2012

Responses to *Varroa destructor* and *Nosema ceranae* by several commercial strains of Australian and North American honeybees (Hymenoptera: Apidae)

Thomas E. Rinderer
USDA-ARS, tom.rinderer@ars.usda.gov

Benjamin P. Oldroyd
University of Sydney, benjamin.oldroyd@sydney.edu.au

Amanda M. Frake
USDA-ARS, Mandy.Frake@ars.usda.gov

Lilia I. de Guzman
USDA-ARS, Lilia.DeGuzman@ars.usda.gov

Lelania Bourgeois
USDA-ARS, Lanie.Bourgeois@ars.usda.gov

Follow this and additional works at: http://digitalcommons.unl.edu/usdaarsfacpub

Responses to *Varroa destructor* and *Nosema ceranae* by several commercial strains of Australian and North American honeybees (Hymenoptera: Apidae)

Thomas E Rinderer,[1]* Benjamin P Oldroyd,[2] Amanda M Frake,[1] Lilia I de Guzman[1] and Lelania Bourgeois[1]

[1]*Honey Bee Breeding, Genetics, and Physiology Laboratory*, USDA-ARS, 1157 Ben Hur Road, Baton Rouge, LA 70820, USA.
[2]*Behaviour and Genetics of Social Insects Laboratory*, School of Biological Sciences A12, *University of Sydney*, Sydney, NSW 2006, Australia.

Abstract   The potential impact of varroa (*Varroa destructor*, Anderson & Trueman) on Australian beekeeping and agriculture depends in part on the levels of resistance to this parasite expressed by Australian commercial honeybees (*Apis mellifera*). The responses of seven lines of Australian honeybees to *V. destructor* were compared with the responses of a stock of Italian honeybees from the United States known for its susceptibility to *V. destructor* and two stocks known for their resistance to *V. destructor*, Russian honeybees (RHB) and a stock expressing the varroa sensitive hygiene trait (VSH). The experiment began in May with uniform colonies having uniform infestation of *V. destructor*. *V. destructor* infestations measured as the percentage of adult bees infested in the Australian lines and the Italian stock rose from less than 10% in August to over 25% in October. From August to November, 44% of both the Australian and Italian colonies died while strongly exhibiting symptoms of parasitic mite syndrome. In contrast, RHB and VSH colonies displayed comparative resistance to *V. destructor*. Their infestation rates rose from about 5% in August to 10% (RHB) and 14% (VSH) in October. Likely, some of this increase resulted from invasion pressure by mites from the dying Australian and Italian colonies. During the August to November period, 4.4% of the RHB and 14.3% of the VSH colonies died. In comparisons of the seven Australian lines, only nonsignificant and trivial differences were found for infestation and mortality rates. All Australian lines were highly susceptible to *V. destructor*. Additionally, evaluations of rates of *Nosema ceranae* infections were made throughout the course of the experiment. Although high levels of infection were found across all stocks and lines, no stock or line exhibited an adverse effect from *N. ceranae* infection.

Key words   Australian honeybees, mite resistance, *Nosema ceranae*, Russian honeybees, *Varroa destructor*, varroa sensitive hygiene.

INTRODUCTION

Varroa destructor (Anderson & Trueman 2000) is an external parasitic mite that, until recently, infested only the Eastern hive bee, *Apis cerana* (Peng *et al*. 1987; Oldroyd 1999). Although *V. destructor* feeds on both adult bees and brood, it persists in colonies of *A. cerana* without causing serious harm (Koeniger *et al*. 1983; Peng *et al*. 1987; Oldroyd 1999; Rath 1999). However, *V. destructor* has successfully extended its host range to the Western honeybee, *A. mellifera,* and has spread through much of this honeybees' Old and New World ranges (Oldroyd 1999). *V. destructor* is highly virulent on *A. mellifera* as infestations result in the death of colonies within 1–2 years of being infested without beekeeper intervention (Shimanuki *et al*. 1994) and from 8 months to a year in areas where brood rearing is year-round (Branco *et al*. 1999).

Various methods have been developed to protect colonies from *V. destructor*, including biotechnical methods to several acaricides. Biotechnical methods, principally drone brood removal, are effective but time-consuming and are only suitable for use with a small number of colonies (Boot *et al*. 1995; Calis *et al*. 1999). For the most part, large scale apiculture has relied on acaricides to control the parasite. However, several problems attend the use of acaricides: mite populations have developed resistance to acaricides, honey can be contaminated if acaricides are used during a nectar flow, and the timing of nectar flows can compete with the timing of acaricide treatments necessary to effectively control the mites. Additionally, properly timed treatments will eventually contaminate the hive to damaging levels, and acaricides decrease the reproductive potential of drones (Lodesani *et al*. 1995; Elzen *et al*. 1998; de Guzman *et al*. 1999).

Honeybees that resist *V. destructor* are an attractive alternative to the continued used of acaricides. 'Russian' honeybees (RHB), a strain developed by the USDA (Rinderer *et al.* 2001) have several mechanisms of resistance to varroa that act in concert to provide commercially important levels of resistance (de Guzman *et al.* 2007). Honeybees with the 'varroa sensitive hygiene' (VSH) trait detect and remove varroa-infested brood (Harris *et al.* 2009). Some beekeepers use RHB or VSH without reliance on acaricides, while other beekeepers use only 'soft' chemical treatments such as thymol.

V. destructor has not yet reached Australia. However, considering varroa's successful occupation of both the Old and New World ranges of *A. mellifera*, it seems likely that varroa will soon spread to Australia. Although they may have retarded the spread of varroa, Pacific Ocean isolation and regulations against honeybee importations have not protected either New Zealand (infested with varroa since 2000) or Hawaii (infested with varroa since 2007).

Considering the likelihood that *V. destructor* will eventually reach Australia, it is prudent to know the response of commercial strains of Australian *A. mellifera* to this parasitic mite. This study compared responses of a total of 240 queens from seven strains of commercial Australian honeybees and three strains of North American honeybees. The seven Australian strains have not been bred for resistance to *V. destructor*, while two of the American strains (RHB and VSH) have documented resistance to *V. destructor* (Rinderer *et al.* 2001; Harris *et al.* 2009). The third American strain is a variety of Italian honeybees that has been bred in the presence of *V. destructor* but has not been artificially selected for resistance (Rinderer *et al.* 2004a; de Guzman *et al.* 2007).

Additionally, we evaluated the rates of infestation of the colonies in the experiment by *Nosema ceranae*. This microsporidian parasite has recently invaded the honeybee colonies of both Australia and the United States. No stock has yet been found to have strong resistance to this parasite, and finding one would be highly desirable.

MATERIALS AND METHODS

Honeybee stocks

Australian queens came from seven queen breeders that were more than 1000 km from the *A. cerana* exclusion zone. Cages were marked with letters (A through G) corresponding to queen breeders. However, line identity for queens was unknown to the persons collecting data. The queens were given water everyday during shipment from queen breeders to their introduction to colonies in apiaries in Kansas, USA, with a history of good nectar and pollen availability. The shipment of queens was approved and regulated by both the Australian Quarantine and Inspection Service (AQIS) and the USDA – Animal and Plant Health Inspection Service (APHIS). These services established various pathogen- and parasite-free certification, and transportation procedures, and

facilitated and assured compliance with these regulations. Experimental apiaries were quarantined in compliance with APHIS requirements.

Resistant queens (RHB = 25 and VSH = 29) and susceptible Italian queens (25) from the United States were all obtained from commercial sources. They were shipped to the USDA, Honey Bee Breeding, Genetics and Physiology Laboratory in Baton Rouge where they were re-caged without attendants, placed in queenless colonies and transported to the experimental apiaries in Kansas.

Upon arrival in Kansas, all queens were marked with acrylic paint and wing clipped to assure later identification. Paint-marking did not identify specific stocks or lines. Small (four frames of brood with adhering bees and six frames with adhering bees) queenless colonies were prepared 1 or 2 days prior to queen introduction. For each of four apiaries, colonies were randomly assigned to queens of the four groups, such that each apiary contained a proportional representation of each group. Apiaries were separated from the next nearest apiary by at least 3.6 km, and contained 54, 57, 60 and 80 colonies. Colonies were in standard 10-frame Langstroth hives placed on four-way migratory pallets. Colonies were individually marked, and the specific queen type (Australian A through G, RHB, VSH or Italian) was recorded at introduction. On 17 May 2011, queens were introduced in cages with a candy-release system. Four days after, colonies were inspected, and successful introductions as indicated by the presence of the marked queen and eggs were recorded (Table 1).

Varroa mite inoculations

Colonies used to produce the queenless colonies had followed a commercial schedule for acaricide treatment for the previous 12 months. They were treated 8 months prior to the beginning of the experiment with the intention of producing test colonies with low and reasonably uniform numbers of mites. Samples of adult worker bees (300–500 bees) taken from the brood nest of each of the queenless colonies detected low infestations of varroa averaging 2.65 ± 0.22 (mean ± SE) mites. In order to assure a reasonably uniform infestation, adult worker bees from highly infested colonies were pooled in a large cage. Once inside the cage, these bees were allowed to settle for 3 h to facilitate an even distribution of mites. Samples of bees from different places within the cage were then sampled to determine their infestation levels. The bees were then divided into groups that were placed in cages formed with a standard frame and eight-mesh screen. Each experimental colony was given a cage that held 1200 worker bees, with an estimated 37.3 ± 5.0 mites. The mites were able to exit the cages through the screen and infest the experimental colonies. Cages were removed from the colonies 1 month later when the caged bees had died and the mites had gained entrance to the colony.

Colony evaluations

Colonies were evaluated on 14 June, 12 July, 23 August, 5 October and 3 November 2011. These evaluations consisted of

Table 1 Original queen survival, supersedure and death rates for four stocks of honeybees and seven lines of the Australian honeybees

Line	May 17 # Queens introduced	May 21 # Queens survived and accepted	June 14 % Original queen	% Supersedure	% Dead or queenless	July 12 % Original queen	Supersedure	% Dead or queenless	August 23 % Original queen	% supersedure	% Dead or queenless	October 5 Original Supersedure queen	% Supersedure	% Dead or queenless	November 3 % Original queen	% supersedure	% Dead or queenless
A	28	28	78.6	17.9	3.6	67.9	21.4	10.7	50.0^{ab}	25.0^{ab}	25.0	10.7^{bc}	25.0^{ab}	64.3	0.0^{b}	25.0^{ab}	75.0
B	22	21	76.2	9.5	14.3	52.4	19.0	28.6	23.8^{b}	28.6^{ab}	47.6	0.0^{c}	33.3^{ab}	66.7	0.0^{b}	33.3^{ab}	66.7
C	23	23	78.3	21.7	0.0	69.6	26.1	4.3	52.2^{ab}	39.1^{a}	13.0	21.7^{abc}	39.1^{a}	43.5	4.3^{ab}	39.1^{a}	60.9
D	25	21	95.2	4.8	0.0	81.0	9.5	9.5	66.7^{a}	14.3^{ab}	19.0	42.9^{ab}	19.0^{ab}	38.1	4.8^{ab}	19.0^{ab}	76.2
E	24	24	91.7	4.2	4.2	87.5	4.2	8.3	58.3^{ab}	8.3^{ab}	33.3	50.0^{a}	8.3^{ab}	41.7	29.2^{a}	8.3^{ab}	62.5
F	25	25	80.0	16.0	4.0	68.0	16.0	16.0	52.0^{ab}	16.0^{ab}	32.0	24.0^{ab}	20.0^{ab}	56.0	4.0^{ab}	20.0^{ab}	76.0
G	24	22	86.4	4.5	9.1	77.3	4.5	18.2	63.6^{a}	4.5^{b}	31.8	45.5^{a}	4.5^{b}	50.0	13.6^{ab}	4.5^{b}	81.8
Australian	171	164	83.5	11.6	4.9	72.0	14.6	13.4	52.4	19.5	28.7	27.4	21.3	51.8^{a}	7.9^{b}	21.3	71.3^{a}
Russian	25	23	69.6	13.0	17.4	60.9	13.0	26.1	52.2	17.4	30.4	52.2	17.4	30.4^{b}	43.5^{a}	21.7	34.8^{b}
VSH	29	28	64.3	21.4	14.3	60.7	25.0	14.3	50.0	28.6	21.4	42.9	32.1	25.0^{b}	28.6^{ab}	32.1	39.3^{b}
Italian	25	25	88.0	4.0	8.0	80.0	4.0	16.0	64.0	12.0	24.0	40.0	20.0	40.0^{ab}	12.0^{ab}	20.0	68.0^{ab}

Values with the same letter are not significantly different ($P > 0.05$).
VSH, varroa sensitive hygiene.

first determining queen survival. Dead colonies and colonies that had no brood suitable to use for queen rearing and with laying workers were considered dead. The presence of original queens was also determined. As the goal of the experiment was to compare the response of genetically different stocks to *V. destructor* infestation, infestation and colony size data were collected only from colonies that retained their original queen. The survival and courses of varroa and nosema infestations were not determined for colonies with supersedure queens.

For surviving colonies with original queens, samples of *ca.* 300–500 worker bees were collected from the surfaces of two brood frames, and estimates were made of the populations of bees according to procedures commonly used for grading colonies rented for almond pollination in California (Rinderer *et al.* 2011). Briefly, estimates are made from counts of the number of spaces between frames filled by bees from both the top and bottom sides of hive bodies. Bee population counts were only conducted in May, August, October and November. Bee samples were frozen on dry ice for transport to the laboratory and stored frozen (-20°C) until processing.

Determinations of varroa and nosema infestation

Fifty bees were removed from the samples for evaluations of *N. ceranae* infestation levels. The remaining bees were washed with soapy water to remove varroa mites (Rinderer *et al.* 2004b), then the mites and bees were counted to determine the percentage of adult bee infestation.

N. ceranae infestation was determined with a real-time quantitative PCR assay (Bourgeois *et al.* 2012). Briefly, 30 bees from each colony were dissected, and midguts were removed. Midgut tissue was pooled per colony and homogenised. Genomic DNA was extracted, then amplified with species-specific primers and probe targeted for *N. ceranae.* Detection and quantification were performed on a StepOne™ real-time PCR System (Applied Biosystems, Carlsbad, CA, USA). Protocols for FAST PCR and reagent specifications followed those described by Bourgeois *et al.* (2010). Modifications to the protocol were made by elimination of primers and probe reagents for *N. apis* and addition of distilled water to adjust the reaction volume to 12.5 μL. All other reagents and specifications remained the same. All samples were run in duplicate and were directly quantified by comparison with a standard curve of known levels of *N. ceranae* DNA copy number. Standards were generated from serial dilutions of a cloned PCR product of the target sequence from *N. ceranae* (Bourgeois *et al.* 2010). Data were converted from copy number to nosema spores per bee based on the formula defined in Bourgeois *et al.* (2010).

Data analyses

All analyses done for the four groups of honeybees (Australian, Italian, RHB and VSH) were also conducted for comparisons of the Australian lines. As very few Australian and Italian
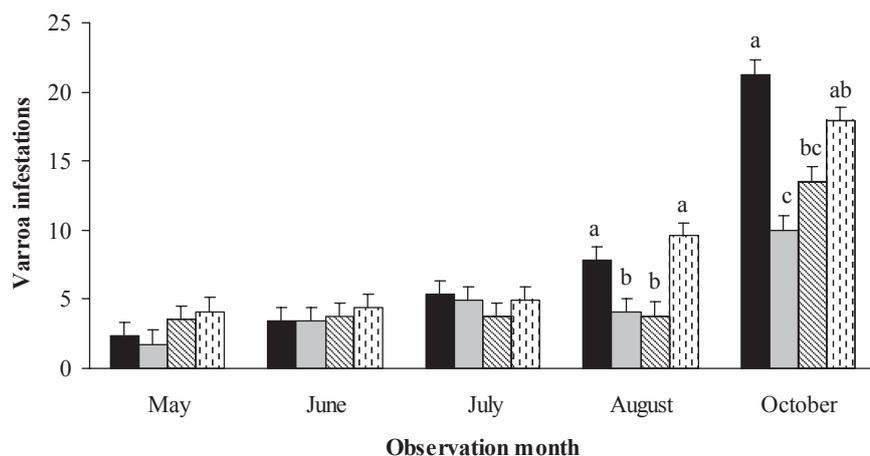
Fig. 1. Percentage (mean ± SE) of varroa mite infestations on adult bees of four stocks of honeybees. For each month of observation, bars with different letters are significantly different ($P < 0.05$). (■) Australian; (▨) Russian; (▨) VSH; (▨) Italian.

colonies were surviving in November, only analyses related to survival were conducted for November. SAS Institute (2008) was used for all analyses.

Varroa infestation

Prior to analysis, data on varroa infestation were transformed with an arcsine square-root transformation to more closely approximate normality. To control for variation among the apiaries, a randomised block design was used to determine the effect of stock and observation date on infestation levels. Mean separations were determined with post-hoc *t*-tests (SAS Institute 2008).

Queen survival, supersedure and colony survival

Queen longevity was calculated as the number of days a queen was known to be in the colony. Ten of the 250 queens (4%) did not survive introduction and were not included in this comparison. Dates of last observation of a queen were used as mortality dates, although all queens died sometime during the observation interval following this date. Prior to analysis, data on queen survival days were transformed with a square-root transformation to better approximate normality. Proc Lifetest was run to determine if there were differences in longevity among the stocks and lines (SAS Institute 2008). A randomised block design was then used to determine the nature of the differences. The proportions of original queen survival, supersedure rates and death/queenless rates were compared among the stocks for each observation date using the Marascuillo procedure (http://www.itl.nist.gov/div898/handbook/prc/section4/prc474.htm).

Colony sizes

Prior to analysis, data on colony size were transformed with a square-root transformation to more closely approximate normality. To control for variation among the apiaries, a randomised block design was used to determine the effect of stock and observation date on infestation levels. Mean separations were determined with post-hoc *t*-tests.

Nosema infestation

Prior to analysis, data on *N. ceranae* levels were transformed with a natural log transformation to more closely approximate normality. To control for variation among the apiaries, a randomised block design was used to determine the effect of stock and observation date on infestation levels. Mean separations were determined with post-hoc *t*-tests.

RESULTS

Varroa infestation

Numbers of *V. destructor* rose in all stocks (Australian, RHB, VSH and Italian) through time (Fig. 1). There was a significant interaction ($P < 0.0001$) between honeybee stock and date of observation. Hence, infestation, defined as adult mites per 100 adult worker bees, was evaluated for each observation. All stocks began with colonies having similarly low infestation on adult bees in May (overall average = $2.63 \pm 0.23\%$) ($F_{3,711} = 2.33$, $P = 0.073$). Infestation levels rose modestly and remained similar through June ($3.57 \pm 0.22\%$) and July ($5.10\% \pm 0.26\%$) ($F_{3,749} = 0.59$, $P = 0.623$ and $F_{3,769} = 1.37$, $P = 0.250$, respectively). August showed an increase in varroa mite infestations in Australian ($7.80\% \pm 0.54\%$) and Italian ($9.51\% \pm 1.36\%$) colonies that were significantly higher than the mite infestations in RHB ($4.09\% \pm 0.50\%$) and VSH ($3.79\% \pm 0.82\%$) colonies ($F_{3,800} = 7.06$, $P = 0.0001$). By October, overall, mite infestations had more than doubled, and the differences between stocks were consistent with August data: Australian ($21.28\% \pm 1.93\%$) and Italian ($17.90\% \pm 3.30\%$) colonies had significantly higher levels of infestation than the RHB ($10.01\% \pm 1.46\%$) colonies and Australian colonies had higher levels than the VSH ($13.57\% \pm 2.83\%$) colonies ($F_{3,800} = 7.06$, $P = 0.0001$).

The same general trends were found in comparisons of the seven Australian lines (Fig. 2). There was an interaction between lines and observation date ($P < 0.0001$). In May, infestations were low ($2.37\% \pm 0.24\%$), with some differences between the lines: lines G and E had the greatest infes-
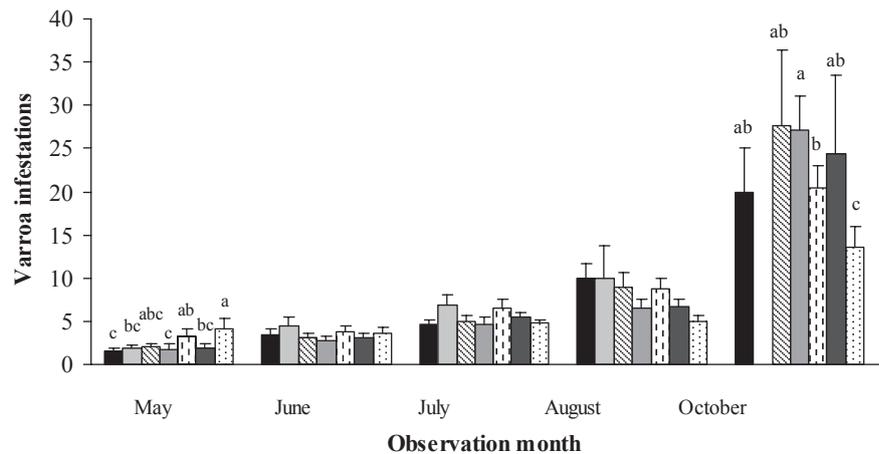
Fig. 2. Percentage (mean ± SE) of varroa mite infestations on adult bees of seven lines of Australian honeybees. For each month of observation, bars with different letters are significantly different ($P < 0.05$). (■) A; (□) B; (▨) C; (▨) D; (▥) E; (■) F; (▨) G.

tations, and lines A, B, D and F had the lowest ($F_{6,450} = 2.23$, $P = 0.039$). Overall, infestations rose but were similar in the seven lines in June (3.43% ± 0.25%), July (5.34% ± 0.30%) and August (7.80% ± 0.54%). Overall, by October, varroa infestation (21.52% ± 1.96%) had increased 3.2-fold. In October, differences among lines were observed: lines C, D and F had the greatest infestations, and line G had the lowest ($F_{5,520} = 5.56$, $P < 0.0001$). No colonies of line B survived until October.

Queen survival and supersedure

Lifetest results showed that the four groups (Australian, RHB, VSH and Italian) had insignificant differences in the average number of days queens survived ($\chi^2_3 = 7.24$, $P = 0.065$). Italian queens had the highest average survival of 95 ± 11 days, followed by RHB (94 ± 16 days), then VSH (83 ± 14 days) and lastly, Australian queens (80 ± 4 days). Nonetheless, these insignificant trends in survival days reflect the death of many colonies or the supersedure of original queens after the October inspection (Table 1). In November, survival of colonies with original queens was: RHB queens (43.5%) ≥ VSH queens (28.6%) ≥ Italian queens (12.0%) ≥ Australian queens (7.9%), with the difference in the survival of RHB and Australian queens being significant ($P = 0.05$). Interestingly, supersedure rates for the four groups were similar.

There were significant differences ($\chi^2_6 = 16.57$, $P = 0.011$) in the Lifetest results for queen survival among the Australian lines (Table 1). Line E had the longest survival (105 ± 12 days), followed in descending order by D (97 ± 11 days), G (96 ± 12 days), F (75 ± 11 days), C (74 ± 11 days) and A (67 ± 9 days). Queens of the B line lived the shortest period (47 ± 8 days). By October, all colonies of line B had died or superseded. Likewise, queens of lines A, C and F had poor survival compared with lines D, E and G (Table 1). By November, line E had significantly higher original queen survival rates (29%) than lines A (0%) and B (0%). Lines G (14%), D (5%), C (4%) and F (4%) were not significantly different. The supersedure rate did differ significantly ($P < 0.05$) among the lines with line C (39%) having a higher rate than line G (5%).

Colony survival

Colony deaths were similar for all four groups until October when varroa infestations were at high levels (Table 1). Between August and October, colony deaths in Australian and Italian lines nearly doubled. In October, death rates were: Australian colonies (51.8%) ≥ Italian colonies (40.0%) ≥ RHB colonies (30.4%) > VSH colonies (25.0%) ($P = 0.05$). Colony deaths continued through October and into November, especially for Australian and Italian colonies: Australian colonies (71.3%) ≥ Italian colonies (68%) > VSH colonies (39.3%) ≥ RHB colonies (34.8%) ($P = 0.05$).

Mortality in the colonies of the seven Australian lines was not significantly different. Line G had the highest death rate (82%), followed in descending order by D (76%), F (76%), A (75%), B (67%), E (63%) and C (61%). The supersedure level differed significantly ($P < 0.05$) among the lines with line C (39%) being higher than line G (5%).

Colony sizes

In the analysis of colony size data, there was an interaction between stock and observation date ($F_{9,303} = 2.11$, $P = 0.029$) (Fig. 3). In May, at the beginning of the experiment, all colonies were of similar size (10.79 ± 0.18 frames) ($F_{3,432} = 1.12$, $P = 0.342$), although RHB colonies were numerically the smallest. By August, the Australian colonies were significantly smaller (8.14 ± 0.34 frames) than the Italian colonies (9.84 ± 0.94 frames), while the RHB (9.71 ± 0.69 frames) and VSH colonies (9.29 ± 0.93 frames) were similar ($F_{3,465} = 2.67$, $P = 0.047$). In October, the RHB (8.96 ± 0.79 frames) and VSH colonies (8.13 ± 0.72 frames) were significantly larger than Australian colonies (6.74 ± 0.42 frames), and the Italian colonies (7.63 ± 1.22 frames) were similar to the other groups ($F_{3,468} = 3.34$, $P = 0.019$).

In the comparison of the seven Australian lines, there was no significant interaction between line and observation date ($F_{15,163} = 1.26$, $P = 0.234$). There was no difference in colony sizes between the lines ($F_{6,228} = 1.57$, $P = 0.158$), but there were differences between observation dates ($F_{3,168} = 44.86$, $P < 0.0001$). Colonies were largest in May (10.91 ± 0.23
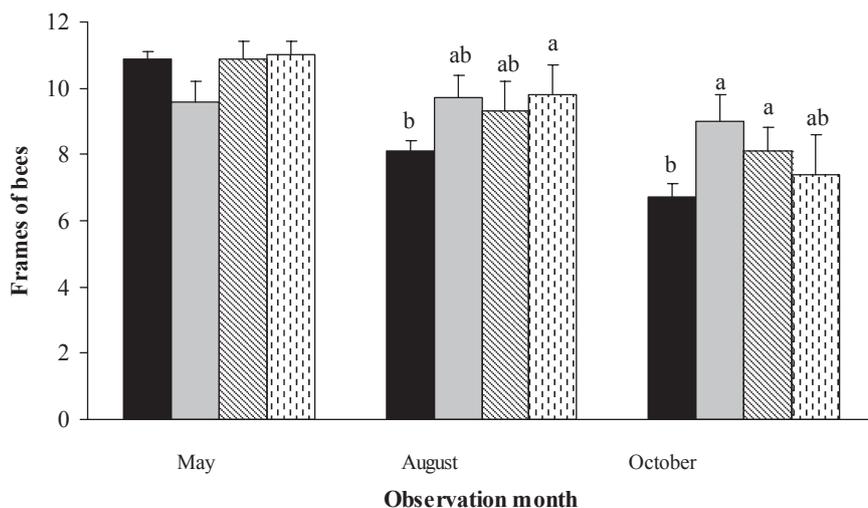
Fig. 3. Colony size (mean ± SE, in frames of bees) for four stocks of honeybees. For each month of observation, bars with different letters are significantly different ($P < 0.05$). (■) Australian; (□) Russian; (▨) VSH; (⊡) Italian.

frames), then decreased in August (8.14 ± 0.34 frames) and October (6.80 ± 0.43 frames). In November, they marginally decreased (6.18 ± 0.63 frames).

Nosema infestation

The analysis of *N. ceranae* infection also indicated a significant interaction between stock and observation date ($F_{15,616} = 2.53$, $P = 0.001$) ($F_{15,163} = 1.26$, $P = 0.234$). In May and June, all stocks had similar and high levels of *N. ceranae* (13.04 ± 0.17 ln spores; $F_{3,690} = 2.29$, $P = 0.078$ and 16.74 ± 0.14 ln spores; $F_{3,690} = 1.30$, $P = 0.275$, respectively), although infections in June were slightly higher than in May. In July, the VSH colonies had more *N. ceranae* (14.03 ± 0.33 ln spores/bee) than the Italian (11.91 ± 0.76 ln spores/bee), Australian (11.66 ± 0.31 ln spores/bee), and RHB colonies (11.63 ± 0.94 ln spores/bee) ($F_{3,690} = 3.36$, $P = 0.018$). In August, the stocks had similar rates of infection ($F_{3,691} = 1.15$, $P = 0.328$). In October, Italian colonies had more *N. cerane* (9.78 ± 1.60 ln spores/bee) than Australian (7.44 ± 0.56 ln spores/bee) and RHB colonies (6.06 ± 0.47 ln spores/bee), and VSH (9.84 ± 2.45 ln spores/bee) had more than RHB colonies ($F_{3,690} = 3.28$, $P = 0.021$).

In the analysis comparing the levels of *N. ceranae* infection among the Australian lines, lines and observation time once again interacted ($F_{26,400} = 2.14$, $P = 0.001$). In May, colonies of all lines have similarly high (12.83 ± 0.20 ln spores/bee) infection ($F_{6,454} = 0.72$, $P = 0.636$). Overall, infection rose in June but remained similar among lines ($F_{6,454} = 0.20$, $P = 0.98$). In July, overall infection declined and remained equal among the lines ($F_{6,454} = 1.80$, $P = 0.097$). In August, infections varied among the lines ($F_{6,454} = 2.94$, $P = 0.008$): colonies of line E were the most infected, followed by those of lines B, F, A, C and D, with colonies of line G having the lowest numbers of spores. In October, colonies of line A had significantly higher levels than colonies of lines C, D, E, F and G ($F_{5,454} = 2.37$, $P = 0.029$).

DISCUSSION

Overall, our results strongly suggest that the Australian lines and the Italian stock were similarly susceptible or intolerant to varroa and parasitic mite syndrome (PMS). Varroa infestations in the Australian lines and the Italian stock rose from rates below economic thresholds (Delaplane & Hood 1999) in August to very high levels (above 20%) by October. After August, 43.9% of the Australian colonies and 44% of the Italian colonies died. Varroa infestation was similar for Australian and Italian colonies in October. During this period, the colonies that were most heavily infested died, and the majority of the colonies that remained were so infested that they were dying and incapable of supporting additional mite population growth.

These observations contrast with the results from RHB and VSH colonies, which displayed comparative resistance to *V. destructor*. Their infestation levels in October were 10% (RHB) and 14% (VSH) that rose to about 18% for both stocks in November. Although these levels are substantially lower than those of the Australian and Italian colonies, they are unusually high for colonies that are resistant to varroa (de Guzman *et al.* 2007). During the August to November period, 4.4% of the RHB and 14.3% of the VSH colonies died. Doubtlessly, the high infestations and colony deaths experienced by the Australian and Italian colonies from October to November were conducive to producing apiaries that had heavy mite invasion pressure (Rinderer *et al.* 2004a). This invasion pressure probably accounted for much of the October to November increases in varroa numbers in the resistant stocks. Typically, RHB colonies in mixed apiaries with susceptible colonies have higher levels of infestation than RHB colonies that are not in mixed apiaries (Rinderer *et al.* 2004a).

Generally, the varroa infestation rates suggest that there were a few differences in resistance to varroa or tolerance to the viruses they transmit (Chen *et al.* 2005) between the Australian lines. Colonies with queens of lines A, C, D and F had both high levels of varroa infestation and high mortality, indicating a

strong susceptibility to varroa mites. While colonies of line B had low levels of varroa in August, by October, 66.7% had died, and 33.3% had superseded. These line B colonies died with lower levels of varroa. They may have been comparatively more susceptible to varroa, less tolerant of the viruses that are transmitted by varroa (Chen *et al*. 2005) or their survival was less owing to an unknown cause. Colonies of line G had a similar comparatively low number of varroa but had the lowest supersedure rate and the highest death rate. This line suffered from susceptibility to varroa and perhaps a heightened intolerance to transmitted viruses. Line E also supported a comparatively low population of varroa mites and had a reduced supersedure rate and somewhat lower death rate compared with most of the other lines. Of all the Australian lines, line E appears to be very marginally more resistant to varroa mites. However, none of the Australian lines displayed any commercially useful levels of resistance to *V. destructor*.

Conclusions concerning the relative susceptibility of the stocks to *V. destructor* infestations based on the numbers of infesting mites and colony survival are supported by colony size data. Although all stocks began the experiment in May with similar populations, by October, the Australian and Italian colonies were respectively significantly and numerically smaller. Colonies heavily infested with varroa mites typically will dwindle as they die.

All the stocks had high levels of *N. ceranae* infestation throughout most of the experiment. Although a few significant differences in infestation level were observed in July, nosema infestations were not unambiguously associated with differences in mortality. For example, Italian colonies had more varroa, more nosema and higher mortality than RHB colonies in November. However, although minor differences were noted in measurements of spores per bee, no stock or line showed any apparent resistance to nosema. Likewise, nosema infestation showed no apparent association with diminished colony health during the course of this experiment. A similar pattern was observed in *A. m. carnica* colonies in Switzerland (Dainat *et al*. 2012) in contrast with the results of Higes *et al*. (2009) in Spain. The chief causes of colony mortality in this experiment were *V. destructor* and, as evidenced by the widespread PMS, the viruses that it transmits.

It is widely thought that despite regulatory controls Australia will one day be invaded by *V. destructor*. Clearly, the current populations of *A. mellifera* in Australia are highly susceptible to *V. destructor*. Certainly, acaricides will be the first strategy to attempt to cope with *V. destructor* once it reaches Australia. However, the mites may be already resistant to at least some acaricides. It is highly desirable to develop and implement strategies to obtain or develop *A. mellifera* that is resistant to *V. destructor* for Australian apiculture prior to the parasite's arrival.

REFERENCES

Anderson D & Trueman JWH. 2000. *Varroa jacobsoni* (Acari: Varroidae) is more than one species. *Experimental and Applied Acarology* **24**, 165–189.

Boot WJ, Schoenmaker J, Calis JNM & Beetsma J. 1995. Invasion of *Varroa jacobsoni* into drone brood cells of the honey bee, *Apis mellifera*. *Apidologie* **26**, 109–118.

Bourgeois AL, Rinderer TE, Beaman LD & Danka RG. 2010. Genetic detection and quantification of *Nosema apis* and *N. ceranae* in the honey bee. *Journal of Invertebrate Pathology* **103**, 53–58.

Bourgeois L, Beaman L, Holloway B & Rinderer TE. 2012. External and internal detection of *Nosema ceranae* on honey bees using real-time PCR. *Journal of Invertebrate Pathology* **109**, 323–325.

Branco MR, Kidd NAC & Pickard RS. 1999. Development of *Varroa jacobsoni* in colonies of *Apis mellifera iberica* in a Mediterranean climate. *Apidologie* **30**, 491–503.

Calis JNM, Boot WJ & Beetsma J. 1999. Model evaluation of methods for *Varroa jacobsoni* mite control based on trapping of honey bee brood. *Apidologie* **30**, 197–207.

Chen YP, Higgins JA & Feldlaufer MF. 2005. Quantitative real-time reverse transcription-PCR analysis of deformed wing virus infection in the honeybee (*Apis mellifera* L.). *Applied and Environmental Microbiology* **71**, 436–441.

Dainat B, Evans JD, Chen YP, Gauthier L & Neumann P. 2012. Predictive markers of honey bee colony collapse. *PLoS ONE* **7**, e32151.

Delaplane KS & Hood WM. 1999. Economic threshold for *Varroa jacobsoni* Oud. in the southeastern USA. *Apidologie* **30**, 383–395.

Elzen PJ, Eischen FA, Baxter JR, Pettis J, Elzen GW & Wilson WT. 1998. Fluvalinate resistance in *Varroa jacobsoni* from several geographic locations. *American Bee Journal* **138**, 674–676.

de Guzman LI, Rinderer TE, Lancaster VA, Delatte GT & Stelzer JA. 1999. *Varroa* in the mating yard: III. The effects of formic acid gel formulation on drone production. *American Bee Journal* **139**, 304–307.

de Guzman LI, Rinderer TE & Frake AM. 2007. Growth of *Varroa destructor* (Acari: Varroidae) populations in Russian honey bee (Hymenoptera: Apidae) colonies. *Annals of the Entomological Society of America* **100**, 187–195.

Harris JW, Danka RG & Villa JD. 2009. Hygienic activity toward Varroa mites in capped brood is not dependent on mite reproductive status. *American Bee Journal* **149**, 587–588.

Higes MR, Martin-Hernandez E, Garrido-Ballon AV *et al*. 2009. Honey-bee colony collapse due to *Nosema ceranae* in professional apiaries. *Environmental Microbiology Reports* **1**, 101–113.

Koeniger N, Koeniger G & Delfinado-Baker M. 1983. Observations on mites of the Asian honeybee species (*Apis cerana*, *Apis dorsata*, *Apis florea*). *Apidologie* **14**, 197–204.

Lodesani M, Colombo M & Spreafico M. 1995. Ineffectiveness of Apistan treatment against the mite *Varroa jacobsoni* Oud. in several districts of Lombardy (Italy). *Apidologie* **26**, 67–72.

Oldroyd BP. 1999. Coevolution while you wait: *Varroa jacobsoni*, a new parasite of Western honeybees. *Trends in Ecology & Evolution* **14**, 312–315.

Peng Y, Fang Y, Xu S & Ge L. 1987. The resistance mechanism of the Asian honey bee, *Apis cerana* Fabr. to an ectoparasitic mite, *Varroa jacobsoni* Oudemans. *Journal of Invertebrate Pathology* **49**, 54–60.

Rath W. 1999. Co-adaptation of *Apis ceranae* Fabr. and *Varroa jacobsoni* Oud. *Apidologie* **30**, 97–110.

Rinderer TE, de Guzman LI, Delatte GT *et al.* 2001. Resistance to the parasitic mite *Varroa destructor* in honey bees from far-eastern Russia. *Apidologie* **32**, 381–394.

Rinderer TE, de Guzman LI & Harper C. 2004a. The effects of co-mingled Russian and Italian honey bee stocks and sunny or shaded apiaries on varroa mite population growth, worker bee population and honey production. *American Bee Journal* **144**, 481–485.

Rinderer TE, de Guzman LI & Sylvester HA. 2004b. Re-examination of the efficacy of a detergent solution for varroa mite detection. *American Bee Journal* **144**, 560–562.

Rinderer TE, De Guzman LI, Frake AM, Stelzer J, Bourgeois L, Wagnitz J. 2011. The effects of pollen-enriched protein food and the prevalence of *Nosema ceranae* on winter clusters of Russian honey bee colonies. *Science of Bee Culture* **3**, 9–10, supplement to *Bee Culture* **139**.

SAS Institute. 2008. *SAS User's Guide*, version 9.2. SAS Institute, Cary, North Carolina, USA.

Shimanuki H, Calderon NW & Knox D. 1994. Parasitic mite syndrome 1: The symptoms. *American Bee Journal* **134**, 827–828.