An Evaluation of ARS Russian Honey Bees in Combination with Other Methods for the Control of Varroa Mites

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Abstract

The effects of several management tools for the control of *Varroa destructor* were evaluated. Both domestic and Russian honey bee (ARS Primorsky) colonies were provided with: 1) a single application of formic acid in gel formulation in hives with solid bottom boards, 2) a single application of formic acid in gel formulation in hives with screened bottom boards, 3) no chemical treatment to hives with screened bottom boards, or 4) no chemical treatment to hives with solid bottom boards. Overall, Russian honey bees had about half the number of *V. destructor* found in domestic colonies at the end of the experiments. Formic acid reduced the numbers of mites in colonies regardless of the type of bottom board. Screened bottom boards did not reduce the number of mites in colonies. The combination that resulted in the fewest number of mites was Russian colonies in hives that were treated with formic acid.

Introduction

Honey bee parasitic mites are potentially devastating to honey bee colonies and most beekeeper pest management expenses and activities are directed to controlling them. Since it is more noticeable, both directly and through its extreme effects on a honey bee colony, the external parasitic mite *Varroa destructor* probably receives more attention from most beekeepers (de Jong *et al.* 1982, Engels and Schatton 1986) than tracheal mites or other disease problems.

For the most part, beekeepers rely on the application of costly acaricides to control mite pests. The time required to apply and then remove acaricides, up to three times a year for some beekeepers for *V. destructor* control, reduces the numbers of colonies that can be effectively maintained. Despite these applications, colony and honey production losses occur, further increasing the overall economic costs caused by this mite. Unfortunately, *V. destructor* has proved its potential to develop resistance to acaricides (Lodesani *et al.*, 1995, Elzen *et al.*, 1998, 1999), creating the need for the constant development of new acaricides that are difficult to find and register for legal use.

Formic acid, provided in a gel formulation, is registered and provides good control of tracheal mites and as much as 70% control of *V. destructor* (Feldlaufer *et al.*, 1997). However, formic acid has drawbacks. Its use reduces drone production and adult drone survival (de Guzman *et al.* 1999). In vapor form, formic acid can be explosive. Also, it is highly caustic so strict safety procedures are required to prevent injury to beekeepers.

Screened bottom boards provide a partial control of *V. destructor* by retarding their population development (Pettis & Shimanuki, 1999). Living mites fall from colonies through the screen, preventing their return to the brood nest which occurs in hives with a solid bottom board.

Efforts to breed *A. woodi* resistant stocks have met with substantial success (Gary *et al.*, 1990, Milne *et al.*, 1991, Rinderer *et al.*, 1993, Danka *et al.*, 1995, Williams *et al.*, 1994, Lin *et al.*, 1996, de Guzman *et al.*, 1998 a,b). Several *A. woodi* resistant stocks are now commercially available to the beekeeping industry. Efforts to breed *V. destructor* resistant stocks have been generally less successful. However, a line of bees that possesses the suppression of mite reproduction (SMR) has been developed (Harbo & Harris, 1999, Harbo & Hoopingarner, 1997). The SMR line is a valuable breeding tool since it is a source of resistance for commercial breeding programs. For this reason, the line has been released to the industry. Currently, honey bee breeders are making efforts to incorporate the trait into their own stocks and several of them are marketing their results. Also, a stock of bees has been found and improved through selective breeding which is resistant to both *V. destructor* and *A. woodi* (Rinderer *et al.*, 1997, 1999, 2001a,b, de Guzman *et al.*, 2001a,b). This stock, known as Russian or ARS Primorsky honey bees, has also been released to the industry through a breeder-queen program (Rinderer *et al.*, 2000). Several queen breeders are producing queens for sale that are nearly "pure" Russian stock or are hybrids.

The growing availability of resistant stocks now makes it possible to develop an Integrated Pest Management (IPM) Plan with resistant stocks as the centerpiece. It may be possible to develop recommendations for managing the parasitic mite populations in resistant colonies with no or only a very occasional use of acaricides. This experiment is part of a larger project of the USDA-ARS Honey Bee Breeding, Genetics and Physiology Laboratory in Baton Rouge, Louisiana, to reach the goal of providing recommendations for the effective, low cost, long-term control of the major pests and diseases in honey bee colonies, especially the parasitic mites. While resistant stock is helpful in reaching this goal, learning and developing the best ways to manage resistant stocks may prove equally helpful. This experiment explored the effects of Russian stock in combination with screened bottom boards and formic acid on the development of *V. destructor* populations. A companion report (de Guzman *et al.*, 2001a) describes the response to these treatments by *A. woodi*.

Materials and Methods

Domestic honey bee colonies in Carencro, Louisiana, were divided into five-frame nucleus colonies in April 2000. ARS Russian queens (naturally mated on a coastal Louisiana island to ensure that matings were exclusively with Russian drones) and

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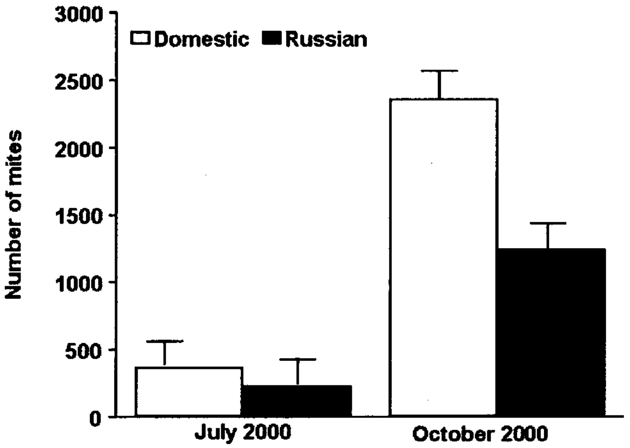

Figure 1. Numbers of adult *Varroa destructor* in Russian and domestic colonies in July and October. Error bars indicate standard errors.

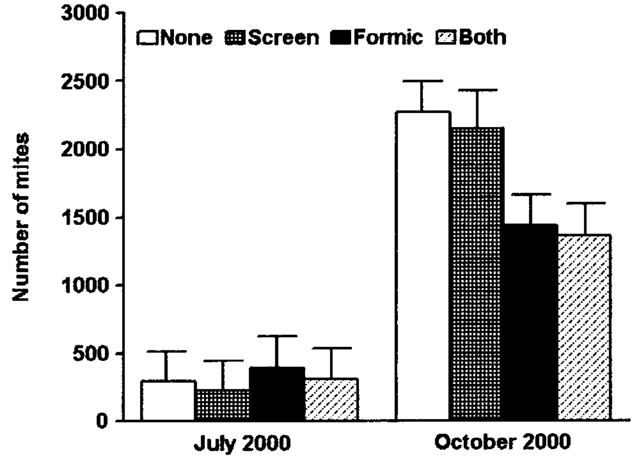

Figure 2. Overall average number of mites in all colonies treated with screened bottom boards, formic acid, screened bottom boards and formic acid or no treatment as a control in July and October. Error bars indicate standard errors.

domestic queens (purchased from a commercial queen breeder) were randomly assigned and installed into the nuclei. The nucleus colonies were placed in a holding yard for two weeks until queens were accepted. The colonies were then moved into standard Langstroth hives in May 2000. At that time, modified screened bottom boards with attached 8-mesh hardware cloth screens similar to those described by Pettis and Shimanuki (1999) were installed for test groups designated as the screened group (n = 12) for each stock, and the screened plus formic acid group (n=12) for each stock. A total of 96 colonies were then relocated to form three apiaries of 32 colonies each. The colonies were set on 4-way pallets with randomized assignment of treatments to pallets. In order to reduce the effects of drifting bees, each pallet had only one type of queen and one type of treatment. Infestations of *V. destructor* in all colonies were determined in July 2000 prior to treatment with formic acid. (July was chosen as a start time for the experiment in order to allow colonies to have a complete population turnover.) The formic acid and the screened plus formic acid treatment groups received a treatment of one pack of Beltsville formic acid gel (200g of 65% formic acid) on July 26, 2000 which was removed 11 weeks later. *V. destructor* populations were again determined in October 2000. The timing of the formic acid treatment was determined according to beekeeper need rather than by optimum temperature conditions. Non-resistant colonies in south Louisiana must be treated in February and March prior to the nectar flow and again in July after the main nectar flow. A July treatment prevents colonies from having damaging infestations of *V. destructor* in late September and October during a period that may have a substantial nectar flow.

V. destructor populations were estimated for each colony using a comprehensive suite of measurements. These estimates were derived from: (a) counts of mites in 200 worker brood cells (50 from each side of two combs), (b) counts of mites in 100 drone brood cells (50 from each side of one comb or from more than two nest areas when drone brood was available), (c) estimates of mites per 100 adult worker bees derived from counts of mites removed from 300 to 600 adult worker bees by the bees being washed in ethanol, (d) comb by comb estimates (to the nearest 5%) of the numbers of sealed and potentially infested worker and drone brood cells in the hive, and (e) comb by comb estimates of the number of adult bees (to the nearest 5%) comprising the colonies. Both mother mites and their nearly mature daughters were included in the estimates. They were individually recognized according to the methods of Ifantidis (1983).

Data on mite numbers, worker brood populations and adult bee populations were analyzed using ANOVA of a factorial experiment in a completely randomized design. In each site, a split plot was used with the main unit being honey bee type, replication being colony and sampling date as the subunit. Honey bee type, treatment type, and sampling date were modeled as fixed effects using SAS proc mixed. Colony within type and apiary site were modeled as random effects. Degrees of freedom were estimated using the Kenward-Roger method (SAS Institute, 1997).

Results

Mite numbers in colonies increased between July and October at different rates in the two stocks (Fig. 1) as indicated by a significant month-by-stock type interaction (P = 0.02). Overall, domestic colonies supported a larger increase in mite populations. They averaged 374 mites in July and 2362 mites in October. In contrast, Russian colonies saw less mite population growth, going from an average of 232 mites in July to an average of 1244 mites in October, or about half the average number of mites observed in domestic colonies.

Treatments of screened bottom boards, formic acid, and screened bottom boards plus formic acid had similar effects for the two stocks as indicated by a non-significant stock type by treatment interaction (P = 0.35). Hence, the overall results were pooled across stocks to evaluate treatment effects (Fig. 2). No treatment resulted in a 7.8 fold increase in mite numbers in colonies of both stocks from an average of 290 mites in July to an average of 2267 mites in October. Screened bottom boards alone resulted in a 9.5 fold increase in mite numbers from 224 mites to 2149 mites. This is a numerical but not a significant increase when compared to the increase in the no treatment control group. Formic acid treatment resulted in a significantly less (P= 0.001) 5.4 fold increase from 392 mites to 1432 mites. Screened bottom boards plus formic acid resulted in a significantly less (P= 0.001) 4.4 fold increase in mites from 307 mites to 1364. While the screened bottom board itself did not result in reduced mite populations, the formic acid treatment (P = 0.001) and the screened bottom board plus formic acid combination (P = 0.001) did reduce mite populations.

Analysis of the amount of sealed brood numbers indicated that the two stocks had sealed brood that varied differently between the months (P =0.01). Domestic colonies averaged 3.7 frames of sealed brood in July and 1.8 frames in October. Russian colonies averaged 2.9 frames of sealed brood in July and 2.5 frames in October. Generally, the domestic colonies reduced their number of frames of brood by half from July to October, while Russian colonies reduced their number of frames of brood only slightly.

Analysis of the numbers of adult bees in colonies was similar to the analysis for sealed brood. Colonies of the two stocks had adult populations that varied differently between the months (P = 0.05). Domestic colonies averaged 8.4 frames of adult bees in July and 7.5 frames in October. Russian colonies averaged 6.4 frames of adult bees in July and 6.8 frames in October. The domestic colonies reduced their adult population from July to October, while the Russian colonies increased their adult population.

Treatments had no detectable effect on either the amount of brood or the size of adult bee populations. The month-by-stock-type-by-treatment interaction was non-significant for both brood ($P = 0.82$) and adult bee numbers ($P = 0.13$).

Discussion

Clearly, a core ingredient in the development of IPM recommendations for mite control is the use of resistant stock. Overall, the largest effect on mite populations was brought about by stock type. Russian colonies, as they have in previous experiments (Rinderer *et al.*, 1999, 2001a,b), reduced the growth of mite populations. Regardless of treatment, the numbers of mites in domestic colonies approached levels that require the use of acaricides.

The treatment combination which resulted in the lowest mite population growth was use of Russian colonies given a formic acid treatment. This is interesting for two reasons. First, it seems that the screened bottom board did not interfere with the effectiveness of the formic acid. This result is counterintuitive. Since formic acid vapors are heavier than air, it would seem that the formic acid would not be present in the colonies for long enough to be as effective as formic acid given to colonies with solid bottom boards. The data indicate that this intuition is wrong. The presence of a screened bottom board did not impair the effectiveness of formic acid to a measurable degree. Second, although the screened bottom board alone resulted in the highest number of *V. destructor* of all the treatment groups with the Russian colonies, the combination of screened bottom board and formic acid resulted in the numerically lowest number of *V. destructor*. We also saw similar effects for *A. woodi* (de Guzman *et al.*, 2001a). Since Russian colonies have a greater proportion of their mites on adults, (Rinderer *et al.*, 2001a) the formic acid treatment aided by the loss of mites through the screened bottom board may have been enhanced slightly in Russian colonies.

Experiences with the use of screened bottom boards proved illuminating beyond the observations regarding mite control. The screened bottom boards were on warehouse pallets that had large openings under the screens which permitted light and air to enter the colonies. For both domestic and Russian colonies, the screens remained entirely free of propolis. No debris remained on the bottom boards: the bees kept them very clean. Small clusters of bees formed under the screens. These bees stayed with the colonies when they were moved with a forklift. The clusters of bees built typical bottom board burr comb under the screen on boards that formed the pallet.

Many other methods to control *V. destructor* have been suggested. While they may not provide sufficient mite control when used alone, they may combine very well with other methods. Many more combinations of *V. destructor* control methods, some of which involve using slightly different beekeeping practices such as using screened bottom boards should be studied. A few methods in combination may provide enough control that monitoring mite population levels or periodically using acaricides may become unnecessary. To be most useful, such research should also consider the effects of candidate control procedures on other pests and diseases of honey bees, as well as considering the effects on fundamental beekeeping concerns such as honey production.

Conclusions

1. The resistance of Russian honey bees to *V. destructor* strongly reduces mite populations. Use of this or another resistant stock is central to an IPM approach to *V. destructor* control.

2. Formic acid reduced mite populations.

3. Screened bottom boards did not interfere with the effectiveness of formic acid treatments.

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References

Cox, R. L., Moffet, J. O., Wilson, W. T., & Ellis, M. 1989. Effects of late spring and summer menthol treatment on colony strength, honey production and tracheal mite infestation levels. *Am. Bee J.* 129: 547-549.

Danka, R. G., Villa, J. D., Rinderer, T. E., & Delatte, G. T. 1995. Field test of *Acarapis woodi* (Acari: Tarsonemidae) infestation and of colony production by four stocks of honey bees (Hymenoptera: Apidae). *J. Econ. Entomol.* 88: 584-591.

Engels, W. & Schatton, K. 1986. Changes in hemolymph and weight loss in worker bees due to *Varroa* parasitization. *Varroa* Workshop Feldafing. Abstract 5.

Elzen, P. J., Eischen, F. A., Baxter, J. R., Pettis, J., Elzen, G. W., & Wilson, W. T. 1998. Fluvalinate resistance in *Varroa jacobsoni* from several geographic locations. *Am. Bee J.* 138: 674-676.

Elzen, P. J., Baxter, J. R., Pettis, J., Spivak, M., & Wilson, W. T. 1999. Amitraz resistance in *Varroa:* new discovery in North America. *Am. Bee J.* 139: 362.

Feldlaufer, M. F., Pettis, J. S., Kochansky, J. P., & Shimanuki, H. 1997. A gel formulation of formic acid for the control of parasitic mites of honey bees. *Am. Bee J.* 137: 661-663.

Gary, N. E., Page, R. E. Jr., Morse, R. A., Henderson, C. E., Nasr, M. E., & Lorenzen, K. 1990. Comparative resistance of honey bees (*Apis mellifera* L.) From Great Britain and United States to infestation by tracheal mites (*Acarapis woodi*). *Am. Bee J.* 130: 667-669.

de Guzman, L. I., Rinderer, T. E., & Beaman, L. D. 1998a. Attractiveness to infestation by tracheal mites, *Acarapis woodi* (Rennie) (Acari:Tarsonemidae) in three stocks of honey bees and two of their hybrids. *BeeScience* 4: 87-91.

de Guzman, L. I., Rinderer, T. E., & Delatte, G. T. 1998b. Comparative resistance of four honey bee (Hymenoptera: Apidae) stocks to infestation by *Acarapis woodi* (Acari: Tarsonemidae). *J. Econ. Entomol.* 91: 1078-1083.

de Guzman, L. I., Rinderer, T. R., Lancaster, V. A., Delatte, G. T., & Stelzer, J. A. 1999. Varroa in the mating yard: III. The effects of formic acid gel formulation on drone production. *Am. Bee J.* 139: 304-307.

de Guzman, L. I., Rinderer, T. E., Delatte, G. T., Stelzer, J. A., Beaman, L., & Harper, C. 2001a. An evaluation of far-eastern Russian honey bees and other methods for the control of tracheal mites. *Am. Bee J.* 141: 737-741.

de Guzman, L.I., Rinderer, T.E., Delatte, G.T., Stelzer, J.A., Williams, J. L., Beaman, L.D, Kuznetsov, V., Bernard, S.J., Bigalk, M., & Tubbs, H. 2001b. Multi-state field trials of ARS Russian honey bees 3. Responses to *Acarapis woodi* 1999, 2000. *Am. Bee J.* 141: 658-661.

Harbo, J. R., & Harris, J. W. 1999. Selecting honey bees for resistance to Varroa. *Apidologie* 30: 183-196.

Harbo, J. R., & Hoopingarner, R. A. 1997. Honey bees (Hymenoptera: Apidae) in the United States that express resistance to *Varroa jacobsoni* (Mesostigmata: Varroidae). *J. Econ. Entomol.* 90: 893-898.

Ifantidis, M.D. 1983. Ontogenesis of the mite *Varroa jacobsoni* Oud. in the worker and drone brood cells of the honey bee. *J. Apic. Res.* 22: 200-206.

Jong, D. de, Goncalves, L. S., & Morse, R. A. 1982. Weight loss and other damage to developing worker honey bees (*Apis mellifera*) due to the infestation with *Varroa jacobsoni*. *J. Apic. Res.* 21: 65-167.

Lin, H., Otis, G. W., & Scott-Dupree, C. D. 1996. Comparative resistance in Buckfast and Canadian stocks of honey bees (*Apis mellifera* L.) to infestation by tracheal mites *Acarapis woodi* (Rennie). *Exp. Appl. Acarol.* 20: 87-101.

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.

Milne, C. P., Otis, G. W., Eischen, F. A., & Dormaie, J. M. 1991. A comparison of tracheal mite resistance in two commercially available stocks of honey bees. *Am. Bee J.* 131: 713-718.

Pettis, J. S., & Shimanuki, H. 1999. A hive modification to reduce varroa populations. *Am. Bee J.* 139: 471-473.

Rinderer, T. E., de Guzman, L. I., Kulincevic, J. M., Delatte, G. T., Beaman, L. D., & Buco, S. M. 1993. The breeding, importing, testing and general characteristics of Yugoslavian honey bees bred for resistance to *Varroa jacobsoni*. *Am. Bee J.* 133: 197-200.

Rinderer, T.E., Kuznetsov, V.N., Danka, R.G., & Delatte, G.T. 1997. An importation of potentially Varroa-resistant honey bees from Far-Eastern Russia. *Am. Bee J.* 137: 787-789.

Rinderer, T.E., Delatte, G.T., de Guzman, L.I., Williams, J., Stelzer, J.A., & Kuznetsov, V.N. 1999. Evaluations of the varroa-resistance of honey bees imported from Far-Eastern Russia. *Am. Bee J.* 139: 287-290.

Rinderer , T. E., de Guzman, L. I., Harris, J., Kuznetsov, V., Delatte, G. T., Stelzer, J. A., & Beaman, L. 2000. The Release of Russian honey bees. *Am. Bee J.* 140: 305-307.

Rinderer, T. E., de Guzman, L. I., Delatte, G. T., Stelzer, J. A., Lancaster, V. A., Kuznetsov, V., Beaman, L., Watts, R., & Harris, J. W. 2001a. Resistance to the parasitic mite *Varroa destructor* in honey bees from far-eastern Russia. *Apidologie* 32: 381-394.

Rinderer, T. E., de Guzman, L. I., Delatte, G. T., Stelzer, J. A., Williams, J. L., Beaman L. D.,Kuznetsov, V., Bigalk, M., Bernard, S. J., & Tubbs, H.. 2001b. Multi-state field trials of Russian honey bees 1. Responses to *Varroa destructor* 1999, 2000. *Am. Bee J.* 141: 658-661.

SAS Institute, Inc. 1997. SAS User's Guide, SAS Institute, Cary, NC.

Williams, K. R., Sugden, E. A., & Webster, T. C. 1994. Effects of honey bee queen type and age on tracheal mite infestation in Kentucky. *Am. Bee J.* 134: 838.