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Scientific note

A scientific note on the fungus *Beauveria bassiana* infecting *Varroa destructor* in worker brood cells in honey bee hives*

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***Beauveria bassiana* / *Varroa destructor* / *Apis mellifera* / entomopathogenic fungi / natural occurrence**

We report on the isolation of the mitosporic fungus *Beauveria bassiana* from varroa mites, *Varroa destructor*, in capped worker brood cells of honey bees, *Apis mellifera*. To our knowledge, this is the first record of *B. bassiana* from mites collected from bee brood. The brood chamber is the area of the hive where bees maintain high constant temperatures of 33–36 °C (Le Conte et al., 1990). An entomopathogenic fungus may be less efficacious because of poor germination at the high temperatures inside brood cells.

Natural enemies of the varroa mite are few and, until recently, included no records of fungal pathogens (Chandler et al., 2001). However, a number of studies have shown that varroa mites are highly susceptible to infection by a range of entomopathogenic fungi, including *B. bassiana* (James, 2009). Many entomopathogenic fungi have a ubiquitous distribution and a wide host range, thus one might expect foraging bees to frequently carry fungus conidia into the bee hive from their environment. The lack of observed natural infections of varroa mites by these fungi could be due to a combination of hygienic behaviour of worker bees and the harsh environmental conditions in bee colonies. Nevertheless, Meikle et al. (2006) isolated *B. bassiana* from approximately 0.2% of varroa mites collected from a number of apiaries in southern France and documented that natural infections could indeed be found. *B. bassiana* has also been reported from varroa mites in southern Spain (García-Fernández et al., 2008).

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Due to the collection method used by Meikle et al. (2006), infected mites could have originated from adult bees, frames, or combs where temperatures and relative humidity levels are likely to differ from those inside capped brood cells. We collected varroa mites from capped worker brood cells from each of 46 hives (in a total of 24 apiaries located in different parts of Denmark). Pupae were sampled from a piece of worker comb (11 × 11 cm) from June to November 2008. Between one and 183 adult female mites were collected from the hives. Mites were surface-disinfected in 5% sodium hypochlorite (2 min), followed by two rinses (2 min each) in sterile water and incubation on moist filter paper in Petri dishes for 10 days at room temperature. Fungus growing from mite cadavers was identified with a stereo microscope, *B. bassiana* was transferred with a sterile glass needle to plates with 2% Sabouraud dextrose agar for cultivation, and isolates were later stored in our culture collection.

A total of 16 infected mites (overall prevalence: 0.9%) were found in three of the 24 apiaries (Tab. I). Fungus prevalence in varroa mites varied from 4.4% (N = 45) to 33% (N = 3) among hives. All fungus was found during the autumn months. Additional mites were collected from an apiary where the fungus was found in early September. Dead mites that fell through the bottom wirescreen in each of four hives were collected at the end of October from plastic trays placed under the hives for a few days. These mites were surface-disinfected and processed as above. *B. bassiana* was found in dead varroa mites from all four hives at comparatively high infection levels (hive 1: 10.1%, N = 198; hive 2: 15.4%, N = 78; hive 3: 40.7%, N = 366; hive 4: 17.2%, N = 493). No *B. bassiana* was found in adult

Table I. *Beauveria bassiana* found in *Varroa destructor* from capped brood cells in honey bee hives.

Month	Number of				
	Apiaries	Hives	Mites	Hives with fungus	Infected mites
June	6	6	578	0	0
July	2	2	9	0	0
August	7	11	90	0	0
September	7	23	1041	1	1
October	1	3	52	2	3
November	1	1	73	1	12
Total	24	46	1843	4	16

bees (N = 139) sampled in November from the three apiaries where the fungus had been detected in varroa mites.

Our data show that varroa mites infected with *B. bassiana* can be found in worker brood cells. The natural occurrence of the fungus appear to be neither rare nor widespread. Nevertheless, the finding of the fungus in all four hives in an apiary sampled in October indicates that the fungus may spread from hive to hive by bee drift and therefore might become common at a local scale. It remains to be seen whether the fungus resides over winter in the hive or whether it disappears when mite populations decline to a minimum. Infected mites collected from bee pupae may have been infected before entering the brood cell. It still needs to be determined if infection affects reproduction of the mites. The fact that the fungus was present in varroa mites in brood cells emphasizes that the temperatures encountered in this warm area of a nest is not detrimental to the fungus while colonizing its host. Thus mites infected elsewhere in the hive cannot escape infection by entering brood cells. The fungus isolates originating from varroa mites in brood cells may be well adapted to the hive environment and may have good potential for varroa control.

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Note scientifique sur le champignon *Beauveria bassiana* infectant *Varroa destructor* dans les cellules de couvain d'ouvrières à l'intérieur des ruches.

Eine wissenschaftliche Notiz über den Pilz *Beauveria bassiana*, der *Varroa destructor* in Arbeiterinnenbrutzellen von Honigbienenvölkern befällt.

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