Efficacy of a Bottom Screen Device, Apistan™, and Apilife VAR™, in Controlling *Varroa destructor*

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Abstract

This study tested the efficacy of a hive bottom screen device in controlling varroa mites, when used alone or in conjunction with the miticides Apistan™ and ApilifeVAR™. Thirty-six colonies were equalized and each assigned to one of six treatments: (1) no treatment, (2) bottom screen, (3) Apistan, (4) Apistan + screen, (5) Apilife, and (6) Apilife + screen. Adult bee populations were not affected by treatment, but the number of brood cells was significantly reduced in colonies treated exclusively with Apilife compared to that of colonies treated with Apistan or exclusively with a bottom screen. Brood production was numerically highest in colonies treated exclusively with a bottom screen. Varroa populations were significantly reduced in colonies receiving acaricide compared to non-treated colonies. Varroa populations in colonies treated exclusively with a bottom screen were 14.9 % lower than that of non-treated colonies, but this difference was not significant. The bottom screen did not affect the percentage of varroa mite population phoretic on adult bees. Apistan provided 100% mite control in South Carolina, whereas in Georgia it provided 0% control in colonies treated exclusively with Apistan. With the addition of a bottom screen, Apistan-treated colonies in Georgia experienced an average mite control of 44.3%. This suggests that fluvalinate resistance exists in Georgia varroa mites. It also indicates that a bottom screen may help compensate for reduced acaricide efficacy. Average efficacy of Apilife ranged from 65.2 - 97.1%.

Keywords: *Apis mellifera* / *Varroa destructor* / integrated pest management / chemical resistance management

There is growing interest in the use of screened bottom boards as a tool for managing the varroa mite (*Varroa destructor* Anderson & Trueman). Specific designs vary, but most feature a floor comprised of #8 hardware screen (3 mesh per cm). The device can be either a standard bottom board whose solid floor has been replaced with screen, or a rim with screen made to fit between a Langstroth hive body and standard bottom board (Fig. 1). A screened bottom board has been used in conjunction with paper collecting sheets as a method for monitoring varroa levels (Szabo 1998, 1999). But the chief merit of the device is its presumed ability to hinder or prevent mites from re-mounting their hosts once the mites fall off the bees and through the screen. Even without miticide, it is not uncommon for mites to drop off their hosts, and it is possible for up to 51% of these individuals to be alive (Webster *et al.* 2000). Thus, any technology that prevents these mites from returning to the brood nest would be an important part of an integrated program for mite control.

In spite of the interest in bottom screens, they have received relatively little quantitative examination. In Russia, Rodionov & Shabarshov (1986) claimed that mite populations in the presence of bottom screens "may be considerably reduced." In the United States, the number of mites retrieved on sticky sheets was consistently lower in hives with screened bottoms compared to that in non-modified hives (Pettis & Shimanuki 1999, Ostiguy *et al.* 2000), although a statistically significant benefit was realized only by Ostiguy *et al.*. The observation by Pettis & Shimanuki that bottom screens significantly increase brood production further justifies continued research on the technology.

We were interested in building upon the work of previous investigators by examining the efficacy of the bottom screen device when used alone or in conjunction with two acaricides, Apistan™ (fluvalinate) and Apilife VAR™ (76% thymol, 16.4% eucalyptol, 3.8% menthol, and 3.8% camphor).

MATERIALS AND METHODS

General

On 7-10 June 1999 the experiment was set up with 36 colonies, 18 in one apiary near Athens, GA and 18 in another apiary near Clemson, SC, USA. Bees, brood, and queens were collected within each state from a variety of sources. Bees were collected into pre-weighed cages, each of which was sampled in order to determine average weight (mg) per bee and average number of phoretic varroa mites per bee. Frames of brood were used if each con-

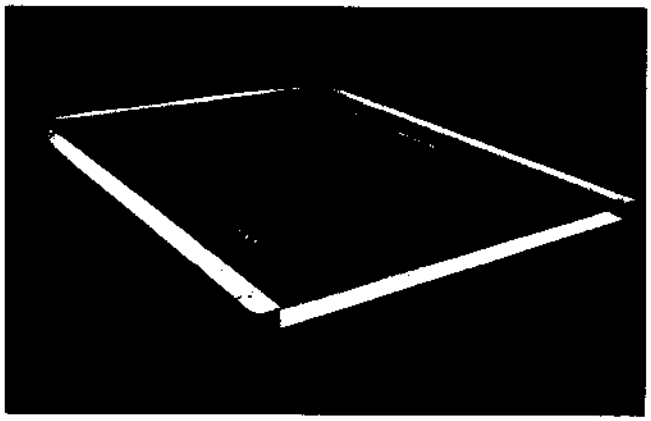

Figure 1. This bottom screen is designed to fit between a Langstroth-style hive body and standard bottom board.

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Figure 2. Apilife VAR™ wafers were enclosed in #8 mesh screen to prevent bees from removing the material from the hive.

tained at least 100 cm^2 sealed brood on each side. Samples of brood combs were measured in order to determine average brood area (cm^2) and number of adult varroa mites per sealed brood cell. These data permitted us to calculate for each colony within an apiary the starting bee population, brood area, and varroa mite population (Delaplane & Hood 1997, 1999). Because bees and brood were pooled together from a variety of colonies, queens were installed in the reconfigured experimental colonies without regard to their former workers or brood. Queens were clipped, marked, and individually caged within each colony. Queens were released 3-11 days after colony installation, depending on the degree of queen rejection behavior being displayed by the colony. Colonies were treated with Terramycin™ antibiotic to prevent foulbrood disease. All Georgia colonies were fed sugar syrup at one time when it was judged that dearth conditions warranted supplemental feeding. Additionally, 7-19 days after colony installation, each colony was given one super of honey above a queen excluder. Queen cells were removed from colonies weekly to discourage swarming.

Each colony within an apiary was assigned one of six experimental treatments: (1) no treatment, (2) bottom screen, (3) Apistan, (4) Apistan + screen, (5) Apilife, and (6) Apilife + screen. There were three replicates per treatment per apiary for a total of six replicates per treatment across the experiment. The day that treatments were administered constituted day zero. Apilife-treated colonies were each given two Apilife wafers which were replaced with fresh wafers on days 14, 28, and 42 for South Carolina and days 14, 23, and 37 in Georgia. The wafers were enclosed in a protective packet of #8 mesh screen (Fig. 2) except for days 14-31 in South Carolina and days 14-23 in Georgia when we left wafers uncovered over concern that the bees were propolizing the packets. We returned the protective packets, however, because we noticed that bees were removing wafer particles from the colonies. Apistan-treated colonies each received two new strips, according to manufacturer's instructions, which remained in the colonies for the duration of the experiment. Bottom screens were each positioned between the hive body and conventional bottom board (Pettis & Shimanuki 1999).

Brood survivorship

Percentage brood survivorship was appraised during weeks 5 and 6. Each week, one comb containing open larvae was labeled per colony, a sheet of transparent acetate placed on one side the comb, and the position of two groups of ten cells (20 cells) each containing a live larva was indicated on the acetate with a permanent marker. Three days later the acetate was returned to the corresponding side of a comb and the number of remaining live brood cells counted. If the larvae had become capped, the cells were opened to confirm that the pre-pupae/pupae were still alive. Forty brood cells were appraised per colony (2 groups per comb x 10 cells x 2 samplings).

Dismantling

The experiment was dismantled on days 50-51 and the number of brood cells, bee populations, colony varroa mite populations, and number of mites retrieved on bottom board adhesive sheets appraised. The methods for making these determinations are explained in detail elsewhere (Delaplane & Hood 1997, 1999). Our data also permitted us to calculate percentage varroa mite control, according to *100-((ending mite population ÷ beginning mite population) x 100)*, and percentage of varroa mite population phoretic on adult bees.

Analyses

The effects of treatment on number of brood cells, percentage brood survivorship, bee populations, colony varroa mite populations, percentage varroa mite control, percentage of varroa mites

Table 1. Treatments administered to Varroa mite-positive honey bee colonies included (1) no treatment, (2) bottom screen, (3) Apistan, (4) Apistan + screen, (5) Apilife, and (6) Apilife + screen. Values are mean ± standard error; n = 6 except for % varroa phoretic for which n is given in parentheses. Row means followed by the same letter are not different at the $\alpha \leq 0.05$ level.

	(1) no treatment	(2) bottom screen	(3) Apistan	(4) Apistan + screen	(5) Apilife	(6) Apilife + screen
ending no. brood cells	7434 ± 1679ab	10413 ± 647a	9895 ± 1186a	9492 ± 566a	5325 ± 780b	7734 ± 635ab
brood survivorship (%)	99.2 ± 0.5a	99.6 ± 0.4a	99.6 ± 0.4a	98.3 ± 0.5a	93.3 ± 5.7a	90 ± 7.3a
ending bee population	22103 ± 958a	22370 ± 1338a	20864 ± 1202a	22054 ± 910a	16769 ± 1893a	20528 ± 2123a
ending varroa population	2202 ± 597a	1874 ± 787ab	131 ± 70b	34 ± 17b	42 ± 24b	47 ± 16b
% varroa phoretic	54.6 ± 8a (6)	59.6 ± 9.4a (6)	74.6 ± 13.8a (3)	100a (3)	33.3 ± 33.3a (3)	65.1 ± 21.7a (5)
no. varroa on adhesive sheet	41 ± 11a	23 ± 7ab	4 ± 1b	8 ± 2b	3 ± 1b	1 ± 0.3b

(1) no treatment	(2) bottom screen	(3) Apistan	(4) Apistan + screen	(5) Apilife	(6) Apilife + screen
			Georgia		
0b	0b	0b	44.3 ± 21.4ab	65.2 ± 32.6a	90.8 ± 5.1a
			South Carolina		
0b	0b	100a	100a	97.1 ± 2.9a	89.3 ± 6.3a

Table II. Percentage varroa mite control in colonies treated as explained in Table I and calculated according to 100-((*ending mite population + beginning mite population) × 100).* There was a treatment × state interaction for this variable, so analyses were run separately by state. Values are mean ± standard error, $n = 3$. Row means followed by the same letter are not different at the $\alpha \leq 0.05$ level.

phoretic, and number of varroa mites retrieved on adhesive sheets were tested with a randomized design analysis of variance, blocked on state (GA or SC) (SAS Institute 1985). The effects of treatment, state, and interactions of treatment x state were tested against residual error. When treatment and state interacted (as was the case with percentage varroa mite control), analyses were run separately by state. Means were separated with Tukey's multiple range test, and differences were accepted at the $\alpha \leq 0.05$ level. We tested beginning colony varroa mite populations as a covariate because this variable seemed to exhibit considerable variation in spite of our equalizing efforts. Beginning varroa mite population was retained as a covariate for the only variable for which it was significant, percentage varroa mite control.

RESULTS AND DISCUSSION

General

There were treatment effects for number of brood cells, colony varroa mite populations, percentage varroa mite control, and number of varroa mites retrieved on adhesive sheets ($P \leq 0.046$). There were state effects ($P = 0.0001$) as well as a state x treatment interaction ($P = 0.0026$) for percentage varroa mite control. There were no treatment effects for percentage brood survivorship, bee population, and percentage varroa mites phoretic. The covariate beginning varroa mite population significantly affected percentage varroa mite control ($P = 0.0015$). Treatment means and mean separation tests are presented in Tables I and II.

Treatment effects on adult bees and brood

Adult bee populations were not affected by treatment, but the number of brood cells was significantly reduced in colonies treated exclusively with Apilife compared to that of colonies treated with Apistan or exclusively with a bottom screen. It is also noteworthy that brood survivorship was numerically smallest in Apilife-treated colonies (Table I). Although the brood survivorship means were not statistically different, the total data set suggests that the effects of Apilife on brood are not innocuous. Published data are not congruent on the toxic effects on bees of thymol-based acaricides. Chiesa (1991) found evidence for increased adult bee mortality in thymol-treated colonies, and Bunsen (1991) documented increased brood mortality in the presence of thyme. However, Mattila *et al.* (2000) failed to detect differences in brood mortality between non-treated colonies and colonies treated with the thymol-based acaricide Apiguard™. There is evidence that negative effects of thymol may be mitigated by management. In the present study the addition of a bottom screen elevated brood production in Apilife-treated colonies to a level comparable to that of other treatments (Table I).

Brood production was numerically highest in colonies treated exclusively with a bottom screen (Table I), an observation noted before by Pettis & Shimanuki (1999). This is a noteworthy feature of the bottom screen and justifies further research to confirm if it is a general phenomenon and to determine its mode of action and implications for the nesting biology of honey bees.

Treatment effects on varroa mites

Compared to non-treated controls, ending colony varroa populations were significantly reduced in colonies receiving acaricide (Table I). Average ending varroa populations in acaricide-treated colonies ranged from 34-131, well below established economic thresholds for the region (Delaplane & Hood 1997, 1999); conversely, mite populations in non-treated colonies (2202 per colony) were approaching the threshold level of 3172 (Delaplane & Hood 1997, 1999). Ending varroa populations in colonies treated exclusively with a bottom screen (1874) were about 14.9 % lower than that of non-treated controls (2202). Although this difference was not statistically significant, it is congruent with the findings of Pettis & Shimanuki (1999) and Ostiguy *et al.* (2000) and suggests that the bottom screen does exert a negative influence on mite populations. The number of mites retrieved on adhesive sheets was about 44% lower in colonies treated exclusively with a bottom screen compared to non-treated controls, a difference outside the range of 14-28% reported by Pettis & Shimanuki (1999).

In Table II we report percentage varroa mite control which represents the percentage change in mite populations from the beginning to the end of the experiment. This allows us to compare treatments, while accounting for differences in beginning mite populations. There was a treatment x state interaction for this variable, so it was analyzed separately by state. The interaction is explained by a pronounced difference in the performance of Apistan in Georgia compared to South Carolina. Apistan provided 100% mite control in South Carolina whereas in Georgia it provided 0% control in colonies treated exclusively with Apistan. In the presence of a bottom screen Apistan-treated colonies in Georgia experienced an average mite control of 44.3%. This suggests that Apistan (fluvalinate) resistance exists in Georgia varroa mites, a phenomenon documented in Europe and other regions of the USA (Milani 1999). The present data also suggest that in cases of resistance a bottom screen may help compensate for reduced acaricide efficacy. The range of average efficacy for Apilife across both states was 65.2 - 97.1% (Table II) which is similar to the range of 63.9 - 99.5% reported for Apilife in Europe (Imdorf *et al.* 1999) and to the range of 70 - 96.7% reported for similar thymol-based blends in North America (Calderone 1999, Calderone & Spivak 1995). In Georgia the bottom screen numerically increased mite control in Apilife-treated colonies, but this benefit was not apparent in South Carolina (Table II).

There were no differences among treatments for the percentage of varroa mites phoretic on adult bees (Table I). The hypothesized mode of action for the bottom screen is that it hinders dislodged mites from re-mounting adult bees (Rodionov & Shabarshov 1986, Pettis & Shimanuki 1999). We reasoned that if this hypothesis is true, then we may expect to see in colonies with bottom screens an average reduction in the fraction of the mite population on adult bees, which did not occur. It remains to elucidate the mode of action for the bottom screen.

Conclusions

Although there is evidence that it reduces brood production, Apilife VAR can provide varroa mite control in North America at a level comparable to that of Apistan. Our data support the conclusion of Pettis & Shimanuki (1999) that the bottom screen increases brood production. Additionally, our data suggest that bottom screens can enhance varroa mite control in colonies with chemical resistant mites. The addition of bottom screens to colonies lowered average mite population numerically by 14.9% compared to non-treated controls, but it is important to stress that this effect was not statistically significant.

To our knowledge, in North America only Ostiguy *et al.* (2000) have reported a statistically significant benefit of using bottom screens against varroa; however, the trends of all studies (Pettis & Shimanuki 1999, Ostiguy *et al.* 2000) including ours have been consistently favorable. It is reasonable to conclude tentatively that the effects of bottom screens are subtle, but real. The fact that their effects are small does not necessarily diminish the value of screens

because it is common in integrated pest management to incorporate many diverse control strategies, any one of which may be insufficient when used alone. In this respect bottom screens may prove useful when used with other practices such as mite removal (Dung *et al.* 1995, Fakhimzadeh 2000), genetically resistant bees (Spivak 1996, Harbo & Harris 1999), and apiary isolation (Delaplane & Hood 1999). A concerted use of such strategies holds promise for slowing the achievement of treatable mite thresholds (Delaplane & Hood 1997, 1999) and for reducing the overall quantity of chemicals used in beekeeping.

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REFERENCES

Bunsen J.D. 1991. Experimentelle Untersuchungen zur Bekämpfung der Milbe *Varroa jacobsoni* Oud., einem Ektoparasit der Honigbiene (*Apis mellifera* L.) mit Stoffen natürlicher Herkunft, Dissertation, Justus-Liebig-Universität Giessen.

Calderone N.W. 1999. Evaluation of formic acid and a thymol-based blend of natural products for the fall control of *Varroa jacobsoni* (Acari: Varroidae) in colonies of *Apis mellifera* (Hymenoptera: Apidae), *J. Econ. Entomol.* 92(2): 253-260.

Calderone N.W., Spivak M. 1995. Plant extracts for control of the parasitic mite *Varroa jacobsoni* (Acari: Varroidae) in colonies of the western honey bee (Hymenoptera: Apidae), *J. Econ. Entomol.* 88(5): 1211-1215.

Chiesa F. 1991. Effective control of varroatosis using powdered thymol, *Apidologie* 22: 135-145.

Delaplane K.S., Hood W.M. 1997. Effects of delayed acaricide treatment in honey bee colonies parasitized by *Varroa jacobsoni* and a late-season treatment threshold for the southeastern USA, *J. Apic. Res.* 36: 125-132.

Delaplane K.S., Hood W.M. 1999. Economic threshold for *Varroa jacobsoni* Oud. in the southeastern USA, *Apidologie* 30: 383-395.

Dung N. van, Tan N.Q., Huan L.V., Beetsma W.J. 1995. Biotechnical manipulations used in Vietnam to control *Varroa jacobsoni* and *Tropilaelaps clareae* in colonies of *Apis mellifera*. *Bee Science* 4: 11-13.

Fakhimzadeh K. 2000. Potential of super-fine ground, plain white sugar dusting as an ecological tool for the control of varroasis in the honey bee (*Apis mellifera*). *Am. Bee J.* 140(6): 487-491.

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

Imdorf A., Bogdanov S., Ochoa R.I., Calderone N.W. 1999. Use of essential oils for the control of *Varroa jacobsoni* Oud. in honey bee colonies, *Apidologie* 30: 209-228.

Mattila H.R., Otis G.W., Daley J., Schulz T. 2000. Trials of Apiguard, a thymol-based miticide part 2. Non-target effects on honey bees, *Am. Bee J.* 140(1): 68-70.

Milani N. 1999. The resistance of *Varroa jacobsoni* Oud. to acaricides, *Apidologie* 30: 229-234.

Ostiguy N., Sammataro D., Finley J., Frazier M. 2000. An integrated approach to manage *Varroa jacobsoni* in honey bee colonies, *Am. Bee J.* 140(11): 906-907.

Pettis J.S., Shimanuki H. 1999. A hive modification to reduce varroa populations, *Am. Bee J.* 139(6): 471-473.

Rodionov V.V., Shabarshov I.A. 1986. The fascinating world of bees. Mir Publishers, Moscow.

SAS Institute. 1985. SAS/STAT User's guide, version 6, 4th ed., SAS Institute, Cary, North Carolina, USA.

Spivak M. 1996. Honey bee hygienic behavior and defense against *Varroa jacobsoni*, *Apidologie* 27: 245-260.

Szabo T.I. 1998. Progress report on selective breeding of honey bees for resistance to parasitic mites, *Am. Bee J.* 138(6): 464-466.

Szabo T.I. 1999. Selective breeding of honey bee colonies for resistance to *Varroa jacobsoni* and the effects of management techniques on varroa infestation levels, *Am. Bee J.* 139(7): 537-540.

Webster T.C., Thacker E.M., Vorisek F.E. 2000. Live *Varroa jacobsoni* (Mesostigmata: Varroidae) fallen from honey bee (Hymenoptera: Apidae) colonies, *J. Econ. Entomol.* 93(6): 1596-1601.