A Study of Chalkbrood Susceptibility in Russian and Domestic Honey Bees

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Abstract

Chalkbrood susceptibility levels were determined in selected Russian and domestic honey bee lines. A baseline chalkbrood infection level was first determined for each colony, followed by three chalkbrood inoculations, each one week apart. Chalkbrood mummies were counted one week after each inoculation and removed. Both Russian and domestic post-inoculation chalkbrood infection means increased an average of five fold when compared to the baseline infection level. However, the pre- and post-inoculation levels were not significantly different (P=0.13), probably due to the high number of colonies exhibiting 0% chalkbrood infection throughout the entire study. No significant differences in chalkbrood susceptibility were found between the two stocks (P=0.31), or between any lines (P=0.60). However, colonies showing extreme chalkbrood resistance or susceptibility were identified. They will be used as parents in a breeding study designed to study the genetic control of chalkbrood susceptibility.

Introduction

Chalkbrood, caused by *Ascosphaera apis* Maassen ex Claussen (Olive et Spiltoir), is considered to be the most serious of all honey bee fungal diseases (Gilliam, 1990). Despite the broad range of research that has been conducted to develop chalkbrood control strategies, no specific control strategy has been universally accepted or adopted by beekeepers (Hornitzky, 2001).

Over the past two decades, reports of chalkbrood-resistant bee strains have been made. Most of these reports have focused on hygienic behavior as the cause of resistance (Milne, 1982; Gilliam *et al.*, 1983; and Spivak and Reuter,1998). Little research has been done on other genetically based mechanisms of chalkbrood resistance in honey bees.

Varroa and tracheal mite resistance has been reported in selected lines of honey bees from far eastern Russia (Rinderer *et al.*, 2001; de Guzman *et al.*, 2002). Although mite resistant stock is highly valued by the beekeeping industry, enhanced resistance to other pests and diseases is also valued. Hence, other characters such as chalkbrood resistance should be investigated in these selected Russian lines. This study was undertaken for two purposes: 1) to determine whether differences in susceptibility exist between Russian and selected domestic honey bee stocks in the United States and between lines within each stock and 2) to identify extremely susceptible and resistant colonies to be used as parents in a breeding program designed to elucidate the genetic basis of chalkbrood susceptibility.

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Methods

Test colonies (n=131) were established in the Baton Rouge, LA area between April 1 and April 19, 2004. These colonies were produced by splitting larger domestic and Russian colonies, which had been treated with CheckMite+ (coumaphos;Bayer, Shawnee Mission, KS, USA) for varroa mites in the winter of 2003-2004. A standard colony size of 4-5 frames of deep brood and 5-6 deep frames of bees was established. Russian queens were introduced into 61 colonies (53 domestic splits, 8 Russian splits), and domestic queens were introduced into 70 colonies (36 domestic splits, 34 Russian splits).

Russian queens were produced from existing Russian breeding colonies at the USDA Honey Bee Breeding Lab. Seven different lines were produced. Five of these lines (R1, R2, R5-R7) were selected Russian lines, chosen for their varroa and tracheal mite resistance and overall vigor. Two lines (R3 and R4) were bred from colonies exhibiting chalkbrood susceptibility in fall 2003. These two lines were chosen as possible sources of chalkbrood-susceptible parents to be used in a breeding study.

Domestic Italian and Carniolan queens were purchased from seven different queen breeders across the United States. Approximately nine queens from each Russian and domestic line were introduced. Due to queen rejection, 71 queen right colonies from 13 lines remained six weeks after introduction. Each line contained approximately 5 colonies each.

The populations were analyzed for existing chalkbrood infection during the first week of July, 2004. All colonies were inspected for proper queen presence and chalkbrood infection. All chalkbrood mummies were counted and removed from all colonies. Colonies (35 domestic and 36 Russian) were then inoculated with a chalkbrood inoculation mixture. All colonies received the same concentration of inoculation mixture. The inoculation mixture was prepared according to Koenig *et al.* (1987). Approximately 5 sporulating, black mummies (0.12 g) were pulverized, suspended in 75 mL of a 50% sucrose solution, and poured into a 100 mL plastic bag. A small corner of the plastic bag was cut and the bag was then placed on the top bars of the colonies. Test colonies were reinoculated at one-week intervals. Three inoculations were performed, and a final mummy count was performed one week after the last inoculation. During the final mummy count, the total capped brood area in the colony was determined using a 2.54 x 2.54-cm wire grid. Brood area was then converted to total numbers of capped worker brood cells (brood area x 3.7 worker cells per square centimeter). The percent infection of chalkbrood was determined as the number of mummies / capped brood cells= % chalkbrood infection.

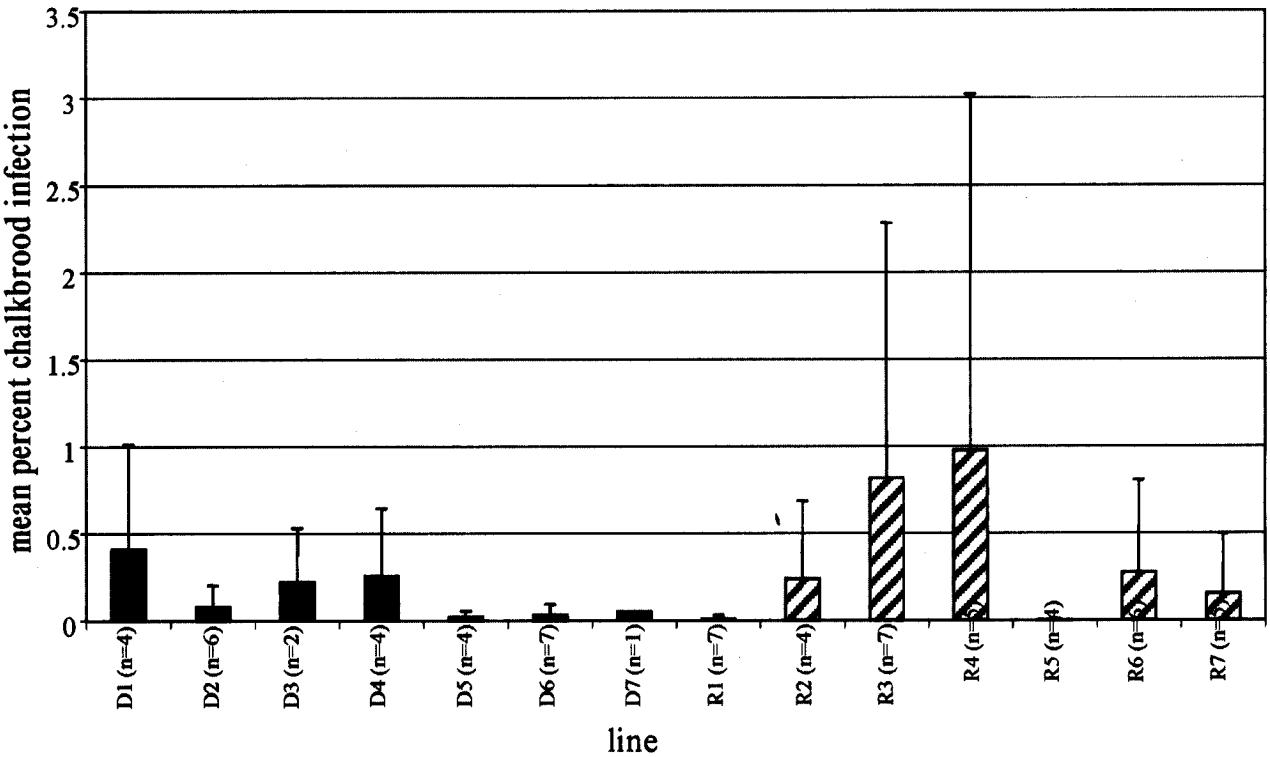

Figure 1. Mean post-inoculation chalkbrood infection levels given by line. D=domestic line, R= Russian line. Note: 0.1% chalkbrood infection= approx. 8 mummies/colony

Statistical Analyses

Comparison of chalkbrood infection levels and of amounts of sealed brood between stocks and among lines and inoculation times was made using an analysis of variance (ANOVA) repeated measures analysis. The mixed model included: 1) stock as a fixed effect 2) line as a fixed effect 3) yard as a random effect and 4) the random error term (PROC MIXED; SAS Institute 2000). Degrees of freedom were adjusted using the Kenward-Roger estimation.

Results

Mean post-inoculation chalkbrood infection levels for the 13 genetic lines are shown in Fig. 1. Although Russian lines R3 & R4 appear to have higher mean infection levels, no significant differences between lines were found (P=0.60). Also, 12 of 29 (41%) of the domestic colonies and 16 of 38 (42%) of the Russian colonies had 0% chalkbrood infection throughout the inoculation study.

Mean chalkbrood infection levels by stock are shown in Fig. 2. No significant differences in chalkbrood susceptibility were found between Russian and domestic honey bee populations (P=0.31). The overall chalkbrood infection means (post-inoculation) increased from baseline levels. Russian colonies exhibited a 5-fold mean increase in chalkbrood levels (0.05% to 0.25% mean post-inoculation infection). Domestic colonies also exhibited a 5-fold mean increase in chalkbrood levels (0.03% to 0.15% mean post-inoculation infection). However, the pre- and post- inoculation levels were not found to be significantly different (P=0.13). Mean levels of capped brood/colony are shown in Table 1. Mean capped brood/colony was not significantly different between the two stocks (P=0.52).

Discussion

Although the inoculation procedure increased the mean chalkbrood infection level of the colonies, pre- and post-inoculation infection averages were not found to be significantly different (P=0.13). This is probably due to a combination of the high number of colonies exhibiting 0% chalkbrood infection throughout the entire study and the small number of colonies representing each line. The overall low level of chalkbrood infection may have been due in part to the hot, dry climate conditions during the time of year the study was performed (July).

Since no significant differences in chalkbrood susceptibility were found between Russian and domestic stocks, nor between individual lines in this experiment, the chalkbrood susceptibility of Russian bees appears to be comparable to that of other US stocks.

This experiment is the first phase of a project designed to investigate the genetic basis of chalkbrood susceptibility in honey bees. The purpose was to inoculate a diverse group of colonies with chalkbrood, and select the colonies showing the highest levels of chalkbrood susceptibility and resistance, to be used as parents in a breeding study. Colonies showing comparative chalkbrood susceptibility and chalkbrood resistance were identified. Collectively, the data from this study offer no support for the hypothesis that honey bees have genetic variance for resistance to chalkbrood. However, variation in response to the inoculations was found which had some consistency within lines. Identifying colonies with contrasting responses to the inoculation creates the possibility for experiments designed to determine if the differences have a genetic component.

Table 1. Capped brood levels for Russian and domestic colonies

Stock	Capped brood (cm^2)
Russian	544 ± 165*
Domestic	596 ± 263*

* Data are shown in mean ± SD capped worker cells per colony, taken after the final chalkbrood mummy count

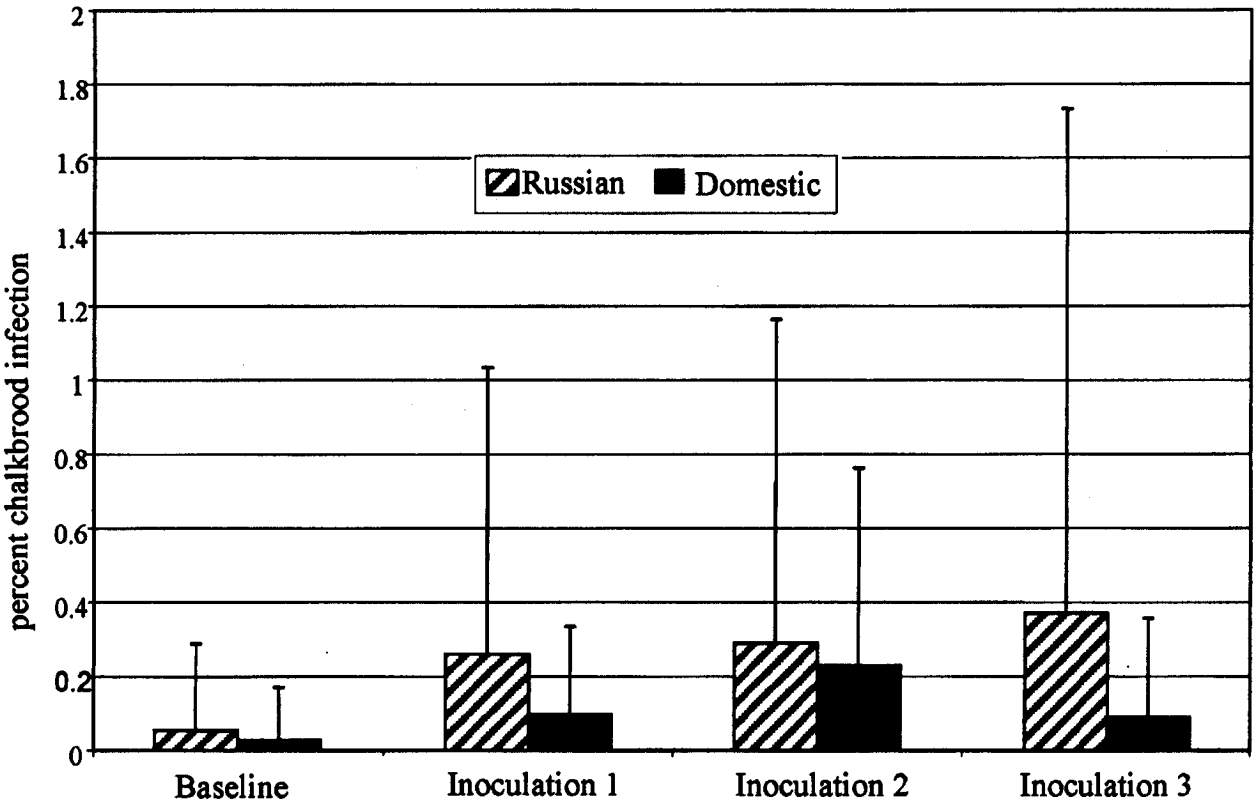

Figure 2. Mean chalkbrood infection levels by stock.

Acknowledgements

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