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ORIGINAL PAPER

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Development of the Mite, *Varroa jacobsoni* Oud., in the Honeybee, *Apis mellifera* L., in Michigan, USA, and a Comparison of Diagnostic Methods for Detection of the Mites

Abstract: The study was carried out with fifteen newly established packages of honeybees that were divided into three groups. Each group was inoculated with 5, 10, and 25 *Varroa* mites as an initial inoculum, respectively. The groups were widely separated from each other to reduce drifting between treatments. The development of the mite infestation was monitored every other week from May until October, 1994. Estimation of the different mite populations was based on a 72-hour mite downfall on a wire-protected sticky board, and a sample of 100 adult bees, plus 100 worker and 100 drone pupae. The colony population of adult bees and brood cells was estimated at each sample period as well. Over the period of one summer, the mite population increased 81, 188, and 193-fold for the groups that were infected with 5, 10 and 25 mites, respectively. Based on 72-hr. mite downfall, the population estimates were 2,032, 1,880 and 968 mites for the groups that started with 25, 10, and 5 mites, respectively. The estimate of the number of mites from the adult bee population was larger than the estimate obtained from sticky boards. However, variation in mite populations between the colonies was large. The sticky board method was better than adult bees and brood samples for the initial detection of mite populations at low infestation levels.

Keywords: Honeybee, *Varroa jacobsoni,* mites, Michigan, *Apis mellifera L., sticky board method*

تطور تجمع حلم الفاروا *Varroa jacobsoni* في طوائف نحل العسل *Apis mellifera* في ولاية ميتشجان بالولايات المتحدة ومقارنة طرق التشخيص لمعرفة وجود الحلم في بداية الإصابة

أحمد الغامدي و روجر هوبنجارنر

المستخلص: تم تقسيم عدد خمسة عشر خلية جديدة من النحل المرزوم إلى ثلاث مجموعات ثم أعديت هذه المجموعات بـ 5 ، 10 و25 حلم كلقاح أولى على التوالي. وضعت المجموعات في مناطق بعيده عن بعضها البعض حتى لا يتم التداخل بين التجارب. تمت ملاحظة زيادة نسبة الإصابة كل أسبوعين من شهر مايو حتى أكتوبر 1994م حيث قدرت أعداد الحلم في الطوائف بطريقتين، الأولى أعتمدت على أعداد الحلم الميتة موت طبيعي، المتساقط كل 72 ساعة على لوح شبكي لاصق ، والثانية أعتمدت على أعداد الحلم الموجودة على 100 نحلة بالغة و100 حضنه مغلقه تحتوي على عذارى شغالات و 100 حضنة مغلقة تحتوي على عذارى وذكور . تم أيضاً تقدير كل من أعداد النحل والحضنة في كل خلية بحيث يسهل تقدير العدد الكلي للحلم بالطوائف. خلال فترة صيف واحدة تضاعفت أعداد الحلم 81 ، 188 ، 193 مرة للمجموعات التي تم تلقيحها (عدواها) بـ 5 ، 10 ، 25 حلم على التوالي وذلك عند التقدير بناء على أعدد الحلم الميتة التي تسقط كل 72 ساعة. أما عند استخدام طريقه النحل الحي فكانت التقديرات 2032 ، 1880 و 968مرة للمجموعات التي بدأت بـ 25 ، 10 ، 5 حلم على التوالي. كانت تقديرات أعداد الحلم الناتجة عن استخدام طريقة النحل الحي أعلى من التقديرات الناتجة من استخدام طريقة الحلم الميت على اللوح اللاصق وعموماً كانت هناك اختلافات كبيرة بين أعداد الحلم بين الطوائف. اتضح أن طريقة اللوح الشبكي اللاصق كانت افضل من طريقة عينات النحل الحية لمعرفة أعداد الحلم في حالة الإصابة المبدئية.

كلمات مدخلية: نحل العسل، ، حلم الفاروا، طوائف، ولاية ميتشجان، طرق التشخيص

Introduction

The mite *Varroa jacobsoni* Oudemans (Acari: Mesostigmata) is a parasite of the honeybee *Apis cerana* and *Apis mellifera* L. It is present in more than 85 countries and is currently considered one of the most serious pests of *A. mellifera* colonies in most of the world (Matheson, 1993, 1994 and 1995).

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There are a number of factors that affect the population growth rate of the mite. Reproduction occurs in sealed honeybee brood cells. Hence, mite population growth occurs only in the presence of brood. Other factors include host-specific effects of the bee (Moritz and Hanel 1984, Buchler and Drescher 1990, Rosenkranz *et al.* 1990, Moretto *et al.* 1991b, Otten 1991, Kulincevic *et al.* 1992), geographic and climatic factors (De Jong *et al.* 1984, Moretto *et al.* 1991a) and possibly *Varroa* genotypes (Delfinado-Baker and Houck 1989, De Guzman *et al.* 1996).

In the Mediterranean climate of California, the initial population increased 300-fold during one

year (Kraus and Page 1995). In colder, temperate climates the increase averages about 10-fold per year (Ritter 1984, Fries *et al.* 1991a, Korpela *et al.* 1992) but can increase up to 100-fold within one summer (Fries *et al.* 1991b). In tropical climates the parasite seems to be less virulent (Ritter and De Jong 1984). In sub-tropical climates the infestation rate is lower than in temperate climates (Moretto *et al.* 1991b). If the population is not controlled, colonies infested with *Varroa jacobsoni* die in three to four years (Ritter 1984).

An important part of control is the initial detection of the mite. Early detection with low infestation rates is important in bee colony management. Ritter (1981) and De Jong (1984) suggested the following diagnostic method: count mites that fall from the bee due to natural causes, examine bee samples for phoretic mites, and check capped brood samples for mites while they are reproducing. For low infestation levels (below ten mites), the use of acaricides may be the only effective method for detecting the mite with acceptable levels of precision in broodless colonies (Ritter, 1984). If the mite population is between ten and 100, then the examination of hive debris should allow for detection (Ritter, 1984). In fact, Ritter (1984) reported that adult bee or brood examinations are insufficient for population levels below 100 mites per colony. Liebig *et al.* (1984) reported a close correlation between mites collected in hive debris and the size of the *Varroa* mite population. Fries *et al.* (1991a) also compared different diagnostic methods for detection of *Varroa* mite infestation at low levels and found that examining debris was more reliable than the brood itself. They said it was preferable to other methods because of its simplicity and efficacy.

The objective of the study was to investigate the population growth of *Varroa jacobsoni* under Michigan conditions and investigate methods of detecting mite infestations at low rates and correlate it to mite population levels in Michigan honeybee colonies.

Materials and methods

Fifteen packages *of Apis mellifera* L. bees (0.9k of bees per package) were classified into three groups, each containing five packages. They were installed into single chamber Langstroth hives containing honey and comb foundation on May 1, 1994 in East Lansing, Michigan. Each package was treated with two Apistan® strips (10% fluvalinate). In addition, the packages had also been treated with an Apistan® strip during shipment. The three groups were placed in separate locations on the Michigan State University Farm in an effort to reduce drifting between groups. Robbing screens were also installed on the colonies to prevent robbing and drifting (Hoopingarner, 1982). All groups were managed optimally as for honey production and were treated with fumagillin in May, 1994 and with terramycin antibiotic in May and July, 1994. Then all the three groups were inoculated with 5, 10 and 25 mites, respectively. The mites were removed from their host bees using CO_2, collected in small tubes and introduced directly upon the bees on May 15th, 1994.

Total mite population was estimated using adult bees and sealed brood samples (workers and drones) and hive debris. Adult bee estimates were taken every two weeks, beginning May 29th. Between 100 and 200 live adult bees were taken from brood combs and stored in a deep freezer. The mites were separated from the bees by vigorously shaking the bees in 70% ethanol for 3 to 4 minutes. The mites were washed from the bees using a hand-shower over a double wire screen. Number of bees and mites were counted to determine the level of infestation on adult bees. Data were adjusted to the number of mites/100 bees.

Samples of 100 sealed worker and 100 drone brood cells were examined on a 12-day cycle when brood was present. The cells were opened and adult mites present were counted. The total amount of brood in the colonies was estimated in each colony using the double-sampling technique described by Rogers *et al.* (1983). The adult bee population was estimated as described by Burgett and Burikam (1985).

The hive debris was collected on a weekly basis from the sticky board placed on the bottom of the hive to monitor the natural mortality. A wire screen prevented bees from gaining access to debris or mites (Ritter, 1981). The sticky board was placed in each colony for three days/week and adult female mites were counted directly from the sticky board.

Total number of mites in each colony with brood was estimated using the following two methods: (1) daily downfall: average daily mite downfall x 120 (Liebig *et al.* 1984); and (2) mites on bees and brood: the infestation rate of sampled bees x total number of adult bees/colony + infestation rate of sampled brood x total number of brood cells/colony (Fuchs and Koeniger, 1984).

Results

The entire five colonies that were inoculated with 25 mites were lost during the winter following the analysis. Their deaths could not be attributed completely to *Varroa*, but may be also due to the cold winter. The maximum number of mites recovered from the sticky board was recorded on the September 17th sampling date. The number of dead mites ranged from 40 to 63 and the adult bee infestation was between 24 to 34%.

Four of the five colonies that were inoculated with 10 mites survived the first winter. The colony that died did not have any more mites than the colonies that survived. The range of mites recovered from sticky boards on September 24th ranged from 25 to 71. The number found in the dead colony were 65 and it had 28% infestation of the adults in November.

Three of the five colonies that were inoculated with 5 mites died over winter. Once again, there were no significant differences in the number of mites recovered on the sticky board among the colonies. The two that survived had 26 and 29 mites on the sticky board in September, while the three that died had 18, 25, and 62 mites on their boards and entered the winter with 14, 17 and 21% adult bee infestation rate. Two of the colonies that died had more foul brood infection than the rest, although they received the same medication treatments in May and July as the other colonies.

When we used the sticky board method of calculating mite density (Fig. 1), the population ranged from 1,640 to 2,520 mites, with an average of 2,032 in the 25 mite inoculated treatment. The 10-mite inoculated treatment had a smaller peak, ranging from 1,200 to 2,200, with an average of 1,880 mites. The last treatment (5 mites) had an average of 968 mites, with a range from 520 to 1,480.

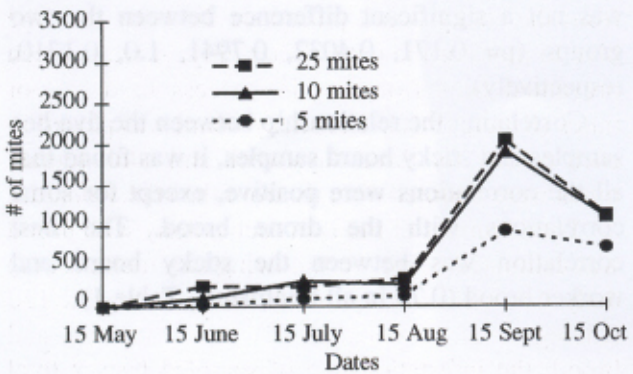

Figure 1. Average number of varroa mites per treatment group (5, 10, 25 mites in initial inoculation), calculation based on average daily mite downfall * 120 (Liebig *et al.* 1984). The last sampling date had no brood in the colonies.

The mite estimate from the adult-bee population plus brood mites (Figure 2) was larger than the mite estimate obtained from sticky board counts for all treatments. It averaged 1,760 (range 578-2,375), 2,247 (range 1,347-2,775), and 3,119 (range 2,130-3,834), for the 5, 10, and 25 mite treatments, respectively.

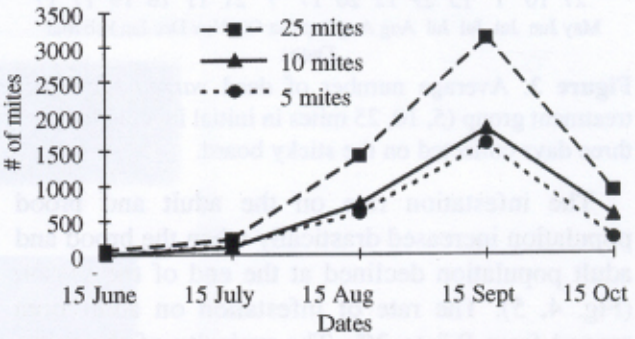

Figure 2. Average number of varroa mites per treatment group (5, 10, 25 mites in initial inoculation), calculation based on number of mites per live bee* number of bees + number of mites per brood cell * number of brood cells. Samples on 15th of October from adult bees only.

Over the period of one summer, the mite population increased 193, 188, and 81-fold for the groups that were infested with 5, 10 and 25 mites respectively, with an average of 154-folds when using the sticky board method of estimating mite populations. On the other hand, when estimating the population from the adult bee plus brood method, there was a 352, 225, and 125-fold increase, respectively, with an average of 234-fold.

The colonies that did survive the winter were weak the following spring and only covered one or two combs and they had an average of 50% brood infestation rate on mid May 15th. All of the colonies collapsed in September of the second year.

The number of mites found on the sticky boards (Figs. 1 & 3) increased slowly during the first year until the middle of August for all three treatments. In the middle of August there was a sharp increase in the number of mites, which peaked on September 17th for the 10 and 25 mite inoculated colonies. This peak plateaued for two weeks and then gradually declined until the population of mites started to increase in March and April of the second year. For the third treatment (colonies inoculated with 5 mites), the downfall began to markedly increase at the end of August, similar to the other two treatments. It sharply increased until the 17th of September (where the other two treatments peaked), but continued to increase until the 7th of October. The peak of this group did not have a plateau, but the number of mites declined immediately, until increasing in March and April when the bee colony began its brood rearing.

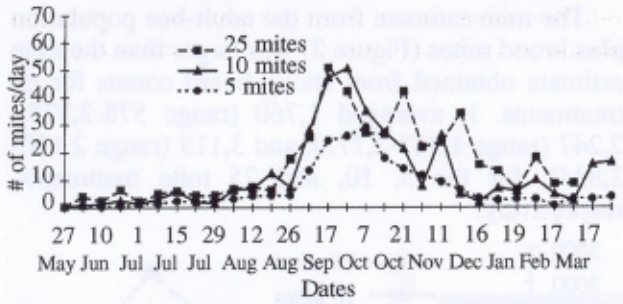

Figure 3. Average number of dead *varroa* mites per treatment group (5, 10, 25 mites in initial inoculation) per three days collected on the sticky board.

The infestation rate on the adult and brood population increased drastically when the brood and adult population declined at the end of the season (Fig. 4, 5). The rate of infestation on adult bees ranged from 0.3 to 3%. The majority of the mites were in summer, while the highest number was at the end of November wintering bees when there was no brood and the bee population was low. The adult infestation rate varied widely between colonies within the same group. For example, in November the infestation rates were 25-34%, 13-28% and 7-17% for the groups with 25, 10 or 5 mites/colony, respectively. Also wide variation occurred in the number of mites recorded in worker cells, *e.g.*, the number of mites ranged between 30-200 mites per 100 worker cells in the group with 25 mites/colony on October 4th.

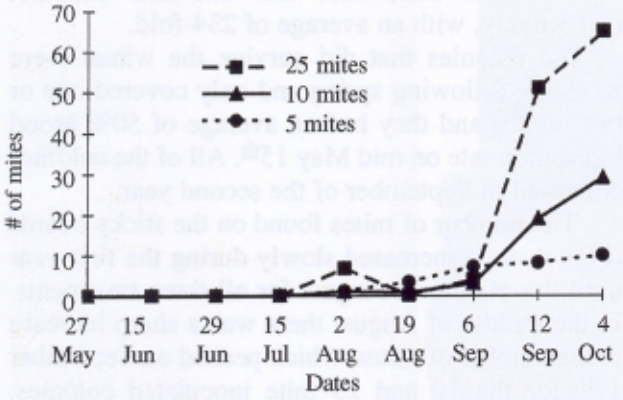

Figure 4. Average number of mites per 100 worker cells per treatment per group (5, 10 and 25 mites in initial inoculation).

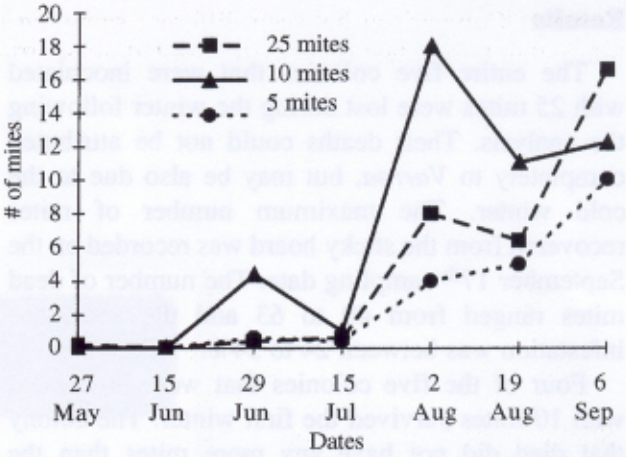

Figure 5. Average number of mites per 100 drone cells per treatment group (5,10 and 25 mites in initial inoculation).

One of the objectives of this study was to compare the two methods for estimating the mite population. The sticky board method is statistically better for the initial detection of mite populations at low infestation levels ($p<0.001$, Colton, 1974). For example, on the first sampling date (15 June), the sticky board detected mites in 12 of the 15 colonies while the adult and brood population detected mites only 2 out of 15 times. When populations grew, by 15 July, the sticky board detected mites in all 15 colonies, whereas the adult and brood population detected mites in 9 of the 15 colonies. Although the sticky board had a higher rate of detection (100%), the two methods were not found statistically different from each other on October sampling date ($p<0.171$). When there was no brood present, there was no significant difference between sticky board and sampling of live bees.

The next question concerned the differences between examining the bee brood vs. the adult bee population. Neither one was good at detecting mite populations at low levels. On all five dates, there was not a significant difference between the two groups ($p= 0.171, 0.4032, 0.7941, 1.0, 0.1710$, respectively).

Correlating the relationship between the live bee samples and sticky board samples, it was found that all the correlations were positive, except for some correlations with the drone brood. The best correlation was between the sticky board and worker brood (0.79 in all treatments, Table 1).

Table 1. Comparison between different sampling methods for detection of *Varroa jacobsoni* at different infestation levels, including their correlation coefficient (r) and the associated level of significance (p)

Sampling Methods	r				p			
	5	10	25	All tmt.	5	10	25	All tmt.
Sticky board v. adult bees	0.74	0. 37	0.32	0.40	0.001	0.111	0.176	0.002
Sticky board v. live bees	0.73	0.83	0.81	0.77	0.001	0.001	0.001	0.001
Live bees v. adult bees	0.90	0.75	0.65	0.74	0.001	0.001	0.002	0.001
Sticky board v. worker brood	0.48	0.91	0.85	0.79	0.033	0.001	0.001	0.001
Mite in worker v. adult bees	0.47	0.44	0.25		0.36	0.053	0.29	
Sticky board v. drone brood	-.13	-.21	-.32	-.14	0.61	0.38	0.21	0.29
Mite in worker v. mite in drone	-.24	-.16	-.26		0.35	0.49	0.31	
[2]Live bees v. adult bees	0.89	0.74	0.59		0.001	0.001	0.013	
Live bees v. drone brood	-.30	0.066	0.69		0.24	0.78	0.002	
Adult bees v. drone brood	-.26	-.5	0.096		0.30	0.83	0.71	
[3]Sticky board v. adult bees	0.51				0.050			

[1] Brood present
[2] Only when drone present
[3] No brood present

Along with the correlations, the data were fit to a series of linear regression models. It was found that the number of mites recovered on the sticky board could be explained by the following linear regression ($r^2 = 0.656$, $p<0.001$):

$$TMS = 199.8 + 0.242 *AB + 0.682 *WB - 9.467 *DB$$

Where:

TMS = Total mites on sticky board
AB = Mites found on adult bees
WB = Mites found in worker brood
DB = Drone brood

Since the p-value associated with the constant and adult bees and worker brood was almost significant ($p\leq0.053$) while the p-value associated with the drone brood was not ($p< 0.341$), the model was revised to exclude the drone brood from the estimate. When the drone brood was removed, the r^2 was still significant ($r^2 =0.518$, $p < 0.001$), and the equation is as follows:

$$TMS = 282.449 + 0.348 *AB + 0.594 *WB$$

Where:

TMS = Total mites on sticky board
AB = Mites found on adult bees
WB = Mites found in worker brood

The p-value associated with each of these coefficients was highly significant ($p<0.005$). The number of mites found in the worker brood alone was the best single predictor of number of mites on the sticky board. This factor explained 46% of the variability in sticky board numbers (p- value < 0.001). Figures 6 and 7 show the brood and bee population, respectively.

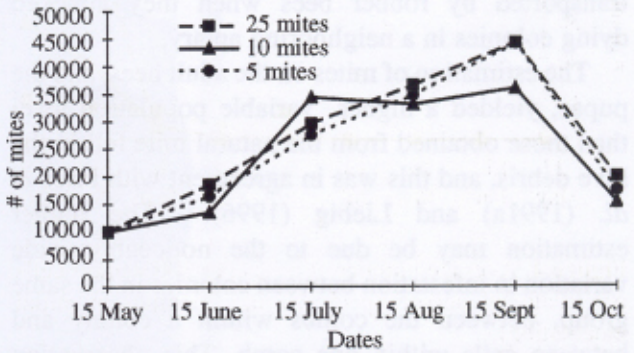

Figure 6. The average bee population per treatment group (5, 10 and 25 mites in initial inoculation).

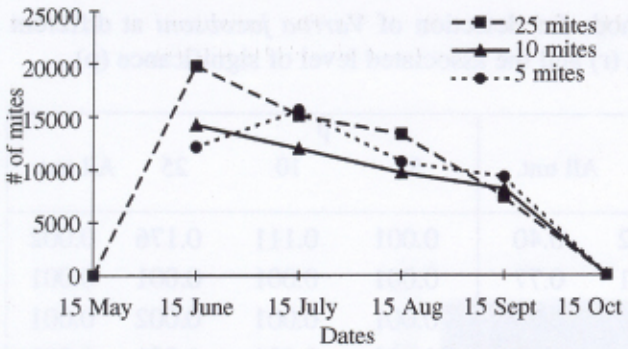

Figure 7. The average total brood population per treatment group (5, 10 and 25 mites in initial inoculation).

Discussion

When mite population estimates were based on the natural mite downfall in the hive debris the data show that the mite population can increase an average of 154-fold during one summer. Fries *et al.* (1991b) reported that mite populations could increase more than 100-fold within one summer in a cold climate. Based on the data from the group inoculated with five mites at the beginning of the experiment, it was noted that the mite population increased at least 50 times faster than in the Fries *et al.* study. This difference may be due to the transport of some mites from outside the experimental unit despite precautions taken to avoid such a situation. This also may explain the large variation between the colonies within the group. Greatti *et al.* (1992) found daily re-infestation rates in high density honey bee areas of up to 100 mites/colony. Imdore and Kilchenmann (1993) estimated between 3000 - 4000 mites/colony were transported by robber bees when they attacked dying colonies in a neighboring apiary.

The estimation of mites on the adult bees, and the pupae, yielded a higher variable population level than those obtained from the natural mite fall in the hive debris, and this was in agreement with Fries *et al.* (1991a) and Liebig (1996). The higher estimation may be due to the noticeably wide variation in infestation between colonies in the same group, between the combs within a colony and between cells within one comb. This observation was in agreement with those of Rosenkranz *et al.* (1984), Fuchs (1985) and Pappas and Thrasyvoulou (1986), who reported that infestation within a single colony varies from one brood comb to another and even from one area of the comb to another. Also the infestation of the adult bees varies from one comb to another. Liebig (1996) reported that adult-bee estimates are more likely to be affected by the part of the hive from which the sample was taken. Ellis and Baxendale (1994) stated that the distribution of mites among adult bees and brood were affecting the results of the sampling method. Both Fries *et al.* (1991) and Liebig (1996) suggested that the natural mite downfall was more reliable for estimating the incidence of *Varroa*.

Colony-mite population was highest in September and this may be due to the high brood production in August and in the first two weeks of September. The sharp increase of mites from mid August to mid September also could be a result of transportation of the mites from the outside tested colonies.

The sticky board is the most reliable method for detecting mite infestations when the population is low. In fact, at zero time of the study the sticky board acted as a superior method for detecting mite populations than the adult bee and brood methods. However, the number of mites found in the worker brood was highly correlated to the mite downfall ($r = 0.79$). This is in agreement with the findings of Boot *et al.*(1994) who reported that 18% of the mites introduced into a colony were found in the sticky board when bees emerged from brood cells. The correlation with the drone brood was negative (-0.14). This was probably due to the small number of drone cells available for the mites in the study colonies.

Detection of mite rates from the sticky board was either greater or equal to those from the examination of adult bee populations. As the mite population grew, the adult bee results became closer to the mite downfall results. This concurs with Fries *et al.* (1991a). Therefore, it is recommended that beekeepers use the sticky board to at least detect initial populations.

Although the examination of the worker and drone brood was not as good a method of detecting mites as examining mite downfall from the sticky board, especially at low population levels, the number of mites found in the worker brood was highly correlated to the mite downfall ($r = 0.79$). It also was significant in both the multiple regression and the simple linear regression model ($r^2 = 0.656$ and $r^2 = 0.460$, respectively).

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