

## Economic threshold for *Varroa jacobsoni* Oud. in the southeastern USA

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**Abstract** – This research was designed to determine economic thresholds for *Varroa jacobsoni* mites in mature overwintered colonies under conditions that encourage or discourage mite immigration. Congruent data from the present study and our earlier work suggest that a true late-season (August) economic threshold for mites in the southeastern USA lies within a range of mite populations of 3 172–4 261, ether roll mite levels of 15–38, and overnight bottom board insert mite levels of 59–187 in colonies with bee populations of 24 808–33 699. Overwintering colonies can benefit from an additional early-season (February) treatment. This benefit was realized in colonies which in February had the following average values: mite populations 7–97, ether roll 0.4–2.8, bottom board inserts 0.6–10.2 and bee populations 12 606–13 500. Continuous acaricide treatment never achieved colony bee populations or brood number significantly higher than in colonies treated more conservatively. There is evidence that minimizing mite immigration has the benefit of delaying the onset of economic thresholds. © Inra/DIB/AGIB/Elsevier, Paris

*Apis mellifera* / *Varroa jacobsoni* / integrated pest management / chemical resistance management

### 1. INTRODUCTION

Synthetic acaricides are the most effective and widely used method for controlling *Varroa jacobsoni* Oud. But recent evidence of acaricide-resistant mites in Italy [18], France [6, 22] and USA [4] has underscored the

need to develop management practices that limit chemical resistance in mites. One way to do this is to use economic thresholds [19], that is, to treat a colony only when the mite population reaches a level at which mites are still tolerable by bees but above which there may be serious and possibly irrepara-

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ble harm to the colony. A corollary to a research-based threshold is a reliable and practical mite sampling method. A program based on accurate sampling and threshold-based treatments can be expected to reduce the overall number of acaricide treatments, relax the selection pressure for mite resistance, reduce the risk of contaminated hive products, and reduce production costs for beekeepers.

Economic threshold recommendations vary by region [2], owing in part to differences in the length of the brood-rearing season, one of the most important regulators of mite population dynamics [14]. Thresholds also may vary depending on the risk of mite immigration [17] and on the management history of colonies. This present paper is part of a project to develop research-based economic thresholds for the Piedmont region of the southeastern USA under a variety of beekeeping conditions. In earlier work we established a threshold for first-year colonies set up from mail-order packages [9]. In this paper we present experimental evidence of an economic threshold for mature overwintered colonies under conditions that encourage mite immigration (1997) or discourage it (1998). We believe that our design and protocols are applicable to workers developing economic thresholds elsewhere.

## 2. MATERIALS AND METHODS

### 2.1. 1997 study: mite immigration encouraged

The 1997 study was designed to develop economic thresholds for overwintered colonies under conditions that encourage mite immigration. On 17–18 February 1997 we organized 60 overwintering colonies of honey bees in the Piedmont region of Georgia and South Carolina, USA (2 states  $\times$  2 apiaries per state  $\times$  15 colonies per apiary). Within each apiary we equalized colonies for initial bee populations, amount of brood, and *V. jacobsoni* mite populations. We did this by shaking all adult bees from one apiary into a common cage, distributing the brood equally

(using visual estimates) among the 15 empty hives, and then distributing equally (by weight) adult bees from that state's sister apiary into the empty hives. By transferring adult bees to their state's sister apiary, we prevented bees from drifting back to their original hive locations and nullifying our equalizing efforts. We further discouraged bee drift by distinguishing hive entrances with a variety of colored geometric symbols [15].

We monitored initial colony mite levels immediately after the equalizing procedure with adhesive bottom board inserts [10]. The number of mites caught per colony in one overnight sampling after the equalizing procedure was  $0.7 \pm 0.9$  (range 0–3) in Georgia and  $6.2 \pm 14.3$  (range 0–77) in South Carolina. Colonies were requeened, treated with Fumidil B (Mid-Con) to control Nosema disease, treated with Terramycin antibiotic (Pfizer) to control brood diseases, treated with vegetable oil/sugar patties to control tracheal mites (*Acarapis woodi* [Rennie]) [8], and managed optimally for honey production.

Each colony within apiary was randomly assigned one of the following treatments: 1) treatment with Apistan® acaricide in February, 2) treatment in August, 3) treatment in February and in August, 4) continuous treatment, and 5) no treatment. Three replicates of each treatment were run in each apiary. This design permitted mites from non-treated colonies to emigrate to treated colonies within the same apiary. Apistan strips inserted in colonies in February were removed at day 56. Strips inserted in the continuously treated colonies were replaced with new strips every 56–69 days, and strips inserted in August were removed at the termination of the experiment, days 40–42 (South Carolina) or 43–44 (Georgia). We replaced failing queens as necessary and equalized brood within treatment group and apiary to minimize swarming and variation within treatment groups. Swarming was minimized in South Carolina with the Demaree method [1].

On 25–26 February, 20, 27, 30 May, and 21, 25 August some colonies were sampled to determine colony bee populations, average body weight of bees, number of sealed brood cells, colony mite populations, and mite levels with an ether roll test and with an overnight adhesive bottom board insert ( $20 \pm 4$  h exposure) using published methods [9]. We did not use acaricide to hasten mite drop on bottom board inserts; however, acaricide unavoidably affected our readings for May and August in the continuously

treated colonies. On the 25–26 February dates, we sampled only colonies scheduled for acaricide treatment that month; however, because colonies had just recently been set up and no treatments were yet in place the February parameters given in table V accurately estimate the initial condition of all colonies. From 25 September to 28 October all surviving colonies were dismantled ( $n = 55$ ) to measure the above parameters plus the percentage of brood cells with visible disease-like symptoms [9] and percentage of colony bees infested with tracheal mites [8]. The following January, nearly 1 year after the start of the experiment, we assessed the condition of the Georgia colonies with a blind subjective survivability score; each of four observers independently examined each colony and scored it, to the nearest half unit, from zero (dead or irrecoverably weak) to three (best condition and greatest probability of surviving winter).

The effects of treatment on colony bee populations, body weight of bees, number of sealed brood cells, colony mite populations, and percentage brood cells with visible disease-like symptoms were tested with a completely randomized design analysis of variance [20] blocked on state (Georgia or South Carolina). The effects of treatment and its interactions with state were tested with the interaction of treatment with apiary nested within state. The effects of state were tested against the effect of apiary nested within state. If the interaction of treatment  $\times$  state was significant, then analyses were run separately by state and the error term was the interaction of treatment with apiary. Least square means was used to adjust for non-equal sample size, and means were separated with a *t*-test. Differences were accepted at the  $\alpha \leq 0.05$  level. The relationship between colony mite populations and mite levels found with ether roll and by bottom board inserts was tested with regression analyses [20]. With bottom board inserts we were interested in measuring natural mite drop, so we excluded colonies from the regression analyses that had Apistan strips in them at the time of sampling.

Forty-one of 55 surviving colonies were positive for *A. woodi*, and infestation levels ranged from 5 to 95%. Tracheal mite levels were significantly higher in South Carolina ( $40.2 \pm 5.3\%$ , mean  $\pm$  standard error) than in Georgia ( $12.5 \pm 3.4\%$ ) ( $F = 47.8$ ;  $df = 1, 2$ ;  $P = 0.0203$ ), but when tracheal mites were included as a covariate in the main analyses they did not explain any variation in our parameters of interest ( $P \geq 0.2025$ ).

## 2.2. 1998 study: mite immigration minimized

The 1998 study was designed to compare the various treatment regimens for overwintered colonies under conditions of minimized mite immigration. The basic protocol was the same as in 1997 with key differences noted below.

We set up 40 colonies (2 states  $\times$  5 apiaries per state  $\times$  4 experimental colonies per apiary). Each of the ten apiaries was at least 0.6 km away from other known bee colonies. Each apiary within state randomly received one of the five experimental treatments as used in 1997, and experimental colonies within apiary received the same treatment. Thus, there was minimized risk of mites immigrating from non-treated colonies to treated colonies within the same apiary. Colonies were removed from the experiment if they died or became queenless and in two cases when acaricide strips were found in colonies past the prescribed treatment interval.

The number of mites caught per colony per day on adhesive bottom board inserts after initial setup was  $0.6 \pm 0.3$  (range 0–1.3) in Georgia and  $0.6 \pm 1.1$  (range 0–4.5) in South Carolina. Apistan strips inserted in colonies in February were removed at day 56; strips inserted in the continuously treated colonies were replaced with new strips every 56–67 days, and strips inserted in August were removed at day 58 (South Carolina) or at days 80–83, the termination of the experiment (Georgia). The experiment was dismantled from 26–30 October ( $n = 36$  colonies).

The effects of treatment on colony bee populations, body weight of bees, number of sealed brood cells, colony mite populations, and percentage brood cells with visible disease-like symptoms were tested with a completely randomized design analysis of variance blocked on state. The interaction of month of treatment  $\times$  state was the error term. Analyses were run separately by state and the parameters tested against residual error when treatment  $\times$  state interaction was significant.

## 3. RESULTS AND DISCUSSION

### 3.1. General

In the 1997 study under conditions that encouraged mite immigration, there were treatment effects in September–October on

colony bee populations and colony mite populations ( $F \geq 6.5$ ;  $df = 4,8$ ;  $P \leq 0.0125$ ), but not for bee weight nor the percentage of brood cells with disease-like symptoms (table I). There were treatment  $\times$  state interactions for the number of sealed brood cells ( $F = 9.63$ ;  $df = 4,8$ ;  $P = 0.0038$ ); treatments affected brood in South Carolina ( $F = 16.4$ ;  $df = 4,4$ ;  $P = 0.0096$ ) but not in Georgia (table III).

In the 1998 study under conditions that minimized mite immigration, there were no treatment effects in October for colony bee populations, bee weight, number of sealed brood cells, nor the percentage of brood cells with disease-like symptoms (table II). There was a treatment  $\times$  state interaction for colony mite populations ( $F = 4.87$ ;  $df = 4,26$ ;  $P = 0.0046$ ) and significant treatment effects in each state ( $F \geq 12.2$ ;  $df = 4,12$ ;  $4,14$ ;  $P \leq 0.0003$ ) (table IV).

### 3.2. Colony bee populations and bee weight

Under conditions encouraging mite immigration, colony bee populations in September–October were highest in colonies treated continuously with acaricide. The populations of continuously treated colonies were not significantly different from those of colonies treated once in February and again in August (table I); however, this is based on a conservative interpretation of a  $P$  value of only 0.0501 ( $t$ -test) between these two means. Nevertheless this supports an argument against treating colonies continuously. Continuous treatment not only risks contaminated hive products and acaricide-resistant mites, but in our study failed to achieve bee populations significantly larger than in the more conservative February + August schedule. Moreover, the satisfactory performance of the February + August schedule in 1997 was achieved in apiaries in which mite emigration from non-treated colonies was an acute threat. In 1998 when apiaries were managed to minimize mite immigra-

tion, the February + August schedule actually yielded the numerically highest average bee populations (table II).

In both years, treatments did not affect average bee weight (tables I and II). Although *V. jacobsoni* can reduce body weight in bees parasitized as immatures [7], a remedial effect of acaricide was not apparent in this study nor in our earlier work with first-year colonies [9].

### 3.3. Number of sealed brood cells and percentage brood cells with disease-like symptoms

In South Carolina in September–October under conditions encouraging mite immigration, the number of sealed brood cells was highest in colonies treated continuously and in colonies treated in February + August (table III). This contrasts with our earlier work with first-year colonies in which brood number was highest in those colonies with the most *V. jacobsoni*-induced dysfunctions, a phenomenon we hypothesized may indicate efforts by bees to compensate for high levels of brood parasitism [9]. Our 1997 results do not support this hypothesis as brood number was significantly highest in those colonies in which mite control was optimized. Instead, our present results support a February + August acaricide treatment schedule. Continuous acaricide treatment has many inherent risks and in our study failed to achieve brood production significantly higher than in the more conservative February + August schedule. Moreover, in 1998 the February + August schedule yielded numerically similar amounts of brood to that in continuously treated apiaries (table II).

Over both years, incidence of brood with disease-like symptoms occurred in the non-treated, February, August, and February + August treatment schedules, but values never differed significantly from zero (tables I and II). *V. jacobsoni* mites vector or activate several bee pathogens [3, 16], and

**Table I.** Effects of different acaricide treatment schedules on colonies in apiaries managed to encourage immigration by *Varroa jacobsoni* (1997 study). Colonies received one of the following treatments: 1) Apistan acaricide in February, 2) August, 3) February + August, 4) continuous treatment, or 5) no treatment.

Colony treatment	Colony bee population	Bee weight (mg)	Colony mite population	Percentage diseased brood	Survivability score (0 = dead, 3 = best)
February	15 506 ± 3 384b (12)	127.8 ± 6a (12)	5 758 ± 1 430a (12)	4.4 ± 2.2a (12)	1.4 ± 0.5 (6)
August	16 515 ± 3 202b (12)	130.7 ± 6.8a (12)	37 ± 17b (12)	0.3 ± 0.3a (11)	2.3 ± 0.2 (6)
February + August	21 258 ± 2 776ab (10)	134.3 ± 6.8a (10)	33 ± 33b (10)	0a (10)	2.3 ± 0.1 (6)
Continuous	29 442 ± 2 220a (12)	138.6 ± 3.3a (12)	39 ± 24b (12)	0a (12)	2.8 ± 0.1 (6)
Non-treated	11 488 ± 2 178b (9)	132 ± 5.4a (9)	5 847 ± 1 215a (9)	5.6 ± 3.7a (8)	0.4 ± 0.2 (6)

The parameters were measured in September–October except for the subjective survivability score which was measured the following January. Values are average ± standard error. Column averages followed by the same letter are not different at the  $\alpha \leq 0.05$  level. Numbers in parentheses = *n*. The survivability score was not analyzed because of its small size and subjectivity.

**Table II.** Effects of different acaricide treatment schedules on colonies in apiaries managed to minimize immigration by *Varroa jacobsoni* (1998 study).

Colony treatment	Colony bee population	Bee weight (mg)	Scaled brood cells	Percentage diseased brood
February	22 534 ± 4 278 (6)	134.5 ± 7.3 (6)	2 426 ± 593 (6)	1 ± 0.8 (6)
August	17 679 ± 1 456 (8)	129.3 ± 4.7 (8)	966 ± 382 (8)	0.2 ± 0.2 (6)
February + August	23000 ± 2270 (7)	123.5 ± 5 (7)	1594 ± 291 (7)	0.3 ± 0.3 (7)
Continuous	17 059 ± 1 494 (7)	140.5 ± 7.8 (7)	1 508 ± 486 (7)	0 (7)
Non-treated	10 959 ± 2 200 (8)	127.2 ± 4.5 (8)	1 067 ± 269 (8)	1 ± 0.6 (8)

Apiaries received one of the treatments described in *table I*. The parameters were measured in October. Values are average ± standard error. There were no significant effects of treatment. Numbers in parentheses = *n*.

**Table III.** Effect of different acaricide treatment schedules on number of sealed brood cells in apiaries managed to encourage immigration by *Varroa jacobsoni* (1997 study).

Colony treatment	Sealed brood cells
<b>Georgia</b>	
February	3 855 ± 933a (6)
August	3 549 ± 1 193a (6)
February + August	2 901 ± 423a (6)
Continuous	3 292 ± 411a (6)
Non-treated	2 737 ± 874a (6)
<b>South Carolina</b>	
February	3 146 ± 723b (6)
August	3 327 ± 1 206b (6)
February + August	7 759 ± 1 623a (4)
Continuous	9 907 ± 1 244a (6)
Non-treated	1 665 ± 1 365b (3)

Colonies received one of the treatments described in table 1. Colonies were dismantled and numerous parameters measured in September–October. There were treatment × state interactions with the number of sealed brood cells, so this parameter was analyzed separately by state. Values are average ± standard error. Column averages followed by the same letter are not different at the  $\alpha \leq 0.05$  level. Numbers in parentheses = *n*.

brood parasitized by mites often display visible disorders [21]. In our study, visible brood pathology was eliminated only in colonies treated continuously with acaricide. We do not believe that this is a compelling defense for continuous treatments considering the low incidence of visible brood pathology in the February + August groups (0 % [1997] and 0.3 % [1998]).

### 3.4. Colony mite populations

In September–October under conditions encouraging mite immigration, colony mite populations were highest in colonies treated in February and in non-treated colonies (table 1). Mite populations did not differ among the August, February + August, nor continuously treated colonies, all of which had Apistan acaricide strips in them at the time of sampling. Our data suggest that one

**Table IV.** Effect of different acaricide treatment schedules on colony mite populations in apiaries managed to minimize immigration by *Varroa jacobsoni* (1998 study).

Colony treatment	Colony mite populations
<b>Georgia</b>	
February	8 890 ± 3 135c (2)
August	22 ± 22a (4)
February + August	0a (4)
Continuous	0a (3)
Non-treated	4 485 ± 1 301b (4)
<b>South Carolina</b>	
February	3 922 ± 661b (4)
August	1 201 ± 274a (4)
February + August	30 ± 30a (3)
Continuous	0a (4)
Non-treated	7 411 ± 1 411c (4)

Apiaries received one of the treatments described in table 1. Colonies were dismantled and numerous parameters measured in October. There were treatment × state interactions with colony mite populations, so this parameter was analyzed separately by state. Values are average ± standard error. Column averages followed by the same letter are not different at the  $\alpha \leq 0.05$  level. Numbers in parentheses = *n*.

February acaricide treatment is not satisfactory under conditions of mite immigration in the southeastern USA. Apistan acaricide strips were removed from February-treated colonies by 23 April, and by September–October mite populations in these colonies had rebounded to the same level as non-treated colonies. This rapid growth occurred from reproducing survivors and also from mites emigrating from non-treated colonies in the same apiary.

A rapid rebound of mites in February-treated colonies also occurred in 1998 under conditions of minimized mite immigration. Acaricide was removed from February-treated colonies by 23 April, and by October mite populations in these colonies had rebounded to a level approaching or exceeding that of non-treated colonies (table IV).

These results imply that if we are to develop an optimally conservative single-

treatment acaricide schedule for the south-eastern USA, it will occur late in the season rather than early. A single treatment early in the season is unsatisfactory because many months of brood rearing remain to support reproduction by surviving mites; reproduction rate of mites is high when the ratio of mites per brood cell is low [11]. A single late-season treatment in August gave optimum results in first-year colonies under conditions of minimized mite immigration [9]. In the present study with mature colonies under varying conditions of mite drift, single August treatments generally gave intermediate results (tables I-IV).

### 3.5. Implications for an economic threshold

In the 1997 study under conditions encouraging mite immigration, satisfactory mite control with minimized use of acaricide was achieved in mature, overwintered colonies treated once in February and again in August. Continuous treatment, a non-sustainable practice included in this study to provide a positive check, failed to achieve bee populations, brood number, and mite populations significantly different from the more conservative February + August schedule. A single February treatment permitted an unacceptably high rebound of mites by September-October which was associated with reduced brood number (in South Carolina) compared to February + August-treated colonies (tables I and III). In 1998 under conditions of apiary isolation, average October bee populations were numerically highest in February + August-treated colonies (table II).

However, there is reason to hypothesize that a more conservative single late-season treatment may work, especially if the beekeeper is able to minimize mite immigration by isolating apiaries from known sources of mite contamination and by treating all colonies in an apiary simultaneously with acaricide. Our 1998 study with isolated

apiaries failed to demonstrate significant differences in October among treatments for all variables except colony mite populations (tables II and IV). But it is worth noting that October bee populations and incidence of diseased brood in colonies treated only in August compared favorably with other treatments; bee populations even exceeded numerically the bee populations in continuously treated colonies (table II).

It is insightful to compare the condition of August- and February + August-treated colonies in the Augusts of both years (tables V and VI). Comparing the February + August-treated colonies, the mite populations, ether roll, and bottom board insert levels were predictably lower in 1998 under conditions of isolation, and this was associated with good colony condition in October (table II). It is possible that the February + August colonies in 1998 were below economic threshold when they were treated in August. However, it is possible that the August-treated colonies in 1998 were in fact at economic threshold when they were treated. Note the similarity between August mite populations in August-treated colonies in 1998 (4 057, table VI) and in February + August-treated colonies in 1997 (4 261, table V).

It is arguable that a single August treatment gave satisfactory results even in 1997 under conditions of mite immigration. August-treated and February + August-treated colonies did not differ significantly in September-October in bee populations, bee weight, and incidence of diseased brood, and August-treated colonies were subjectively scored the following January as equally likely to survive winter (table I). These similarities are paralleled by similar colony mite populations in August (4 144 and 4 261, table V).

If late-season mite populations of ~4 000 represent a tolerable level, we must also identify levels that are damaging. Our earlier work with first-year colonies [9] established that late-season mite populations of 6 662

**Table V.** Colony parameters at different sampling periods during the year in apiaries managed to encourage immigration by *Varroa jacobsoni* (1997 study).

Colony treatment	Colony bee population	Sealed brood cells	Colony mite population	Percentage mite population on adult bees	Ether roll (mites/300 bees)	Bottom board inserts (mites)
<b>February sampling</b>						
NA	12 606 ± 1 145 (12)	4 074 ± 235 (12)	97 ± 33 (12)	52.8 ± 10.2 (7)	2.8 ± 1.1 (12)	10.2 ± 6.1 (12)
<b>May sampling</b>						
February	23 803 ± 13 848 (3)	6 507 ± 3 996 (3)	129 ± 97 (3)	64.5 ± 35.5 (2)	0.3 ± 0.3 (3)	2.3 ± 1.5 (3)
August	27 064 ± 8 222 (3)	12 909 ± 2 245 (3)	192 ± 151 (3)	35.5 ± 35.5 (2)	0.2 ± 0.2 (3)	2 ± 1.5 (3)
February + August	36 763 ± 9 043 (3)	14 177 ± 1 335 (3)	177 ± 177 (3)	31.4 (1)	0.6 ± 0.6 (3)	3 ± 2.5 (3)
Continuous*	29 827 ± 4 240 (3)	11 811 ± 4 046 (3)	0 (3)	NA	0 (3)	0.3 ± 0.3 (3)
Non-treated	26 832 ± 2 372 (3)	8 356 ± 2 252 (3)	299 ± 299 (3)	16.8 (1)	2.8 ± 2.8 (3)	12 ± 9 (3)
<b>August sampling</b>						
February	27 952 ± 2 598 (4)	8 337 ± 853 (4)	3 094 ± 1 172 (4)	58.8 ± 13.4 (4)	8 ± 2.8 (4)	156 ± 75 (4)
August	24 032 ± 8 141 (4)	6 247 ± 1 922 (4)	4 144 ± 1 119 (4)	45.2 ± 8.3 (4)	37.7 ± 17 (4)	139 ± 52 (4)
February + August	24 317 ± 3 018 (4)	7 191 ± 1 155 (4)	4 261 ± 1 585 (4)	72.2 ± 11.9 (4)	13.9 ± 5.8 (4)	187 ± 69 (4)
Continuous*	28 524 ± 476 (4)	8 013 ± 1 496 (4)	111 ± 69 (4)	60.6 ± 39.4 (2)	0.4 ± 0.4 (4)	35 ± 17 (4)
Non-treated	13 882 ± 6 606 (4)	2 895 ± 1 176 (4)	2 666 ± 873 (4)	59.9 ± 13.9 (4)	52.9 ± 45.8 (4)	148 ± 61 (4)

Each colony received one of the treatments described in *table 1*. Values are average ± standard error. Numbers in parentheses = *n*. Colonies were sampled in February before treatments were begun. \* Apistan was present in these colonies at time of sampling.

exceed economic threshold, and in the present study mite populations approaching 6 000 were associated with decreasing bee populations and increasing brood disease in September–October and poor colony condition the following January (table I).

In summary, we believe that in this study only the August-, February + August- (1997) and August-treated (1998) colonies were in fact at economic threshold when they were treated in August. Moreover, the August parameters of these mature colonies (tables V and VI) and the August threshold parameters we developed for first-year colonies [9] are noticeably congruent. Thus, we suggest that a true late-season economic threshold for varroa mites in the southeastern USA lies within a range of mite populations of 3 172–4 261, ether roll mite levels of 15–38, and bottom board insert mite levels of 59–187 in colonies with bee populations of 24 808–33 699. Our present results suggest that overwintering colonies can benefit from an additional early-season (February) treatment. This benefit was realized in colonies which in February had the following average values: mite populations 7–97, ether roll 0.4–2.8, bottom board inserts 0.6–10.2, and bee populations 12 606–13 500 (tables V and VI). And finally, our present results suggest that apiary isolation has the benefit of delaying the onset of economic thresholds; August colony mite levels were considerably lower in February + August-treated colonies in 1998 under conditions of isolation than they were in 1997 under conditions of mite immigration.

Economic thresholds vary by region [2], owing to such differences as brood rearing season and possibly unknown genetic differences in bee and mite populations. Thus, we believe that this type of research should be replicated in other regions. Our protocols described here and earlier [9] were able to identify mite levels that were damaging and levels that were tolerable, and we believe that these protocols can give practical results elsewhere.

### 3.6. Sensitivity of mite sampling methods, 1997 data

The relationship of ether roll mite levels with colony mite populations was described by a model ( $r = 0.74$ ) with a simple positive linear term (coefficient =  $0.013 \pm 0.001$ ). The same relationship with bottom board inserts was described by a model ( $r = 0.76$ ) with linear ( $0.05 \pm 0.01$ ), quadratic ( $-5.2 \times 10^{-6} \pm 1.8 \times 10^{-6}$ ), and cubic ( $1.6 \times 10^{-10} \pm 0$ ) terms. The simple positive linear relationship in the first model means that one can expect increasing ether roll levels to reliably predict increasing colony mite populations. The complicated cubic relationship with bottom board inserts means that high colony mite population may yield high or low insert levels.

These results diametrically contradict our earlier work in which bottom board inserts yielded the more straightforward linear model [9]. Bottom board inserts is generally regarded as the more reliable sampling method [12, 13] because inserts potentially sample the entire adult bee population. We did not use acaricide to hasten mite drop, so our values reflect natural mite drop off adult bees during the sampling interval ( $20 \pm 4$  h). Excluding the continuously treated colonies which contained Apistan strips at the time of sampling, the number of mites retrieved on inserts expressed as a percentage of colony mites on adult bees was 2.8–23.9 % (derived from table V) which is similar to the daily range of 0.6–17.8 % published by others [5].

The ether roll test is easy to do, and our present results suggest that it can reliably predict colony mite populations. Collectively, these results suggest that threshold-based treatment decisions should be based on an average of several samplings. The method of sampling, whether by ether roll or bottom board inserts, may be of secondary importance.

**Table VI.** Colony parameters at different sampling periods during the year in apiaries managed to minimize immigration by *Varroa jacobsoni* (1998 study).

Colony treatment	Colony bee population	Sealed brood cells	Colony mite population	Percentage mite population on adult bees	Ether roll (mites/300 bees)	Bottom board inserts (mites)
February sampling						
NA	13 500 ± 2 507 (12)	5 173 ± 964 (12)	7 ± 7 (12)	100 (1)	0.4 ± 0.2 (12)	0.6 ± 0.2 (12)
May sampling						
February	40 252 ± 9 772 (3)	12 961 ± 1 652 (3)	84 ± 84 (3)	100 (1)	0.6 ± 0.6 (3)	2 ± 2 (3)
August	33 975 ± 7 453 (4)	11 790 ± 3 433 (4)	306 ± 178 (4)	68.7 ± 20.5 (2)	2.4 ± 2.1 (4)	12.5 ± 7.2 (4)
February + August	42 940 ± 7 866 (4)	12 449 ± 2 645 (4)	25 ± 25 (4)	40.2 (1)	0.3 ± 0.3 (4)	4.8 ± 2.8 (4)
Continuous*	48 325 ± 10 741 (3)	15 952 ± 1 233 (3)	45 ± 45 (3)	100 (1)	0 (3)	0.3 ± 0.3 (3)
Non-treated	43 130 ± 7 258 (4)	14 167 ± 1 126 (4)	355 ± 251 (4)	59.6 ± 24.7 (2)	1.8 ± 1.1 (4)	5 ± 2.6 (4)
August sampling						
February	29 738 ± 1 301 (3)	5 415 ± 985 (3)	1 090 ± 836 (3)	57.1 ± 29.7 (3)	3.1 ± 2.2 (3)	8 ± 7.5 (3)
August	33 699 ± 5 101 (4)	5 784 ± 523 (4)	4 057 ± 1 943 (4)	60.7 ± 10.4 (4)	27.2 ± 17.3 (4)	58.8 ± 32.4 (4)
February + August	25 675 ± 5 435 (4)	5 247 ± 1 395 (4)	730 ± 198 (4)	57 ± 10.4 (4)	3.3 ± 0.8 (4)	10.8 ± 6.5 (4)
Continuous*	25 365 ± 2 393 (3)	4 052 ± 1 731 (3)	0 (3)	NA	0 (3)	0.3 ± 0.3 (3)
Non-treated	27 197 ± 4 641 (4)	4 222 ± 1 105 (4)	2 309 ± 853 (4)	53.1 ± 5.5 (4)	12.3 ± 5.1 (4)	47 ± 25.4 (4)

Each colony received one of the treatments described in *table 1*. Values are average ± standard error. Numbers in parentheses = *n*. Colonies were sampled in February before treatments were begun. \* Apistan was present in these colonies at time of sampling.

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**Résumé – Seuil de dégâts économiques pour *Varroa jacobsoni* dans le sud-est des États-Unis.** Dans ce travail nous apportons la preuve d'un seuil économique pour *Varroa jacobsoni* dans des colonies ayant hiverné dans des conditions qui soit facilitent, soit entravent la réinfestation par l'acarien.

En février 1997 nous avons installé dans la région du Piedmont de la Géorgie et de la Caroline du Sud, États-Unis, 60 colonies d'abeilles ayant hiverné. Les ruchers étaient infestés par *V. jacobsoni*. Chaque colonie au sein d'un même rucher a reçu l'un des traitements suivants : 1) traitement à l'Apistan® en février, 2) en août, 3) en février et en août, 4) en continu, 5) pas de traitement. Ce dispositif permettait aux acariens des colonies non traitées de réinfester les colonies traitées d'un même rucher. En février, mai et août, on a déterminé dans certaines colonies les paramètres suivants : taille de la population d'abeilles, poids corporel moyen des abeilles, nombre de cellules de couvain operculé, taille de la population d'acariens et niveau d'infestation à l'aide du « test du rouleau d'éther » [9] et d'un lange adhésif placé sur le plateau de fond. En septembre et en octobre, toutes les colonies survivantes ont été démantelées afin de mesurer ces paramètres ainsi que le pourcentage de cellules de couvain présentant des symptômes visibles de maladie.

Le protocole de base a été répété en 1998, mais cette fois-ci la réinfestation a été réduite au minimum. Nous avons installé 40 colo-

nies (2 états × 5 ruchers/état × 4 colonies/rucher). Chaque rucher était au moins distant de 0,6 km des autres colonies connues. Dans un même état chaque rucher a reçu l'un des cinq traitements et les colonies d'un rucher ont toutes reçu le même traitement. Ainsi le risque de réinfestation des colonies traitées par les acariens des colonies non traitées était réduit au minimum. L'expérience s'est terminée en octobre ( $n = 36$  colonies).

Nos résultats suggèrent que seules les colonies traitées en août 1997, en février + août 1997 et en août 1998 étaient au seuil économique lorsqu'elles ont été traitées en août. En outre les paramètres pour le mois d'août de ces colonies (tableaux V et VI) concordent nettement avec les paramètres du seuil en août que nous avons développés pour des nuclei. Nous estimons donc qu'il existe un véritable seuil économique d'arrière saison pour l'acarien *V. jacobsoni* dans le sud-est des États-Unis, situé dans une fourchette de population d'acariens comprise entre 3 172 et 4 261. Cela correspond à un nombre d'acariens entre 15 et 38 par rouleau d'éther et entre 59 et 87 par lange sur le plateau pour une colonie de 24 808 à 33 699 abeilles. La concordance entre cette étude et la précédente [9] suggère qu'en arrière saison des populations d'acariens ~ 6 000 sont au-dessus du seuil économique. Les colonies qui hivernent peuvent bénéficier d'un traitement supplémentaire d'avant-saison (février). C'était le cas des colonies qui en février avaient les valeurs moyennes suivantes : population d'acariens 7-97, rouleau d'éther 0,4-2,8, langes 0,6-10,2, population d'abeilles 12 606-13 500 (tableaux V et VI). Un traitement acaricide continu n'a jamais réussi à augmenter significativement la population d'abeilles ni la quantité de couvain par rapport au traitement de février + août, plus traditionnel. Enfin nos résultats montrent que l'isolement d'un rucher retarde le dépassement des seuils économiques ; les niveaux d'acariens dans les colonies d'août étaient considérablement plus faibles dans les colonies traitées en février + août dans les conditions d'isole-

ment de 1998 que dans celles de 1997 en conditions de réinfestation. © Inra/DIB/AGIB/Elsevier, Paris

***Apis mellifera* / *Varroa jacobsoni* / lutte intégrée / gestion de la résistance chimique**

**Zusammenfassung – Ökonomische Schadensschwelle durch *Varroa jacobsoni* im Südosten der USA.** In dieser Arbeit belegen wir die ökonomische Schadensschwelle für Schäden durch *Varroa jacobsoni* an überwinterten Wirtschaftsvölkern unter Milbeninfektion fördernden oder behindernden Bedingungen.

Ab Februar 1997 betreuten wir 60 überwinterte Bienenvölker in der Piedmont Region von Georgia und Süd Carolina, USA. Die Bienenstände waren mit *Varroa jacobsoni* infiziert. Jedes Volk pro Stand wurde einer der folgenden Behandlungen unterzogen: 1) Behandlung mit Apistan im Februar, 2) Behandlung im August, 3) Behandlung im Februar und August, 4) Dauerbehandlung und 5) keine Behandlung. Bei dieser Anordnung konnten die Milben von unbehandelten Völkern in behandelte innerhalb des Bienenstandes eindringen. Im Februar, Mai und August wurden einige Völker untersucht, um die Volksstärke, das mittlere Körpergewicht der Bienen, die Zahl der verdeckelten Brutzellen, die Milbenpopulation und den Milbenbefall durch einen 'Ätherrolltest' und mit klebriger Bodeneinlage zu bestimmen. Im September und Oktober wurden alle überlebenden Völker ( $n = 55$ ) aufgelöst, um die oben aufgeführten Parameter und zusätzlich den Prozentsatz der Brutzellen mit sichtbaren Schadenssymptomen zu messen.

Dieser Versuch wurde 1998 wiederholt, nur wurde diesmal die Milbeneinwanderung minimiert. Dazu stellten wir 40 Völker auf (2 Staaten  $\times$  5 Bienenstände pro Staat  $\times$  4 Versuchsvölker pro Stand). Jeder Stand war mindestens 0,6 km von anderen bekannten Völkern entfernt, auf jedem Stand pro Staat wurde eine der 5 Behandlungen angewendet,

und alle Versuchsvölker innerhalb eines Standes wurden gleich behandelt. Dadurch wurde das Risiko einer Rückinfizierung durch Milben aus nicht behandelten Völkern innerhalb eines Standes minimiert. Der Versuch wurde im Oktober aufgelöst ( $n = 36$  Völker).

Nach unseren Daten scheinen nur die 1997 im August bzw. im August + Februar und die 1998 im August behandelten Völker unter der ökonomischen Schadensschwelle zu bleiben. Zusätzlich stimmen die August Parameter dieser Wirtschaftsvölker (Tabellen V und VI) und die August Schwellenparameter, die wir für Ableger entwickelten, deutlich überein. Deshalb nehmen wir an, daß eine reale spätsaisonale Schadensschwelle durch Varroamilben im Südosten von USA bei einer Milbenpopulation von 3 172 – 4 261 besteht. Das entspricht einer Ätherroll-Milbenzahl von 15–38 und einer Milbenzahl auf der Bodeneinlage von 59 – 187 bei einer Volksstärke von 24 808 bis 33 699 Bienen. Übereinstimmende Daten dieser Untersuchung und unserer früheren Arbeit [9] legen nahe, daß eine spätsaisonale Milbenzahl von über 6 000 über der ökonomischen Schadensschwelle liegt.

Überwinterte Völker können von einer zusätzlichen Frühjahrsbehandlung (Februar) profitieren. Dieser Vorteil wurde bei Völkern mit Volksstärken von 12 606–13 500 Bienen erreicht, bei denen im Durchschnitt folgende Werte im Februar vorlagen: Milbenpopulation 7–97, Ätherrolltest 0,4–2,8; Bodeneinlage 0,6–10,2 (Tabellen V und VI). Eine Dauerbehandlung erreichte nie eine signifikant höhere Bienenzahl oder Brutmenge als die herkömmliche Behandlung im Februar + August. Schließlich zeigen unsere Ergebnisse auch, daß die Isolation von Bienenständen das Überschreiten der Schadensschwelle behindert. Im August 1998 war der Milbenbefall unter isolierten Bedingungen bei im Februar + August behandelten Völkern verhältnismäßig niedriger als es 1997 unter Bedingungen mit Rückinfizierung durch Milben der Fall war. © Inra/DIB/AGIB/Elsevier, Paris

***Apis mellifera* / *Varroa jacobsoni* /  
integrierte Behandlung / Management  
der chemischen Resistenz**

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