed concerning conidia dosage, number of applications, and the ecology of entomopathogenic fungi within the beehive under ambient conditions and colony age structures.

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Impact d'un traitement à base de *Beauveria bassiana* (Deuteromycota : Hyphomycetes) sur la santé des colonies d'abeilles (*Apis mellifera*) et sur les acariens *Varroa destructor* (Acari : Varroidae).

***Apis mellifera* / *Varroa destructor* / *Beauveria bassiana* / biopesticide / formulation**

Zusammenfassung – Auswirkung einer Behandlung mit *Beauveria bassiana* (Deuteromycota: Hyphomycetes) auf die Gesundheit von Honigbienenvölkern (Hymenoptera: Apidae) und *Varroa* Milben (Acari: Varroidae). In zwei Experimenten wurden Honigbienenvölker in Südfrankreich mit Konidiensporen eines aus Varroamilben der Region gesammelten Isolates behandelt. Ziel war die Erfassung der Auswirkung der Behandlung auf das Gewicht der Völker, das Gesamtgewicht an adulten Bienen, auf die verdeckelte Brut und auf die Honigmenge, sowie auf den Totenfall von Varroamilben auf klebrige Bodeneinlagen. Die Behandlungen umfassten Formulierungen von Konidiensporen entweder mit Wachspulver oder mit Weizenmehl, sowie Weizenmehl alleine und unbehandelte Kontrollvölker. Die Behandlungen hatten keinen Einfluss auf die Gesundheit der Bienenvölker. Die mit Konidiensporen und Wachspulver behandelten Völker hatten einen höheren Milbentotenfall als die Kontrollvölker, bei den mit Konidiensporen und Weizenmehl behandelten Völkern war dies nicht der Fall. Der Anteil an infizierten Milben im Totenfall war bei beiden Behandlungen mit Konidiensporen über den Zeitraum von etwa einer Woche höher als bei den Kontrollen. Ein erhöhter Milbentotenfall und erhöhte Infektionsraten wurden auch in dem zweiten, kleineren Experiment gefunden. Die Beziehung zwischen der Dosierung und dem Anteil infizierter Milben im Totenfall und zwischen der Umgebungstemperatur und der Infektionsdauer wurden untersucht. Zukünftige Experimente sollen die Möglichkeit einer Behandlung der Varroose mittels Konidiensporen untersuchen.

***Apis mellifera* / *Varroa destructor* / *Beauveria bassiana* / Biopestizide / Formulierungen**

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