UNIVERSITÀ DEGLI STUDI DI TORINO

Journal of Apicultural Research, 53(1): 141-145 (2014) DOI: 10.3896/IBRA.1.53.1.15

ACCEPTED ARTICLE. Published in the Journal of Apicultural Research, 53(1): 141-145 (2014)
Please cite this article as doi: 10.3896/IBRA.1.53.1.15

ORIGINAL RESEARCH ARTICLE

Megaselia rufipes (Diptera: Phoridae): a new cause of facultative parasitoidism in Apis mellifera.

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Received 6 July 2012, accepted subject to revision 17 January 2013, accepted for publication 7 March 2013.
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Summary

Here we present the first report of the Phorid fly Megaselia rufipes as a "facultative parasitoid" of the honey bee Apis mellifera after its identification in a natural colony in the Piedmont Region of Italy. 60 bees with deformed wings probably caused by deformed wing virus and 50 healthy bees were collected. Whilst maintained in the laboratory, parasitoid larvae emerged from the deformed-winged dead bees; further examination revealed that the parasitized bees contained emptied body cavities. Parasitization was not detected in the normal honey bees. This finding suggests that M. rufipes should be considered among the facultative parasitoids of A. mellifera. As the parasite was found only in non-flying bees already destined for death, the M. rufipes damage seems unlikely to be important. However, this finding, and the role of Phorids in beekeeping generally, beg more study given the recent link of the Phorid Apocephalus borealis to its potential of one of the indirect causes of widespread honey bee losses in the USA.
Key words: Apis mellifera, Megaselia rufipes, facultative parasitoid, bees with deformed wings, natural colony in Piedmont (Italy) 2

Running title: Megaselia rufipes: new cause of parasitoidism

Introduction

Our knowledge of Diptera responsible for parasitizing the honey bee Apis mellifera L is still incomplete. This is shown by the recent detection of Apocephalus borealis (Brues, 1924) (Diptera: Phoridae), already well known as a parasitoid of bumble bees in North America (Otterstatter et al., 2002), on the honey bee. It was tied recently to honey bee parasitoidism, and considered a potential indirect cause of "Colony Collapse Disorder" (Core et al., 2012). Specifically, A. borealis larvae have wreaked havoc on honey bee populations by causing disorientation and abnormal behaviour, such as hive abandonment during the night. This behaviour, and the early death that results from it, have led the parasite to be considered a potential cause of record bee losses in the USA. Furthermore, microarray analysis has shown honey bees from infested hives frequently are affected by deformed wing virus (DWV) and by the fungus Nosema ceranae. Both larvae and adult Phorids are often found to be positive for DWV and N. ceranae, which implicates A. borealis as a potential vector or reservoir of these pathogens (Core et al., 2012) as well.

Currently, we know that the handful of fly species responsible for world-wide infestations derive from just five families: Sarcophagidae, Phoridae, Conopidae, Tachinidae, and Calliphoridae (Morse and Nowogrodzki, 1990). In Europe, the species Senotainia tricuspis (Meigen, 1838) (Diptera: Sarcophagidae) is the most common cause of honey bee attack and it has led to major bee losses. Infestation occurs when viviparous females attack foraging bees or drones near the alighting boards of their hives and deposit one or two larvae in the body of its host (Simintzis, 1949; Giordani, 1956; Pape, 1987; Orantes Bermejo et al., 1996; Palmeri et al., 1997.

The Phorid family is comprised of Diptera with very heterogeneous larval food requirements that have developed adaptable feeding behaviours: phytophagous, scavenger, and entomophagous (Tremblay, 1994). Pseudohypocera kerteszi Malloch, (Diptera: Phoridae) is a honey bee predator in Mexico (Reyes, 1983), Colombia (Robinson, 1981) and Brazil (Pérez Gómez, 1975). Among Phoridae, in the tropics of the Americas at least eight species of the genus Melaloncha (Diptera: Phoridae) have 3

been identified as causing heavy damage to honey bee populations (Örösi-Pál, 1938; Ramirez, 1984). They have been known as regional parasitoids of meliponid bees (Borgmeier, 1935) that were passed to European honey bees after A. mellifera spread to the area. Until recently, these species, and M. ronnai Borgmeier, 1935, in particular (Ronna, 1936), were considered the only parasitoids of honey bees belonging to the Phoridae family. In fact, these honey bee infestations became so expected and devastating that they were named "autumn disease" (Knutson and Murphy, 1990). Later, as Africanized bees appeared in these same areas, they too were attacked (Van de Sande et al., 1986).
Species of the genus Megaselia (Diptera: Phoridae) are known to beekeepers as cleptoparasites, which are dependent on stored pollen and other organic remains (Disney, 2008). The most common is Megaselia scalaris (Loew). It was previously identified as a parasitoid both of stingless bees and A. mellifera in South America, Africa, and India (Örösi-Pál, 1938; Macieira et al., 1983; Rocha et al., 1984). Lately, M. scalaris has been found as a scavenger in honey bees hives in Spain (García Fernandez et al., 2010). While it has been known to attack cells within A. mellifera larvae or dead bees, Megaselia preacuta (Schmitz, 1919) has not been regarded as a parasite of the honey bee, but rather as a scavenger in sick colonies (Sammataro, 1997). The Phorid and focus of this paper, Megaselia rufipes (Meigen), has been found in dead honey bees (Clout, 1956) and in the fauna associated with bee hives in Poland (Banaszak, 1980).
In this paper, we will present M. rufipes as a legitimate agent of facultative parasitoidism in Italy. We show the incidence of M. rufipes in honey bees with deformed wings, which probably increases their vulnerability to parasitization due to their inability to fly.

Materials and methods

During July 2011, natural honey bees nests in a rural area near Cuneo (Piedmont, Italy) at 425 m above sea level were inspected. Sixty worker honey bees with markedly deformed wings were collected from a colony located within a stone enclosed empty wall to determine their health status. Fifty bees with the ability to fly were also captured. The deformed wing specimens were captured alive near the hive 4

flight hole or in the soil beneath it with entomological tweezers. Those capable of flight were taken only from near the hole by means of an electric vacuum cleaner with a filter, specifically adapted for bee removal. For transport to the laboratory, the specimens were placed within test tubes plugged with cotton to maintain ambient air exchange. After stereomicroscopic examination in the laboratory, each specimen was introduced into a Petri dish and maintained at a temperature of 24 ± 2° C and humidity of 60%. The honey bees were sustained by a wad of cotton wool soaked in a glucose-saturated solution and monitored daily.
Larvae developed on the bees with deformed wings and the flies that emerged from the adults were examined under a stereoscopic microscope at magnification 5-80 times for systematic determination. We identified the larvae based on the key of Velasquez et al., 2010; Disney (1994) was used for the adult flies. Slide preparations were made for detailed observation of the cephalopharyngeal skeleton and the anterior and posterior spiracles. Following their death, both the apparently healthy honey bees and those with deformed wings were dissected to observe the condition of their internal organs. Photographs of both adult and larva cephalopharyngeal skeletons were taken by a Kodak EasyShare® P880 camera mounted on microscopes Motic® SMZ-168 and Leica Diaplan®, respectively.

Results

Several distinguishing characteristics were found in the deformed wing specimen population. At capture we observed a dark-toned integument compared to that of their healthy counterparts and the stretched ligula. The entire deformed wing population demonstrated limited movement, and 5% (N = 60) were found to be infested by the mite Varroa destructor Anderson & Trueman. Among the non-flying bees, 14 specimens (23%) had some eggs adhering to their abdomens at the level of the intersegmental membranes. At 36-48 hours after Petri dish placement, 87% (52) of these honey bees had died and released Diptera larvae; not parasitized bees (8) survived for 2-4 days longer than the former.
The parasitoid larvae that developed on dead honey bees were classified as belonging to the Phoridae family (Figs 1-2). Pupal metamorphosis took place at about 15 days after the hatching of the eggs; 10 days later, adult flies emerged. 5

Examination of the male genitalia under the light-microscope allowed identification of the flies as M. rufipes (Fig. 3). Microscopic examination of the dissected dead bees indicated that larvae developed in the bee body cavity by consuming the organs. Inspection of the parasitized bee remains revealed complete destruction of the body cavity structures and intersegmental membranes; the thorax and abdomen had been fully emptied and dismembered. Following their death, no other parasite was detected.
In the specimen population with normal wings (and flight capability), 8% (N = 50) were found infested with the V. destructor mite, but no others. Under the additional scrutiny of the stereomicroscope, no other parasitoids were detected.

Discussion

M. rufipes infestation begins with egg deposition on the abdomen of the bee and the ensuing embryonic development. After hatching, the first instar larvae penetrate the intersegmental membranes of the abdomen. At completion of the instar life, larvae leave their host in search of suitable pupation sites. While such parasitoid behaviours have often been observed and reported in species of the Megaselia genus, similar phenomena have also been documented under controlled conditions in laboratory-reared Hemiptera (Costa et al. 2007) of the cockroach species (Disney, 2008), in other insects such as blowflies (Diptera: Calliphoridae) (Batista-Da-Silva, 2012), and Noctuid moths (Disney et al., 1992; McCabe, 1998).
In this study, parasitoid activity was detected only in bees with deformed wings, a condition that pre-destined them to death (Martin et al., 2013). No parasites were detected in the healthy specimens. These findings suggest that M. rufipes is a facultative parasitoid of A. mellifera that exploits the movement difficulties of deformed-winged bees.
Robinson (2005) observed similar behaviours in Megaselia scalaris (Loew) in both laboratory cockroach cultures and in the field. He described females capable of identifying injured individuals and then laying their eggs on them. Phorids have also shown an attraction to injured ants (Brown and Feener, 1993; Silveira-Costa and Moutinho, 1996). On the other hand, Macieira et al., 1983, and Rocha et al., 1984, have suggested that M. scalaris may be an optional parasitoid of the honey bee. Our 6

findings agree with the behaviour of Melaloncha against the honey bee in South America observed by Ramirez (1984).

From a practical perspective, infestation can be determined by collecting honey bees dying close to the hive entrance and then maintaining them individually in Petri dishes in the laboratory. The presence of a black-brown colour of the integument and a stretched ligula are signs indicative of larvae activity inside parasitized specimens. This finding is consistent with the atypical darker colour and stretched proboscis and legs observed in Triatoma brasiliensis Neiva (Hemiptera: Reduviidae) laboratory colonies infested by M. scalaris (Costa et al., 2007).

Prevention of M. rufipes infestation of honey bees and their hives requires maintenance of a high hygienic standard in the bee hive because organic debris is a strong fly attractant. Hive relative humidity is also important for complete development of species of the Megaselia genus (Trumple and Pienkowsky, 1979; Rocha et al., 1984), such as Melaloncha (Ronna, 1936), and must be balanced against proper family distribution with no overcrowding by correctly exposing the structure.

M. rufipes can be considered a facultative parasitoid of A. mellifera; its detection in honey bees with deformed wings from a colony seemingly affected by the presence of DWV, opens new scenarios in the role of Phoridae in honey bee colony health, even though Varroa destructor Anderson & Trueman is still the dominant player in problems of the honey bee (Dietemann et al., 2012). The very recent detection of A. borealis as a honey bee parasitoid, coupled with positive tests for DWV and N. ceranae, might place this Phorid among the possible causes of North American bee disorientation and death (Core et al., 2012). These new data require a thorough investigation on the role of Phoridae with respect to the honey bee. It also highlights the need for inquiry into the action of M. rufipes on healthy and infested bees and its Italian apiary incidence.

Acknowledgements

We are very grateful to Dr Henry Disney (Department of Zoology, University of Cambridge, UK) for its confirmation of the determination of Megaselia rufipes. 7

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Fig. 1. Megaselia rufipes: third instar larva. 12

Fig. 2. Cephalopharyngeal skeleton of Megaselia rufipes. 13

Fig. 3. Adult of the Phorid fly Megaselia rufipes.