The Effects of Co-mingled Russian and Italian Honey Bee Stocks and Sunny or Shaded Apiaries on Varroa Mite Infestation Level, Worker Bee Population and Honey Production

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ABSTRACT

The effects of co-mingling colonies of commercial Russian stock with colonies of Italian stock, and the effects of direct sun exposure and shade on the growth of *Varroa destructor* populations, worker bee populations and honey production were compared. Colonies in six apiaries (treatment combinations) were studied: 1) a Russian apiary that received direct sunlight throughout the day, 2) a Russian apiary that received shade all day, 3) an Italian apiary that received direct sun until mid- to late afternoon, 4) an Italian apiary that received shade all day, 5) an apiary in which half of the colonies were Russian and half the colonies were Italian and that received direct sunlight until mid to late afternoon, and 6) an apiary in which half of the colonies were Russian and half were Italian and that received shade until mid to late afternoon. Russian colonies had substantially smaller *V. destructor* populations than the Italian colonies at the end of the experiment, ranging from 1/10th to 1/3rd depending upon the specific comparison of the two stocks in similar treatment combinations. Exposure to sunlight retarded mite population growth, while prolonged shade accelerated it, causing the death of many of the Italian colonies. The numbers of mites in Russian colonies kept in Russian apiaries were about one-third the numbers of mites in Russian colonies co-mingled with Italian colonies. Italian colonies had larger seasonal changes in honey bee population because of continued brood production beyond the end of the nectar flow. Overall, Russian colonies produced an average of 146 pounds of honey, while the Italian colonies produced an average of 126 pounds. Honey production in apiaries with both Russian and Italian colonies was consistent with this general difference.

INTRODUCTION

The severity of *Varroa destructor* (Anderson and Trueman 2000) parasitism depends upon several factors, which may simultaneously affect the growth of mite populations. Mite-resistant honey bees (*Apis mellifera*) such as the Russian honey bees (Rinderer *et al.* 1999, 2001 a, b) and those that possess the suppression of mite reproduction (SMR) trait (Harbo and Harris 1999) deter mite population growth. Climate is also thought to influence the reproductive success of varroa mites. The Mediterranean climate of California is considered to be favorable for an accelerated varroa mite population growth (Kraus and Page 1995). Temperate climates having prolonged cold winters are considered to be less favorable for varroa mite population growth (Calis *et al.* 1999). In addition, high ambient temperatures or the combination of high ambient temperatures and low relative humidity seem to limit varroa mite population growth (Harris *et al.* in press).

With the presence of parasitic mites in almost every colony in the U.S., managing colonies has been exceptionally difficult. Beekeepers rely on the use of chemicals to control these parasites, but acaricide application is costly and presents a myriad of problems. Thus, other methods of controlling varroa mites are highly desirable.

Honey bee stock that is resistant to varroa can be very helpful in controlling varroa. However, it is important to learn which management approaches will maximize the benefits of having resistant stock. Our general observations of Russian colonies in a variety of settings has led to the hypothesis that Russian colonies that are co-mingled in apiaries with colonies that are susceptible to varroa mites may have larger mite populations than Russian colonies that are not co-mingled with susceptible colonies. This hypothesis rests on the notion that susceptible colonies support the rapid development of large populations of varroa which are a source of mites which produce a strong invasion pressure on co-mingled colonies of Russian bees, taxing their resistance mechanisms. In comparison, an apiary comprised of only resistant honey bees is hypothesized to have smaller mite populations owing to a reduction in invasion pressure from nearby colonies.

Another alternative method to control varroa is the use of heat (Engels 1994). Heat has been used to control varroa mites in Russia with reported efficacies in the range of 85 to 90%. Heat (46-48°C) is applied individually to colonies during the broodless period for 12-15 min when outside temperatures range from 0-12°C (Akimov *et al.* 1988). Heat is also reported to reduce tracheal mite populations in the colonies. Harbo (1993) showed that dark hives exposed to direct sun (inside hive temperature exceeded 40°C) had fewer tracheal mites than white hives (temperature = 38°C). Thus, it is possible that keeping colonies in apiaries that provide direct exposure to sun may also prove helpful in lowering varroa mite numbers.

We evaluated both of these possible management tools on varroa mite infestation level, adult worker bee populations, and honey production. Comparisons of co-mingling or not co-mingling resistant and non-resistant stock, as well as comparisons of apiaries that were mostly in the sun or mostly in the shade were conducted.

MATERIALS AND METHODS

Commercially purchased Russian and Italian queens were installed

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in colonies equalized to similar size (about four deep frames of brood and six frames of bees) near Carencro, Louisiana in October 2001. The colonies were produced by either Russian queens, Russian hybrid queens or Italian queens with generally uncertain histories from commercial honey production apiaries. Queens were installed randomly to the various stocks of bees in the colonies. Candy tube release push-in cages were used for installation. The following spring these divisions were well-established Russian and Italian colonies in brood chambers of one deep and one medium hive body (using 10 combs in each) separated from honey supers with a queen excluder.

Russian queens for the experiment were purchased from a queen breeder in Louisiana. This queen breeder had produced Russian queens for three years and had Russian colonies as drone sources in his queen mating apiary. Hence, while the queens may have mated with some non-Russian drones, most of the drones were likely to have been Russian. Domestic Italian queens were obtained from a queen breeder in California who likely had mostly Italian drones in his queen mating apiary.

Colonies in six apiaries (treatment combinations) were studied: 1) a Russian apiary that received direct sunlight throughout the day (RSN, n = 32), 2) a Russian apiary that received shade all day (RSH, n = 32), 3) an Italian apiary that received direct sun until mid to late afternoon (ISN, n = 32), 4) an Italian apiary that received shade all day (ISH, n = 32), 5) an apiary in which half of the colonies were Russian and half were Italian and that received direct sun until mid to late afternoon (RISN, n = 32, 16 for each stock), and 6) an apiary in which half of the colonies were Russian and half were Italian and that received shade until mid to late afternoon (RISH, n = 32, 16 for each stock). No attempts were made to measure temperature or humidity differences among the apiaries. The colonies were set on 4-way pallets; each pallet in the mixed groups (RISN and RISH) had only one type of queen. All colonies were treated with coumaphos in order to strongly reduce varroa populations in all of the colonies; coumaphos-resistant mites have not yet been found in Louisiana. In May 2002, all colonies that had supersedure queens (25 Russian and 26 Italian colonies) were excluded from the experiment (Table 1). Through the winter and early spring, all colonies were fed with corn syrup as needed. Later in the year colonies were managed for honey production with medium-depth honey supers added to colonies prior to the onset of the main Chinese tallow tree (*Triadica sebifera*) nectar flow and again during the flow as the initial supers became nearly filled with honey and incoming nectar. Honey production for each colony was determined in mid-July shortly after the end of the Chinese tallow tree nectar flow. Although other plant species provide resources for colony development earlier in the spring, Chinese tallow is the almost exclusive source of surplus honey in the area of this experiment. Chinese tallow is strongly influenced by photoperiod with the nectar production period being from mid-May to the first week of July, just prior to our determinations of the honey produced by the colonies. All apiaries were in locations where honey production from Chinese tallow historically has been excellent. Surplus honey was determined for each colony by weighing honey supers in the field using a portable electronic scale. Net weights were calculated by subtracting the average weight of empty equipment, which was estimated by weighing 20 supers with empty frames and calculating the average weight.

Varroa mite infestations for each colony were estimated by counting adult female mites in 200 worker brood cells (50 from each side of two brood frames). Also, the percentage of adult bee infestation was estimated for each colony by collecting 400 to 600 bees from the broodnest, separating the mites from the bees by washing them in soapy water, and counting both bees and mites. Initial infestations of varroa in all colonies were determined in May 2002. Subsequent estimations of varroa mite infestations were made in July and October 2002. Sealed brood production and adult bee population were estimated by summing occupied portions of comb and using standardized units of bees and brood per portions of comb described by Burgett and Burikam (1985). The occurrence of drone brood and hence drone brood infestation rates was insufficient to support a meaningful analysis.

Data on mite numbers expressed as percentage of brood infestation and percentage of adult worker bee infestation, honey production, worker brood and adult bee populations were analyzed using ANOVA as a factorial experiment in a Completely Randomized Design. For each apiary (= treatment), a split-plot was employed where the main unit was honey bee type, colony was replication and sampling month was the subunit. Honey bee type, treatment type, and sampling month were modeled as fixed effects using Proc Mixed. Colony within type and treatment were modeled as random effects. Degrees of freedom were estimated using the Kenward-Roger method (SAS Institute 1997). Before analyses, data on worker brood infestation and number of mites in the colonies were transformed using square root transformation.

RESULTS

The analysis of the infestation of brood showed a significant interaction between colony type, treatment and sampling month ($P < 0.05$). In May, all colonies had similarly low levels of infestation regardless of bee type or treatment (Fig. 1). A slight increase in infestation was observed in July, especially in the RISN and RISH apiaries. In October, one year after the colonies were established, a significant increase in infestation was observed in most colonies.

The October increase in mite infestation was significantly larger in Italian colonies (Fig. 1). This was especially true of Italian colonies kept alone under the shade (ISH) or kept together with the

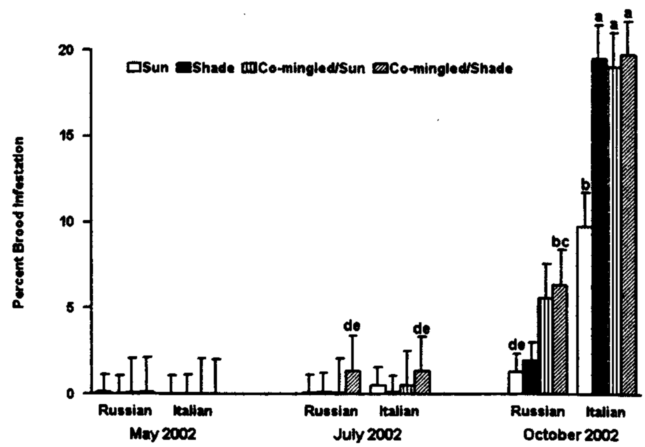

Fig. 1. The average percentage of brood infestation for three months in Russian and Italian colonies kept as non- co-mingled colonies or as co-mingled colonies in either the sun or the shade.

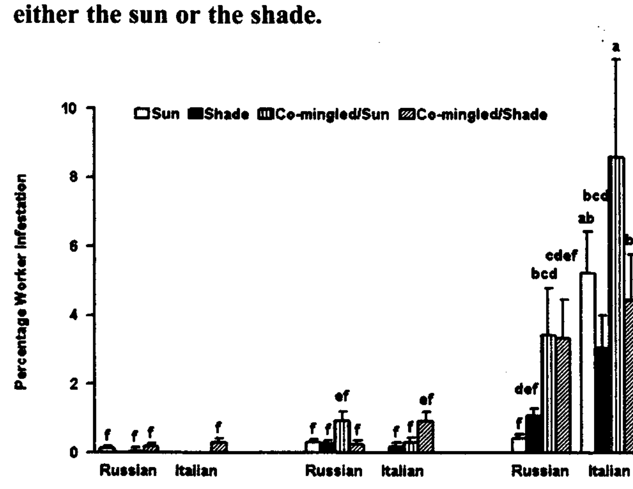

Fig. 2. The average percentage of adult infestation for three months in Russian and Italian colonies kept as non- co-mingled colonies or as co-mingled colonies in either the sun or the shade.

Russian colonies (RISN and RISH). All three of these groups of Italian colonies had Varroa mite infestations averaging about 20% in October (Fig. 1). All Russian colonies, regardless of treatment, had about 3x fewer mites (P<0.0001) than Italian colonies with the highest level of about 6% observed in October in Russian colonies that were co-mingled with Italian colonies.

Although the magnitude of the differences in the analysis of the percentage of worker bee infestation were less than they were for the analysis of the percentage of brood infestation, the general trends indicated by the percentage of brood infestation were reflected in the analysis of the percentages of adult worker bee infestations (Fig. 2). Again, a significant interaction between colony type, treatment and sampling month ($P < 0.05$) occurred. In May, all colonies had similarly low levels of infestation regardless of bee type or treatment (Fig. 2). A slight increase in infestation was observed in July. In October, one year after the colonies were established, a significant increase in infestation was observed in most colonies. Italian colonies had nearly twice the infestation rate of the Russian colonies (P=0.05). Russian colonies in co-mingled apiaries had about three times the mite infestation when compared to the Russian colonies that were not co-mingled (P=0.001).

Exposure of colonies to direct sunlight had a tendency to reduce varroa mite infestations for the percentage of brood infested. Mite infestations increased through time at different rates as a result of an interaction between treatment and sampling month ($P < 0.0001$). The major trends which began to develop in July were clear in October. Colonies in the Italian apiary in the sun (ISN) had smaller infestations (P < 0.05) than colonies in the shaded Italian apiary (Fig. 1). Indeed, by October the Italian colonies in the shaded apiary (ISH) were exhibiting strong levels of Parasitic Mite Syndrome (PMS) (Shimanuki et al. 1994) with many of them already dead or dying from PMS (Table 1). This general trend of smaller infestations in colonies in the sun was also seen in the comparison of Russian colonies in the sunny apiary (RSN) with Russian colonies in the shade (RSH) (Fig. 1) although the contrast is much weaker. Similarly, Russian and Italian colonies in the sunny co-mingled apiary (RISN) averaged numerically fewer mites than their counterparts in the shady co-mingled apiary (RISH) (Fig. 2). In the analysis of the percentage of infested adults, non-co-mingled Russian colonies in the sun numerically (P=0.10) had a lower infestation than similar colonies in the shade. The effects of sun were not evident in the Italian colonies for the percentages of worker bees having mites (Fig. 2).

Co-mingling Russian colonies with Italian colonies resulted in a strong increase (about 3x) in the percentage of mites in brood in Russian colonies (P < 0.05) in October (Fig. 1) and also a strong increase (about 4x) in the percentage of infested Russian worker bees (P=0.05) (Fig. 2). Italian colonies in co-mingled apiaries had high mite infestations in October and hence did not appear to benefit from being co-mingled with Russian colonies (Fig. 1 and 2).

Table 1. Number of Russian and Italian colonies surviving with original queens. (Number of colonies established in October 2001).

Date	Treatment	Russian	Italian
May 2002	Shade	27 (32)	21 (32)
	Sun	24 (32)	22 (32)
	Co-mingled in Shade	10 (16)	14 (16)
	Co-mingled in Sun	10 (16)	13 (16)
October 2002	Shade	22	9*
	Sun	21	18
	Co-mingled in Shade	9	12
	Co-mingled in Sun	9	13

*Surviving colonies were weak and exhibited PMS symptoms in brood and adults. Dead colonies had brood frames showing PMS.

Analysis of the number of sealed brood indicated a significant (P = 0.02) interaction between bee type and sampling month. Both Russian and Italian colonies had similar amounts of brood in May and October (Fig. 3). However, the Italian colonies produced more brood than the Russian colonies in July. A significant (P = 0.003) interaction between treatment and sampling month was also observed (Fig. 4). An examination of Fig. 4 suggests that the clearest trend in this interaction is that, in October, colonies in the shade tended to produce less brood. However, since many shaded Italian colonies were suffering severe PMS in October and thereby had less brood, the effect of shade *per se* on brood production may be minimal.

Similar trends were observed with adult bee populations. Adult bee populations were significantly ($P < 0.03$) affected by the inter-

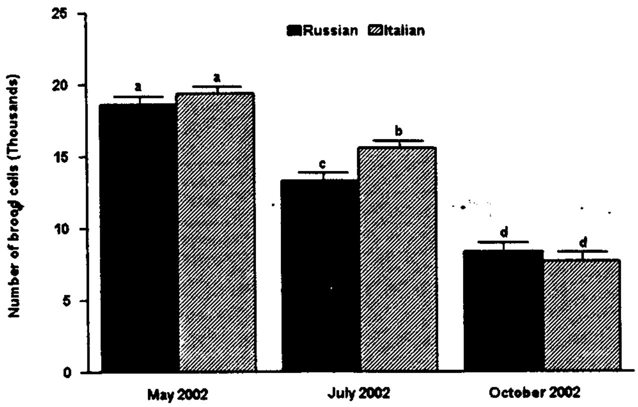

Fig. 3. The average number of sealed worker brood cells for three months for Russian and Italian colonies in all apiaries.

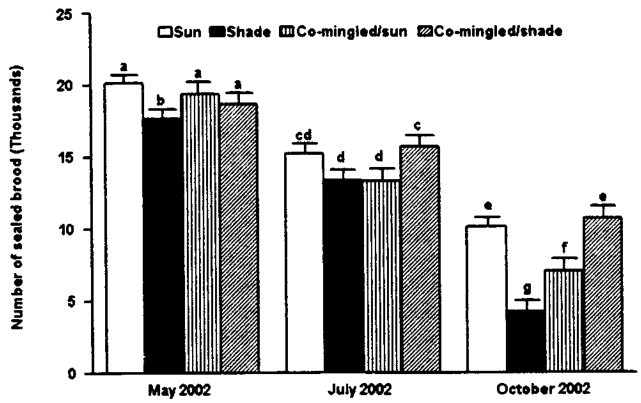

Fig. 4. The average number of sealed worker brood cells for three months for all colonies in apiaries that were in the sun, the shade, co-mingled in the sun, and co-mingled in the shade.

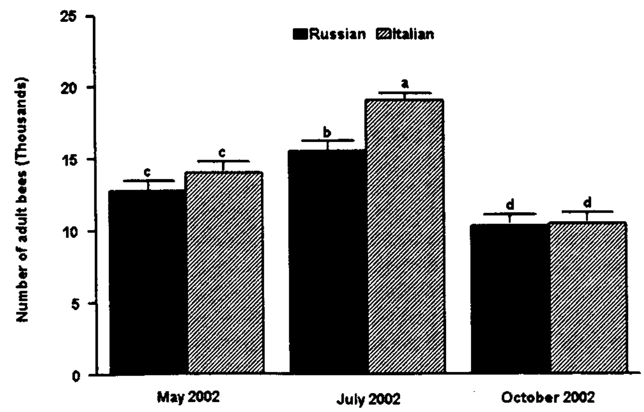

Fig. 5. The average number of adult bees for three months for Russian and Italian colonies in all apiaries.

action of bee type and sampling month. The peak adult bee population was in July with Italian colonies having significantly ($P < 0.05$) more adult bees than the Russian colonies (Fig. 5). In both stocks, adult bee populations declined to similar levels by October.

The Russian colonies produced significantly ($P < 0.04$) more honey than the Italian colonies with an average of 146 and 126 lbs, respectively (Table 2). Of the Russian colonies, 80% (57 colonies) produced 101-200 lbs of honey and 7% (5 colonies) produced 51-100 lbs. Of the Italian colonies, 56% (40 colonies) produced 101-200 lbs. and about 21% (15 colonies) produced 51-100 lbs. The best Russian colony produced 246 lbs, while the best Italian colony produced 10 lbs less (238 lbs). Honey production by Russian colonies was consistently higher regardless of apiary treatment (sun vs. shade). No significant ($P = 0.73$) interaction between stock type and treatment was observed.

Table 2. Number of colonies showing the amount of honey produced in Russian and Italian colonies and overall average production.

Honey Produced	Honey Bee Stock	
	Russian	Italian
0 - 50 lbs	4	8
51 - 100 lbs	5	15
101 - 200 lbs	57	40
> 200 lbs	5	6
Total number of colonies	71	70
Average honey production*	146 lbs	126 lbs

* Significantly different, $P < 0.05$

DISCUSSION

Growth of mite populations infesting honey bee colonies is influenced by a variety of factors. In this study, it is clear that the genetics of the host bees played the most important role in the regulation of the varroa mite populations. Regardless of the treatment, Russian colonies maintained low varroa mite infestations. This observation, made on commercially available Russian honey bees, corroborates our earlier reports on research stocks of pure Russian honey bees (Rinderer *et al.* 1999, 2001 a, b).

The growth of varroa populations in the Italian colonies was accelerated by prolonged (all-day) shade. Nearly half of the Italian colonies in the shaded apiary (ISH) died primarily due to varroa as indicated by the presence of dead brood showing typical PMS. Serious varroa parasitism in the surviving shaded Italian colonies was indicated by the full expression of PMS: a high proportion of infested brood, numerous diseased and starved larvae, the presence of numerous adult bees with damaged wings, a small amount of very scattered brood (mean = 2,579 ± 1373 brood cells) and small populations of adult bees (6,436 ±1600 bees). However, Italian colonies in the RISH apiary were not as severely affected by varroa infestations as those in the ISH apiary. Although the Russian colonies in this apiary had more mites as a consequence of being in an apiary with highly infested Italian stock, the presence of resistant Russian colonies may have reduced the overall pressure of mite invasion in the apiary, relieving the co-mingled Italian colonies from some mite invasion pressure. However, regardless of these potential interactions, our results suggest that apiaries receiving all-day shade should be avoided where possible, especially when keeping susceptible stocks of honey bees.

This study also re-enforces the idea that drifting and robbing honey bees from highly infested colonies spread mites to other colonies, perhaps causing their re-infestation after treatment earlier in the season and increasing their infestation levels later in the season. The arrangement of colonies within an apiary may reduce the drifting of worker bees (Jay, 1969) and the spread of mites by drifting workers or drones. However, mites are also spread by honey bees robbing colonies that have been weakened by varroa. Also, many apiary sites are not amenable to colony arrangements that will strongly reduce drifting and the common practice of using pallets may encourage drifting. Thus, it is important to keep colonies with resistant stock in apiaries that are separated from colonies with susceptible stock. Heavy mite invasion pressure from highly infested colonies will cause even resistant stock to develop larger populations of mites than they would if they were in apiaries having only resistant stock.

These general trends regarding levels of mite infestation are found in both the analysis of the percentage of infested brood and the percentage of infested adults. The strength of differences in the analysis of the percentage of infested adults tends to be less in various comparisons. This in part may be a consequence of Russian honey bees having a higher percentage of the total colony mites on adults rather than in brood (Rinderer *et al.* 2001a), a tendency that dampens the differences between Russian and Italian colonies. Also, in this experiment the variation in the percentage of brood infestation in Italian colonies was less than it was for the variation in the percentage of infested worker bees. It may be that studies of the infestation of honey bee colonies by varroa may generally benefit from including both infestation rates of brood and infestation rates of adults, although both result in similar conclusions in this study.

Comparisons of brood production and adult bee populations in this experiment with commercially available Russian honey bees are similar to results in studies with research stocks of pure Russian honey bees (de Guzman *et al.* 2002, Rinderer *et al.* 2001a). The Russian colonies built strong populations equal to those of the Italian colonies when pollen and nectar were plentiful before and during the main nectar flow as indicated by both the brood and worker bee data from May. After the beginning of the summer dearth, brood production and adult bee numbers in the Russian colonies became comparatively less as indicated by the data from July. In October, pollen and nectar were once again available and the brood and adult bee populations in Russian colonies once again equaled those of the Italian colonies. This comparatively greater responsiveness to nectar and pollen flows is a trait of Russian honey bees that results in smaller food requirements in many circumstances (Tubbs *et al.* 2003).

Likewise, honey production was strongly affected by the honey bee stocks used in the experiment. With a strong nectar flow producing an overall good crop, the full- size over-wintered colonies of Russian honey bees in this experiment produced more honey than the comparable Italian colonies. The apiary conditions (sunny or shady; stock co-mingled or not co-mingled) did not influence the honey production of either the Russian or the Italian colonies. Also, the comparatively lower honey production of the Italian colonies cannot be attributed to them having a higher number of varroa mites. The crop was harvested prior to the collection of mite infestation data in July when the overall number of mites in the experimental colonies was still quite low. The Italian honey bee stock is highly regarded owing to its excellent honey production. The difference in honey production may have resulted in the Italian colonies collecting less nectar in support of honey production, from differential honey consumption since the Italian colonies produced large amounts of brood throughout the nectar flow, while the Russian colonies reduced brood production as the flow lessened, or both.

It is interesting that between October 2001 and May 2002 both the Russian and Italian queens displayed a supersedure rate of about 30%. Because of the timing of the queen introductions, no back checking or cell cutting was done after queen introduction. Hence, we do not know if the supersedure rate resulted from introduction issues or colony events that occurred during winter or spring. Also, since commercial beekeeping generally uses unmarked queens and in many cases does not include back checks after introduction, it is not clear whether or not this supersedure rate is unusual for re-queening without back checking until 5 months later.

CONCLUSIONS

Commercially available Russian colonies are resistant to varroa mite infestations as indicated by their low mite numbers. Infestation rates of Varroa mites trended higher in Russian colonies and lower in Italian colonies when the two stocks were co-mingled, especially in the shade. To optimize the value of resistant stock, we recommend that resistant stock be kept in apiaries not having susceptible stock. Exposure to direct sun tends to impede mite growth and shade tends to accelerate mite growth. However, while sunny apiaries are ideal, the benefits may not override other considerations regarding apiary placement, such as, for example, ease of access or landowner preference.

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