Mycoflora and fungal vector capacity of the parasitic mite Varroa destructor (Mesostigmata: Varroidae) in honey bee (Hymenoptera: Apidae) colonies

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MYCOFLORA AND FUNGAL VECTOR CAPACITY OF THE PARASITIC MITE VARROA DESTRUCTOR (MESOSTIGMATA: VARROIDAE) IN HONEY BEE (HYMENOPTERA: APIDAE) COLONIES

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ABSTRACT - Female adults of the honey bee mite, Varroa destructor, have fungi on their surfaces, and have the potential to disperse fungal spores (conidia) throughout the bee colony. Fungi present are typical of those associated with bees, their combs and provisions, and are common filamentous, soil saprophytes (listed in order of relative abundance): Aspergillus, Penicillium, Fusarium, Trichoderma, Alternaria, Rhizopus and Mucor. These fungi have no biological control implications (i.e., entomopathogenic) because none were recovered internally within the mites; absence of fungi internally also implies that this mite is not a fungivore. Our isolation of Aspergillus flavus, agent of stonebrood disease in honey bees, implicates Varroa as a potential fungal vector of stonebrood disease.

Key words - Acari, Varroidae, Varroa, parasitic mite, honey bee, fungi, USA.

INTRODUCTION

In recent years, the honey bee parasitic mite Varroa destructor Anderson and Trueman (Mesostigmata: Varroidae; ex: V. jacobsoni Oudemans) has displayed increased resistance to acaricides, resulting in serious economic losses to the honey bee (Apis mellifera L.) industry worldwide. Efforts to control V. destructor have been extreme (Bruce and Needham, 1996; Yoder et al., 1999; Yoder and Sammataro, 2003) and generally unsuccessful, prompting the development of alternative methods of control within the framework of integrative pest management (Sammataro et al., 2000). One possible strategy gaining attention is to include entomopathogenic fungi for Varroa control (Kanga et al., 2002; Davidson et al., 2003).

Fungi are found in association with honey bee colonies where they persist on nectar, pollen, in colony debris, and inside bees themselves. Some fungi have been shown to be beneficial to the colony by preserving stored pollen and bee bread, whereas others are pathogenic and contribute to colony losses (e.g., chalkbrood via Ascosphaera apis, stonebrood via Aspergillus flavus) (Gilliam and Vandenbergh, 1997). Recent work has been carried out to determine if Varroa is capable of harboring pathogenic microorganisms, including A. apis (Liu, 1996), viruses (Kleespies et al., 2000), and bacteria (Kanbar et al., 2002; de Rycke et al., 2002). The use of entomopathogenic fungi, however, is regarded as problematic because many strains are not selective to the insects they infect, resulting in their limited use in biological control (Roy and Pell, 2000). This is especially true of V. destructor, whose development and life cycle are closely tied to its bee host through parasitism and phoresy (Sammataro et al., 2000). Thus, it is conceivable that entomopathogenic fungi intended for Varroa control could result in reductions of bee populations when applied to a bee colony. Once established, a fungus prevalent in one bee colony may have the potential to spread to other colonies via asexual spores (conidia) carried by adults (Gliński and Buczek, 2003). One positive feature of entomopathogenic fungi is that many species are most virulent towards their original host, and less so towards distant arthropod relatives (Goettel et al., 1990, Hajek and Butler, 2000). Even so, the use of non-specific fungi aimed at Varroa control, namely species of Beauveria, Hirsutella and Metar-
hizium, has shown their pathogenicity towards A. mellifera (Shaw et al., 2002; Peng et al., 2002). The discovery and use of fungal pathogens lethal to Varroa and not to bees would, therefore, be invaluable.

Recently, an isolation procedure modified for plants was adapted for use in arthropods (Yoder et al., 2003c) including V. destructor, which permitted the recovery of the internal mycoflora. In this study, the internal and external mycoflora of the bee mite in three states (Arizona, Florida and Maine) is described to: (1) explore what types of fungi may be present internally that are pathogenic to mites, and (2) determine whether V. destructor harbors fungi externally that have the potential to spread throughout the bee colony.

**MATERIALS AND METHODS**

Field collections of V. destructor were conducted from December 2002 - May 2003 from mite-infested bee colonies maintained in Arizona (AZ), Florida (FL) and Maine (ME). Only female adults were used in the experiment and the age of the mites was not known. Mites were handled by means of sterile forceps, and all storage materials were sterilized before use. No fungi were visible macroscopically or microscopically (100x) on the mites and all mites appeared healthy.

External fungi were isolated using standard aseptic techniques as described by Currah et al. (1997) and Yoder et al. (2003c). Briefly, mites (in lots of three) were shaken twice for 1 min in 1 ml fresh deionized (DI) water in capped vials, transferred to a 9 cm i.d. Petri dish, and covered with potato dextrose agar (PDA, Difco, Fisher Scientific, St. Louis, MO). The plate was swirled gently to separate the mites and the agar was allowed to cool. Plates were incubated at 22-24°C for 7 d with daily observations for fungal growth. Hyphal tips visible under a dissection microscope were excised using a sterile scalpel, and subcultured onto PDA. Plates were incubated in darkness at 22-24°C until cultural characteristics (e.g., conidiophore) were evident (= 2 wks) that permitted identification using Barnett (1960).

Internal fungi (i.e., parasitic and facultative parasitic forms) were isolated similarly, except mites were first surface sterilized by shaking in a mixture of DI water: ethanol: 5.25% NaOCl (18:1:1, v/v/v) prior to DI water rinses, and the body was dissected into halves with a sterile scalpel before addition of PDA. This surface sterilization pretreatment was employed to strip the mite surface of external fungi that might have been present. To test the effectiveness of our sterilization technique, a separate subgroup of 15 surface-sterilized mites were plated onto PDA, but no traces of fungi were detected after 10 d of incubation at 22-24°C (data not presented). Dissected portions of another set of surface-sterilized mites were then immersed in molten PDA and incubated 1 wk until hyphal tips were visible originating from internal body sections. Permanent cultures of fungi are stored in the Wittenberg Microfungus Collection (specimens number WMC10554-10563).

One hundred mites were taken by random sampling from each population, AZ, FL and ME. Forty of the mites were used for external analysis. The remaining 60 mites were used for internal analysis, which were halved making 120 body sections.

**RESULTS AND DISCUSSION**

A listing of fungal components from adult female V. destructor is presented in Table 1. Fungi present on external bee mite surfaces included seven species of deuteromycetes (= imperfect fungi) in five genera: Alternaria sp., Aspergillus spp. (2), Fusarium sp., Penicillium spp. (2), and Trichoderma sp. Two genera of zygomycetes were also recovered: Mucor sp. and Rhizopus spp. Aspergillus and Penicillium species were recovered most frequently in all of the samples from the three states (AZ, FL, ME), with both genera being equally common. No fungi were recovered internally from any of the mites, ruling out the possibility that V. destructor exists as an occasional fungivore, or harbors pathogenic strains.

The fungal taxa recovered from V. destructor in this study support the findings of Batra et al. (1973) and Glinski and Bucek (2003) who reported on fungi common in beehives. Gilliam and Vandenberg (1997) also reported that Aspergillus and Penicillium were the two most prevalent genera in the honey bee colony. Within the colony, fungi (e.g., Penicillium, Aspergillus, Mucor, Trichoderma) have been isolated from numerous substrates including dead adult bees, combs, bee food, floral nectar, honey, and pollen provisions (Batra et al., 1973). As common opportunistic saprophytes that produce copious conidia, the occurrence of these fungal taxa on various substrates - including mites representing three states (AZ, FL, ME) - is not surprising. Given that the hive microhabitat is universally stable with respect to temperature and relative humidity (Yoder et al., 1999), such conditions likely favor a small number of prolific fungal species, especially anamorph (= asexual stages). Under such conditions, bees apparently tolerate, and perhaps even regulate their fungal co-habitants to some extent. Upon death of bee larvae and prepupae, species of Aspergillus, Penicillium and Mucor have been known to become secondary invaders (Glinski and Bucek, 2003), eventually overgrowing the cells and forming fungal masses (mycelia), also known as "fungal flower" within the brood cells (Batra et al., 1973). On occasion, some fungi (e.g., Fusarium, Penicillium, Rhizopus, Aspergillus) negatively impact the hive by spoiling provisions, leading to natural population declines due to a reduced food supply (Batra et al., 1973). To date, none of these
Table 1. Catalog of the mycoflora of honey bee mite, Varroa destructor, from Arizona (AZ), Florida (FL) and Maine (ME), USA.

<table>
<thead>
<tr>
<th>No. isolates from mite surface*</th>
<th>Fungi</th>
<th>AZ</th>
<th>FL</th>
<th>ME</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Deuteromycota</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Alternaria sp.</td>
<td>0</td>
<td>0</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>Aspergillus flavus</td>
<td>7</td>
<td>7</td>
<td>0</td>
<td>14</td>
</tr>
<tr>
<td></td>
<td>Aspergillus niger</td>
<td>6</td>
<td>6</td>
<td>8</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>Fusarium sp.</td>
<td>0</td>
<td>2</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>Penicillium glabrum</td>
<td>3</td>
<td>3</td>
<td>7</td>
<td>17</td>
</tr>
<tr>
<td></td>
<td>Penicillium sp.</td>
<td>6</td>
<td>4</td>
<td>7</td>
<td>17</td>
</tr>
<tr>
<td></td>
<td>Trichoderma sp.</td>
<td>4</td>
<td>0</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>Zygomycota</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Mucor sp.</td>
<td>2</td>
<td>2</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>Rhizopus sp.</td>
<td>2</td>
<td>0</td>
<td>4</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>30</td>
<td>24</td>
<td>23</td>
<td>77</td>
</tr>
</tbody>
</table>

* Nil in mite body contents (internal)

fungi have been shown to pose a serious threat to the honey bee colony as a whole, possibly because of well-established behavioral and immunoregulatory mechanisms (Gliński and Buczek, 2003).

The exception is Aspergillus flavus, which is suspected of causing epidemics among honey bees (Batra et al., 1973). This species is widely regarded as the causative agent of stonebrood disease in A. mellifera larvae, known to attack and damage the brood by producing a neuropathic aflatoxin (Batra et al., 1973; Gliński and Buczek, 2003). Presumably, the switch to parasitism by this otherwise harmless saprophyte (= facultative parasite) is triggered by some environmental cue, or perhaps high incidence of the fungus, or both (modified from Yoder et al., 2003a and b). Our inability to detect A. flavus internally in V. destructor suggests that this fungus is not a parasite of the mite itself. The presence of A. flavus on external mite surfaces implies that V. destructor serves as an additional, albeit minimal, source of fungal inoculum within the hive as a resident on the adult bee. Of considerable significance, however, is the possibility that A. flavus would gain access into new brood cells via conidia carried on mites that venture out into the hive in search of a new bee host. As such, V. destructor, in addition to causing heavy losses to the honey bee industry by direct infestation (Sammataro et al., 2000), may also promote the spread of stonebrood disease, as first proposed by Liu (1996). For the first time, our study provides evidence for this possibility.

Our revelation that A. flavus conidia are carried readily on Varroa begs the question of whether there is an increased incidence of bee death attributed to this fungus. Stonebrood is a rare disease considered to be of minor importance (Gilliam and Vandenberg, 1997). However, A. flavus not only produces toxins fatal to bee larvae, but has also been reported to kill adult bees on occasion (Batra et al., 1973; Gliński and Buczek, 2003), and may contribute to colony losses, at least in theory. Future studies are encouraged to explore Varroa's potential as a fungal vector, and to isolate additional fungal isolates that may be of significance to both V. destructor and A. mellifera.

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