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A short test evaluating larval attractiveness of honey bees to *Varroa jacobsoni*

LILIA I DE GUZMAN[1]; THOMAS E RINDERER[1]; VICKI A LANCASTER[2]

[1]USDA/ARS, Honey-Bee Breeding, Genetics and Physiology Research Laboratory, 1157 Ben Hur Road, Baton Rouge, Louisiana 70820, USA

[2]Department of Statistics and Statistical Laboratory, Kansas State University, Manhattan, Kansas 66506, USA

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SUMMARY

A controlled experiment which utilized a larval transfer (grafting) technique was used to evaluate attractiveness of larvae from four different stocks of honey bees (*Apis mellifera*) to *Varroa jacobsoni*. The stocks of honey bees were: ARS-Y-C-1 (*A. m. carnica*, from Yugoslavia), Hastings (*A. m. carnica*, from Canada), an F_1 hybrid between ARS-Y-C-1 and Hastings, and a Louisiana stock. Newly hatched larvae (target larvae) from each test stock were grafted into an area at the centre of a brood frame occupying 8 rows of 20 cells (160 cells). After larval transfer, each brood frame containing target larvae was introduced into a *Varroa*-infested colony. Inspection of the frames 2 weeks later showed that ARS-Y-C-1 pupae were less frequently infested than Hastings and Louisiana pupae (20% *vs*. 36% and 40%), while the infestation rate of the hybrids was intermediate (29%). The stocks did not differ in other parameters of *Varroa* infestation (mite load per infested pupa, number of females per infested pupa, number of progeny per female, number of progeny per infested pupa, and proportion of infested cells containing infertile females). The results indicate that larvae of the ARS-Y-C-1 stock, which has been selected for resistance to *V. jacobsoni*, are less attractive to female mites than the larvae of some other honey bee stocks.

Keywords: honey bees, *Apis mellifera carnica*, larvae, attractivenes, *Varroa jacobsoni*, resistance, grafting, host parasite relationships

INTRODUCTION

Any chemical applied in a honey bee colony for the control of *Varroa jacobsoni* raises concerns of hive product contamination. Also, ubiquitous and relentless use of chemicals for several years may produce mite populations that are resistant to acaricides. These considerations have created an immediate need for an alternative control strategy. More efforts are being directed to finding honey bees resistant to varroa.

Several factors have been identified as contributing to the resistance of *Apis mellifera* to *V. jacobsoni* (Buchler, 1994). One of them is the capped period of worker pupae. Honey bees with a shorter capped period will support the production of fewer reproductive females (Moritz & Hänel, 1984; Buchler & Drescher, 1990). Some stocks have an increased ability to detect, remove and kill mites in capped brood and on adult bees (Boecking & Drescher, 1991; Moritz & Mautz, 1990; Ruttner & Hänel, 1992). In addition, the number of infertile females plays an important role in the regulation of the mite population in colonies. So far, no differences have been found in the proportion of infertile female mites in infestations of colonies of different races of European bees (Buchler, 1994). A reduced attractiveness of larvae is also considered to be one of the factors in resistance. By testing pairs of small units of worker brood comb from different strains of European bees (in conjunction with a laboratory test), Buchler (1988) showed that larvae of one *A. m. mellifera* strain were less attractive to *V. jacobsoni* than larvae of the other strains tested, whereas larvae of two strains of Buckfast bees were more attractive. However, these results for one colony may have arisen from bias due to the use of open mated queens in two of the three strains tested, the small experimental size (one *mellifera* colony, two colonies of Buckfast bees and four *carnica* colonies) and the inappropriate method used to assess attractiveness (pairing two strains at a time rather than exposing all strains to mites simultaneously).

Despite significant findings from the studies mentioned above, none of the European bees tested was shown to be resistant to varroa. On the other hand, a stock of *A. m. carnica* from Yugoslavia (ARS-Y-C-1) has been shown to have some degree of resistance (reduced infestation rate) to varroa (Kulinc̆evic̆ & Rinderer, 1988; Kulinc̆evic̆ *et al.*, 1992). However, the mechanisms of its resistance have yet to be identified. This stock was imported into the USA through strict quarantine procedures for further testing (De Guzman *et al.*, 1990; Rinderer *et al.*, 1993).

Typically, assessments of response by honey bees to *V. jacobsoni* involve extended and costly field evaluation. It would be desirable to have a short test which could provide important information on some broad measures of resistance or tolerance. This study was conducted to:

- Develop a simple method for testing larval attractiveness to *V. jacobsoni*.
- To compare the attractiveness to *V. jacobsoni* of ARS-Y-C-1 with selected stocks of *A. mellifera* using this method.

MATERIALS AND METHODS

Daughter queens of ARS-Y-C-1 (*A. m. carnica*), from stock selected for reduced infestation by *V. jacobsoni*, were imported into the USA from Yugoslavia in August 1989. Consequently, ARS-Y-C-1 was compared with three selected stocks of *A. mellifera*: Hastings (*A. m. carnica*) from northern Saskatchewan, F_1 hybrids between ARS-Y-C-1 and Hastings stock, and a general Louisiana stock. Queens were instrumentally inseminated with 8 µl of mixed semen from the appropriate stock.

In order to compare the attractiveness of the four stocks to *V. jacobsoni*, uncapped larvae from each test stock were exposed to mites simultaneously. This method provided larvae of all stocks with similar chances of becoming infested in a common environment and was achieved by grafting or transferring young larvae of all test stocks into a section of a brood frame. We used a grafting technique developed by Harbo (1992) to compare the developmental time of honey bee stocks, with two modifications. First, we used larvae taken from the colonies rather than from frames previously placed in an incubator and second, rather than dry grafting, we primed cells with royal jelly prior to grafting.

Prior to grafting, two colonies with known varroa infestations (20% and 24%) in the pupal cells were established as host colonies. Host colonies received the grafted larvae, referred to here as target larvae. A total of five trials (one colony from each of the four stocks at one time) were done; two trials in the host colony with 20% varroa infestation and three trials in the host colony with 24% infestation. Since mites infest older larvae (L5) that are about to be capped, some frames containing younger uninfested larvae were removed from the host colonies to channel mites available for infestation toward the target larvae.

In each test colony, an empty frame was inserted in the middle of the brood nest for egg laying. Frames were inspected the next day for the presence of eggs. Grafting of target larvae was conducted three days later when the eggs had hatched and the larvae were less than one day old. At this time, a brood frame with young larvae from each host colony was chosen to receive the grafted larvae. Colonies were fed with sugar syrup and pollen patties one week prior to grafting to increase brood rearing.

At the centre of a frame receiving the larvae, an area occupying 8 rows of 20 cells was chosen to receive the target larvae. Two rows were assigned randomly

TABLE 1. Reproductive ability of *Varroa jacobsoni* in four selected stocks of *Apis mellifera* (mean ± between infested cell standard deviation).

Bee stock	Mite load/ infested pupa	Foundress/ infested pupa	Progeny/ infested pupa	Progeny/ foundress	% infested cells with infertile females[1]
Hastings (121[2])	6.40 ± 3.29 (52[3])	1.40 ± 0.66 (52[3])	5.00 ± 2.79 (52[3])	3.67 ± 1.54 (52[3])	22.00 ± 43.82 (73[4])
Louisiana (110[2])	5.77 ± 2.54 (48[3])	1.29 ± 0.58 (48[3])	4.47 ± 2.12 (48[3])	3.62 ± 1.43 (48[3])	2.79 ± 3.85 (62[4])
F_1 hybrid (116[2])	6.34 ± 2.23 (38[3])	1.23 ± 0.59 (38[3])	5.10 ± 1.74 (38[3])	4.32 ± 0.95 (38[3])	0 (47[4])
ARS-Y-C-1 (107[2])	6.50 ± 3.16 (28[3])	1.39 ± 0.63 (28[3])	5.11 ± 2.82 (28[3])	3.85 ± 2.05 (28[3])	7.69 ± 13.32 (39[4])

[1]mean ± between trial standard deviation
[2]number of cells examined
[3]number of examined cells that were infested
[4]total number of females found in infested cells

for each test colony per trial. Larvae inside the cells within the area were discarded and the cells were cleaned using a grafting needle. A drop of royal jelly was then placed at the bottom of each cell and larvae from each stock were then grafted into the cells. Priming of royal jelly was done one row at a time to prevent the material from drying. The positions of the grafted brood were then mapped on a transparent sheet and the frame was placed back into the host colony.

After two weeks, when cells contained dark-eyed pupae (about 16–17 days old), all accepted pupae were examined for the presence of mites. Mites together with the pupae were placed in Eppendorf vials containing 70% ethyl alcohol for later examination under a dissecting microscope. Numbers of mites infesting a pupa were counted and all developmental stages were differentiated.

The primary variable of interest, percent infestation, was described using a one-way treatment structure in a randomized complete block design; trials were the blocks and stocks were the treatments. The variable was first transformed using the Freeman-Tukey double arcsine transformation (Freeman *et al.*, 1950) and then analysed using the General Linear Model procedure in SAS (SAS Institute Inc., Version 6.08, 1989). The Bonferonni procedure was used for stock mean comparisons. There were no departures from the model assumptions of a normal error structure with constant variance.

RESULTS

The proportion of cells infested with *V. jacobsoni* differed significantly among the stocks ($F = 4.37$; d.f. = 3, 12; $P = 0.03$). It was lower in the ARS-Y-C-1 stock (20.65 ± 19.2%; mean ± between trial standard deviation) than in the Hastings (36.04 ± 26.8%) and Louisiana (40.12 ± 14.09%) stocks. Infestation in the F_1 hybrid (ARS-Y-C-1 × Hastings) was intermediate (29.73 ± 22.5%) between its parental stocks. The results of the Bonferroni multiple comparison procedure showed a significant difference between the Louisiana and ARS-Y-C-1 stocks at a probability level of 0.05. Neither of these stocks were significantly different from Hastings and F_1 hybrid colonies.

Descriptive statistics for the reproductive ability of the mites are listed by stock in table 1. Regardless of the stock, an infested pupa contained about seven mites mostly of different stages. At least one foundress mite, which may or may not have been capable of reproducing, was observed on each infested pupa. Those mites which were able to reproduce produced about five progeny. A maximum of five progeny were recorded per infested pupa. The highest proportion of infested cells containing infertile females or females with no progeny at the time of observation was observed in the Hastings stock. No infertile females were recorded in infested cells of the F_1 hybrid. None of these variables however, was useful for identifying differences in larval attractiveness among the stocks used in this study.

DISCUSSION

The population dynamics of *V. jacobsoni* in different *A. mellifera* stocks and subspecies has been studied by several researchers under long-term field conditions. In this study, a short-term test indicated that ARS-Y-C-1 (*A. m. carnica*) stock was the least attractive to mites which were given a choice between larvae of different stocks. The F_1 colonies were intermediate between their ARS-Y-C-1 and Hastings parents, suggesting that the underlying genetic difference between ARS-Y-C-1 and Hastings stocks lacks dominance.

The attraction of mites to host bees may be attributed to the existence of chemicals. Trouiller *et al.* (1992) reported that high levels of esters of fatty acids which served as kairomones were present in the brood prior

to capping in *A. mellifera*. However, this claim was not supported by the results of experiments conducted by Zetlmeisl and Rosenkranz (1994). These authors found that pentane extracts of L5 larvae, and not the esters, attracted mites to the same degree as live L5 larvae, which is the susceptible stage for *V. jacobsoni* infestation. Whether or not the levels of these chemicals were different in the stocks tested is not known.

We observed that once a brood cell was infested, the reproductive ability of mites was similar in all the stocks. After years of selection, the reproductive rate of mites infesting *A. m. carnica* stocks in Yugoslavia ranged from 1.41 to 3.8 (Kulinčević & Rinderer, 1988). The results of our controlled experiment showed a similar reproductive ability by mites in the ARS-Y-C-1 stock of about four progeny per foundress.

Under the conditions of a choice experiment, ARS-Y-C-1 stock demonstrated its potential for resistance to *V. jacobsoni* by being less frequently infested than other stocks. ARS-Y-C-1 was selected for reduced infestation rates (Kulinčević *et al.*, 1992), a trait of the stock confirmed by this test. However, whether or not this stock possesses the other characteristics influencing resistance to *V. jacobsoni* needs to be investigated.

We measured some additional variables relating to the reproductive ability of *V. jacobsoni*. Resistance in some honey bee stocks is reported to be expressed as a reduction in some of these reproductive attributes of the parasite. Resistance of this type was not found to be a characteristic of the ARS-Y-C-1 stock or the other stocks in this study. Nonetheless, the data to make direct comparisons of stocks for their abilities to support reproduction of *V. jacobsoni* were readily available at the end of the short test. Consequently, this short test is ideal for directly comparing stocks in a common environment for these attributes as well as for reduced infestation rates. Such information on larval attractiveness is very important to consider as a potential characteristic which breeders can combine with other qualities conferring resistance to *V. jacobsoni* during their selection programmes.

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