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NOTES AND COMMENTS

Effects of multiple applications of a *Beauveria* based biopesticide on *Varroa destructor* (Acari: Varroidae) densities in honey bee (Hymenoptera: Apidae) colonies

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One or two applications of *Beauveria bassiana* (Balsamo) Vuillemin (Hypocreales: Cordycipitaceae) have been reported to significantly increase the fall of *Varroa destructor* mites in honey bee (*Apis mellifera*) hives without affecting bee health, but satisfactory control using only the biopesticide has not yet been observed (Meikle *et al*., 2007; 2008a, b). Here we evaluated three successive applications of the biopesticide on mite densities and on colony health, estimated by measuring brood area.

Beauveria bassiana 05002 (NRRL 30976; ARSEF 8254) was grown on Sabouraud dextrose agar with yeast (SDAY) and chloramphenicol, and harvested as described in Meikle *et al*. (2008a, b). Three formulations were prepared: *B. bassiana* conidia with carnauba wax powder ("biopesticide"); unformulated conidia ("conidia-only"); and wax powder alone ("powder-only") to control for mite fall due to powder application (Fakhimzadeh, 2001). The biopesticide and powder-only treatments were formulated as in Meikle *et al*. (2008a, b); the per colony dose of biopesticide consisted of 0.6 g conidia mixed with 9.4 g carnauba wax powder (Strahl & Pitsch Inc.; West Babylon, NY, USA) and 0.05 g hydrated silica, and that of powder-only consisted of 10.0 g wax powder and 0.05 g silica. Carnauba wax is permitted as a food additive in the USA (U.S. Code of Federal Regulations, Title 21, part 184, section 1978). The number of colony-forming units (cfu) per g biopesticide was determined at application by suspending samples in distilled water and Tween 80 (Merck; Munich, Germany), plating them at 7- and 8-fold dilution onto potato dextrose agar (PDA), then counting the colonies after 96 h; cfu per g were 1.76×10^{10} for the first application, 1.80×10^{10} for the second, and 3.36×10^{9} for the third. Conidium viability was assessed by incubating conidia on SDAY at 22ºC for 24 h, and microscopically examining >200 conidia for germ tubes; viability was >90% in all samples.

On 13 September sticky boards (Mann Lake Ltd; Hackensack, MN, USA) were placed under each of 35 bee colonies in10-frame Langstroth hives, grouped in pairs on wooden pallets, with at least 2 m between pallets. Each board was replaced every 3-4 days thereafter and all mites counted. Before and after hive treatment, digital photos were taken of all frames and the brood surface area calculated on a computer, as in Meikle *et al*. (2008a, b). Phoretic mite density was estimated by collecting 400 - 600 adult bees per hive into glass jars containing ethanol, agitating the jars, pouring the contents onto trays, and counting all bees and mites. The cfu per bee was measured before, during, and after application period by collecting samples of 15 bees from each colony and freezing them. Two subsamples of five bees were removed from each of four colony samples per treatment group (the same colonies were always used). Each subsample was placed in a 50 ml centrifuge tube, vortexed for 3 min. with 10 ml of a 0.1% aqueous solution of Tween 80, and 100 µl aliquots of the resulting suspension were spread onto petri dishes containing PDA with chloramphenicol (0.4 g/l). After incubation for 14 days at 23°C, the *B. bassiana* cfu were counted.

On 28 September and 8 and 18 October, nine colonies were treated with biopesticide, 10 colonies with powder-only, three colonies with conidia-only and 13 colonies were left untreated. Except in one case, both hives on each pallet were assigned the same treatment.

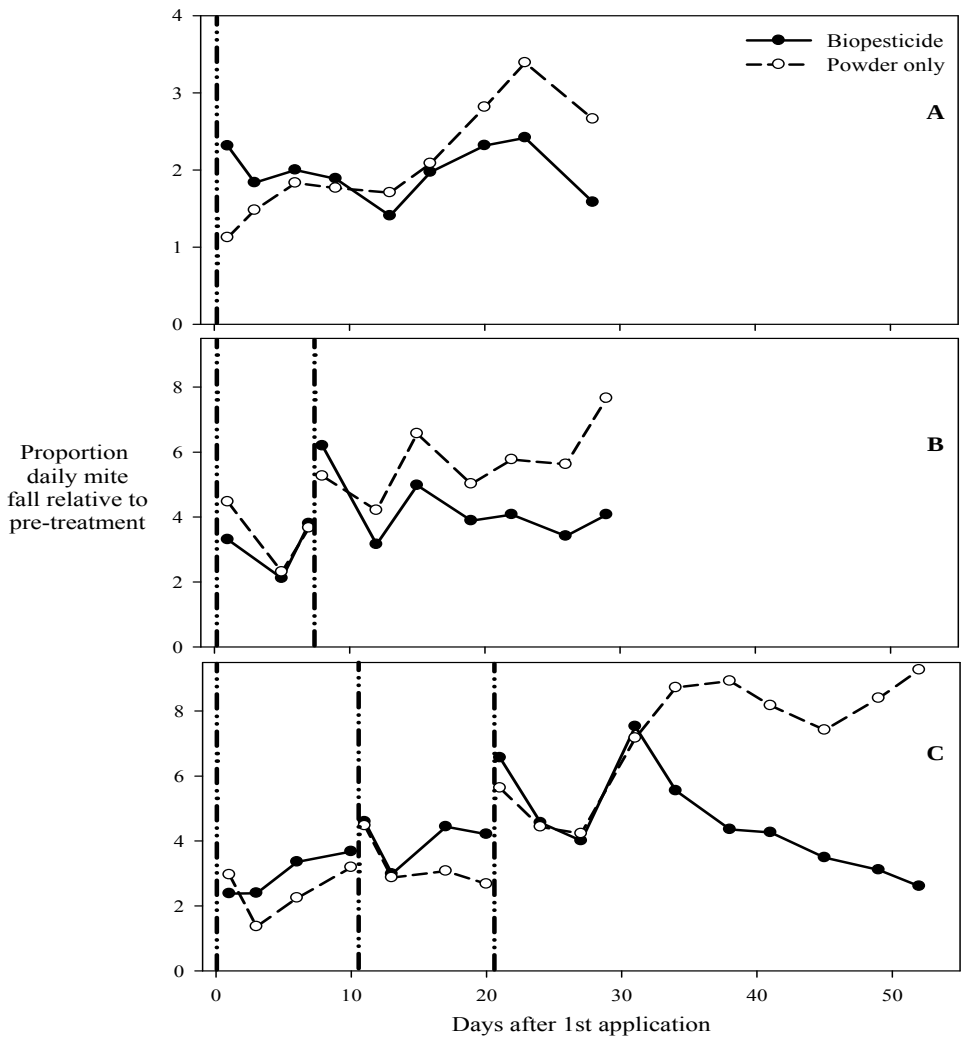

Fig. 1. Average daily mite fall per honey bee colony relative to pre-application levels for three experiments using the same *B. bassiana* based biopesticide and conducted near Montpellier, France: A) one application in an experiment conducted in spring 2006 (data from Meikle *et al.*, 2008b); B) two applications in an experiment conducted in spring 2007 (data from Meikle *et al.*, 2008a); C) three applications (this study) conducted in autumn 2007.

Colonies treated with conidia-only were small and only used to measure dispersal of unformulated conidia. The applications were squirted between all frames, as in Meikle *et al.* (2008a,b), using laboratory wash bottles. Repeated-measure ANOVA were conducted for a linear mixed model (SAS PROC MIXED) with mite fall (log transformed) as the response variable and with three fixed effects: treatment; date; and their interaction (α=0.05) (degrees of freedom calculated using the Satterthwaite method). *Post hoc* contrasts of least squares means differences were conducted with the Bonferroni adjustment.

Three hives lost their queens prior to first application. One hive in the powder-only group remained queenless so those data were removed. *Beauveria bassiana* was the only entomopathogenic fungus found on the mites. With pre-treatment mite fall as a covariate, date x treatment effect was significant ($F_{34,354}$ = 2.31, $p < 0.0001$) overall and, in *post hoc* tests, for all dates after 31 October when mite fall was lower in the biopesticide group ($F_{2,115}$ = 3.58 to 8.07, p = 0.0351 to 0.0005). To compare results with those of Meikle *et al.* (2008a, b), mite fall data after the first application were converted to proportion pre-treatment mite fall by dividing the group average (geometric) for each date by pre-treatment average (Fig. 1). Sealed brood was unaffected by treatment; a month after final application hives in the biopesticide group averaged 0.74 frames of brood, those in the powder-only group 0.70 and those in the control group 0.64 frames.

Cfu per bee varied from 3400 to 5300 for the biopesticide group one day after application, and 1900 to 5300 for conidia-only, but six days later were 30-250 and 20-70 per bee, respectively. There was no significant difference between the two groups (p = 0.99) indicating

that wax powder did not significantly affect conidia persistence, although formulated conidia are easier to apply. Meikle *et al.* (2008a) reported 3400-3800 cfu and Meikle *et al.* (2007) reported 11,000 cfu per bee one day after biopesticide application, in both cases declining to 50 or less in 10 days. The rapid decline in cfu per bee, probably due to cleaning, suggests a shorter period between applications.

Biopesticide treatment did not significantly reduce the number of phoretic mites per 100 bees ($p = 0.58$). Average (geometric) values rose from 4.8 for the biopesticide, 3.6 for the powder-only and 4.4 in the control to 10.6, 14.1, and 14.0, respectively, after final application. Treatment did not significantly reduce the number of phoretic mites per 100 bees, and thus did not provide adequate control, but it did have a significant effect on mite fall compared to pre-treatment levels. Further work is needed on the method and frequency of biopesticide application, as well as conidial dose and formulation.

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References

FAKHIMZADEH, K (2001) Effectiveness of confectioner's sugar dusting to knock down *Varroa destructor* from adult honey bees in laboratory trials. *Apidologie* 32: 139-148.

MEIKLE, W G; MERCADIER, G; HOLST, N; GIROD, V (2008a) Impact of two treatments of a formulation of *Beauveria bassiana* (Deuteromycota: Hyphomycetes) conidia on Varroa mites (Acari: Varroidae) and on honey bee (Hymenoptera: Apidae) colony health. *Experimental and Applied Acarology* 46: 105–117. DOI 10.1007/s10493-008-9160-z

MEIKLE, W G; MERCADIER, G; HOLST, N; NANSEN, C; GIROD, V (2008b) Impact of a treatment of *Beauveria bassiana* (Deuteromycota: Hyphomycetes) on honey bee (Hymenoptera: Apidae) colony health and on varroa mites (Acari: Varroidae). *Apidologie* 39: 1-13. DOI 10.1051/apido:2007057

MEIKLE, W G; MERCADIER, G; HOLST, N; NANSEN, C; GIROD, V (2007) Duration and spread of an entomopathogenic fungus, *Beauveria bassiana* (Deuteromycota: Hyphomycetes), used to treat varroa mites, *Varroa destructor* (Acari: Varroidae), in honey bee hives (Hymenoptera: Apidae). *Journal of Economic entomology* 100(1): 1- 10.