

Controlling Tracheal Mites (Acari: Tarsonemidae) in Honey Bees (Hymenoptera: Apidae) with Vegetable Oil

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J. Econ. Entomol. 87(4): 910-916 (1994)

ABSTRACT Field experiments conducted between 1991 and 1993 demonstrated that treating colonies of honey bees, *Apis mellifera* L., continuously with vegetable oil depressed populations of tracheal mites *Acarapis woodi* (Rennie). In 1992-1993, continuous exposure to oil patties, with or without the antibiotic Terramycin, produced significant control of mites. Five of 11 untreated (control) colonies perished. None of the oil-patty-treated colonies died, and only two of 10 colonies treated with Terramycin patties expired. Oil patties were made from a combination of solid vegetable oil (shortening) and white sugar, with or without the addition of the antibiotic. Two oil patty treatments during the summer of 1991 failed to prevent mite population increases in bees by that fall.

KEY WORDS *Acarapis woodi*, *Apis mellifera*, vegetable oil

SINCE 1984, when the endoparasitic tracheal mite *Acarapis woodi* (Rennie) was discovered in honey bees, *Apis mellifera* L., in Texas, efforts to control it have been intense. Migratory beekeeping practices and sale of package bees and queens rapidly spread the mite throughout the United States, and, by 1988, it had reached Canada. Normal wintering losses vary widely but average $\approx 11\%$ in most states; colony losses attributed to tracheal mites have been reported $>31\%$ in Pennsylvania (Tomasko et al. 1993). Nationally, $>\$165$ million worth of pollination services, bees, and honey has been lost since the mites' detection, jeopardizing a significant component of U.S. agriculture (Robinson et al. 1989).

In general, mite populations flux out of synchrony with bees during the year. That is, when bee numbers increase in spring and reach their height during the summer nectar flow, mite numbers are at their lowest (Dawicke et al. 1992). This decreased proportion of bees with mites is likely caused by a dilution effect from the rapid emergence of large numbers of young, uninfested bees. In addition, the spread of mites may be hindered because infested forager bees would have less regular contact with uninfested younger bees in the months when outside flight activity is highest. During peak mite populations, colonies with moderate to heavy mite infestation levels rear less brood (Eischen 1987), have fewer bees, do not form tight winter clusters, and have increased honey consumption relative to uninfested colonies (Bailey & Ball 1991, Otis & Scott-Dupree 1992).

Control of tracheal mites has been difficult be-

cause they live in the protected environment inside bee trachea. Initially, depopulation of bee colonies was used until it became impractical. Currently, the only registered treatment, Mite-A-Thol (Mann Lake Supply, Hackensack, MN) or menthol crystals, an extract from the plant *Mentha arvensis*, is partially effective because its beneficial qualities are temperature dependent. The other pesticide, Miticur (amitraz, Hoechst-Roussel Agri-Vet, Somerville, NJ), has been withdrawn recently from the market. Formic acid, a potentially effective agent, is not registered for this use. The need for expedient and bee-safe controls for these mites is imperative.

Vegetable oil has long been used in its solid form (shortening) mixed with granulated sugar as a carrier for antibiotics to treat colonies for bee diseases. Beekeepers are seeking products that can be used inside colonies without contaminating honey, pollen, or wax. Serendipitously, vegetable oil was discovered to keep bees mite-free in laboratory experiments (Gary & Page 1987). Also, it has shown potential for controlling mites under field conditions (Delaplane 1992a, Smith et al. 1991). However, researchers treating bees with vegetable oils (Smith et al. 1991) or combinations of oil and other ingredients, such as menthol (Delaplane 1992a), have found variable success. As a result, it is not yet clear whether oil provides satisfactory mite control. The resolution of this inconsistency was the main thrust of our research. Studies were designed to test whether control of mites could be obtained using vegetable oil-sugar patties and to test if exposure conditions were important.

Additionally, the antibiotic Terramycin (Pfizer, New York) was investigated more thoroughly because mite-infested colonies often have associated bacteria and other infections (Bailey & Ball 1991, Otis 1991). Terramycin commonly is applied to colonies for American foulbrood disease control and now is registered for this use.

Material and Methods

Summer Application of Oil Patties. The first study, conducted in 1991, was designed to measure mite infestations throughout a summer season and to determine whether midseason oil patty treatment would prevent mite levels from increasing in the fall. Twenty honey bee colonies from The Ohio State University's Rothenbuhler Honey Bee Laboratory were selected at random from two apiary sites ≈ 20 mi apart around the Columbus metropolitan area. Colonies were divided randomly into two treatments of 10, with each treatment consisting of two deep brood chambers and each containing nine frames. Each colony received either an oil-sugar patty or a sugar-only patty. The 300 g oil-sugar patty was made of 1:2 Crisco (Procter & Gamble, Cincinnati, OH) vegetable shortening; white sugar; the sugar-only patty was made with granulated sugar and corn syrup. Treatments were administered on 6 June and 23 July on top of the frames (top bars) between the two brood-nest chambers to ensure adequate exposure of bees to the treatment. When colonies collected surplus amounts of honey, supers with comb were added on top.

Bees were sampled from the inner covers of the honey supers and at the hive entrance to obtain older bees that usually have more mites, thus facilitating detection. Bees infested with mites are not distributed randomly in a colony (Calderone & Shimanuki 1992). Samples were gathered on several occasions before treatment on 25 March; 1, 11 April; 7, 30 May; 6 June; and 23 July. After treatments began, three samples were collected on 13 August, 17 September, and 18 October. A modified, hand-held vacuum was used to draw bees into a glass vial, after which they were stored in 70% ethyl alcohol in glass vials for later examination.

The potassium hydroxide (KOH) method (Shimanuki & Knox 1991) was used to determine if mites were present. Briefly, the proximal end of the bee's thorax behind the first spiracle was sliced with a single-edge razor blade to obtain a disk of muscle and tracheal tissue. The disks were incubated overnight in a 7% KOH solution and rinsed with water, and the trachea were examined for mites with a binocular dissecting microscope. Thirty bees were sampled from each colony. Occasionally, a single 100-bee sample was collected in addition to the 30-bee sample to confirm the accuracy of the mite population estimate from each colony. Trachea were scored as

positive or negative, and the percentage of infested bees per colony sample was determined.

Continuous Exposure to Patties. In the second study (1992–1993), mite infestations were recorded in colonies exposed continuously to oil patties compared with no patty (control) from spring 1992 through spring 1993. For this study, we chose two apiary sites ≈ 30 mi apart in central Ohio. To ensure similar genetic lines between treatments, colonies were divided, equalized, and requeened with Carniolan—*Apis mellifera carnica* Pollman—queens selected at random from a closed population (Page & Laidlaw 1985) maintained cooperatively between The Ohio State University and the California Bee Breeder's Association. To equalize bee populations, each colony initially was assessed for population by counting frames of brood and bees. In total, 33 colonies were established by June 1992 and assigned each treatment randomly between two apiary sites; i.e. six colonies per treatment at site 1 and five per treatment at site 2.

The Terramycin oil patty was included as a treatment to determine if antibiotic therapy influenced mite control compared with oil treatment alone. The oil patty consisted of 1.35 kg (3 lb.) Crisco vegetable shortening and 2.7 kg (6 lb.) granulated white sugar; an addition of 100 g Terramycin 50 was used for the Terramycin patties (Wilson 1970).

To control *Nosema* disease at site 1, ≈ 3.8 liter (1 gallon) of 1:1 (by wt. water:sugar) syrup plus Fumidil-B (fumagillin [AI], 100 mg/gallon syrup; Mid-Continental Marketing, Overland Park, KS) was fed to each colony on 24 March 1992 (before the study began) and again on 10 October 1992. In addition, these colonies were treated on 4 November 1992 with two Apistan strips (fluvalinate, 10% [AI]; Zoëcon, Dallas, TX) per brood-nest chamber to control varroa mites, *Varroa jacobsoni* Oudemans. On 16 April 1993, colonies were given a supplemental feed of powdered sugar and syrup.

At site 2, Fumidil-B was fed to colonies on 14 April 1992 (before initiation of the experiment) and on 17 October 1992. Apistan strips were inserted on 23 October 1992, and, in April 1993, these colonies also were fed a powdered sugar patty supplement.

Bees were sampled by the same methods and from the same locations within colonies as in the prior study at ≈ 1 -mo intervals. Specimens were collected in glass or plastic vials and placed in a portable cooler for transport to the lab freezer (-20°C) for storage. All colonies were treated equally, and normal hive management practices were followed. To ensure continuous bee exposure to the treatments, a new patty was provided when more than three-quarters of a patty was gone. We also took notes at this time of any noticeable changes between treated or untreated colonies. Also, colonies were weighed at each

sampling period using a hand-held scale (Hanson, Model 8920, Shubuta, MS, The Viking, capacity 200 lb.) to monitor bee population growth and changes in honey storage (Brimhall 1991).

Bees were thawed for 1 min at room temperature, after which the prothoracic tracheal tubes were pulled through the spiracle opening (Smith & Needham 1988). This technique proved faster than the KOH method. We examined each trachea with a binocular dissecting microscope and reexamined them at higher magnification on a glass slide if no mites were visible initially. The percentage of infested bees per sample was calculated, and the mite loads were expressed as low, medium, or high infestation levels. Queens recovered from site 2 in June 1993 were inspected for mites as well.

The number of bees inspected varied, depending on the infestation level. If we detected mites within a sample of five bees, we dissected at least 25 bees; if mites were not visible until after the 10th bee, we examined up to 50 bees.

Statistical Analysis. Because the infestation rates were measured as percentages, an arcsine (square root of the proportion) transformation on all data was performed to normalize the distributions for analysis of variance (ANOVA) (Little & Hills 1978, Sokal & Rohlf 1981). This transformation prevents the variance from being a function of the mean.

Data were analyzed from each site separately using SYSTAT (Wilkinson 1989), with the transformed responses as the dependent variable against treatment and date. Also examined were the treatments of oil patty and Terramycin patty and hive weights as dependent variables, against the response and dates.

Results

Summer Application of Oil Patties. A three-way ANOVA was performed for treatment, date, and site effects. A two-time application of oil patties (treatment effect) during the summer of 1991 had no effect on the mite population ($F = 0.37$; $df = 1, 172$, $P > 0.05$) at either site (Fig. 1). Mite infestation levels in oil-treated colonies were not different from the control colonies. Thus, well-populated, established colonies already infested with mites gained no protection from oil patties when fed twice at peak bee populations. Infestation levels ranged between 10 and 50% from March to April for treatments at both sites. In May, the mite levels fell until August, then rose in September. Because mite levels already were decreasing when the patties were applied in June and July, as seen in the control colonies, the beneficial effects of oil could not be established clearly.

Mite levels varied significantly among dates ($F = 8.8$; $df = 8, 144$; $P < 0.05$) and sites ($F = 17.05$; $df = 1, 144$; $P < 0.05$). Therefore, mite

levels changed considerably over time, and differences between apiary locations greatly affected colony conditions.

Interactions were never significant. If oil patty treatments had kept mite levels from rising in the fall, then, given our experimental design, the interaction terms should have been significant; that is, mite populations would have behaved equivalently in both groups of colonies before oil treatment of one group. However, if oil had affected the mites, then mite loads in the oil-treated colonies should not have increased significantly in the fall compared with control colonies.

Continuous Exposure to Patties. For the second study (1992–1993), we hypothesized that a continuous treatment of the patties may be required to keep mites at low levels throughout the season. Yearly mite population fluctuations, as observed in the 1991 study, led to the supposition that autumn may be the crucial time to treat bees. Perhaps, overwintering colonies exposed to oil patties could reduce the mite levels. Two-way ANOVAs were performed on transformed percentage of infestations to test for effects of treatment, date, and site in the two yards. Treatment was significant at both sites: Site 1, $F = 14.95$; $df = 2, 165$; $P < 0.001$. Site 2, $F = 5.541$; $df = 2, 96$; $P < 0.001$. Oil- and Terramycin-treated colonies had significantly lower mite loads than control colonies. Interaction terms were not significant.

The maintenance of low levels of mite infestation (<14%) was dramatic in oil- and Terramycin-treated colonies at both sites (Figs. 2 and 3). In comparison, mite populations in the control colonies peaked between November and February to >30% before dropping in April when the food resources and bee populations increased. When examining the average infestation rates over 8 mo at site 2 (Fig. 3), the oil- and Terramycin-treated colonies rarely exceeded 10%, compared with 4–36% in control colonies. Again, mite populations are at their highest between August and February. If colonies survive the winter infestations, mite levels appear to decline naturally.

Mite infestations levels were significantly different between sites ($F = 16.8$; $df = 2, 301$; $P < 0.05$). The two apiaries were established 2 mo apart, and weather conditions, forage, and soil conditions were somewhat different. Hive weights did not change much in 1992, and honey production was below normal. The weights of the colonies varied little and were unaffected by treatment ($F = 2.84$; $df = 2, 102$; $P > 0.05$ at site 1. $F = 0.35$; $df = 2, 60$; $P > 0.05$; at site 2).

To determine if Terramycin was more effective than oil, a two-way ANOVA was performed with treatment as the dependent variable. There was no statistical difference between the two patties at either site ($F = 0.18$; $df = 1, 101$; $P > 0.05$ at site 1. $F = 0.107$; $df = 1, 64$; $P > 0.05$ at site 2).

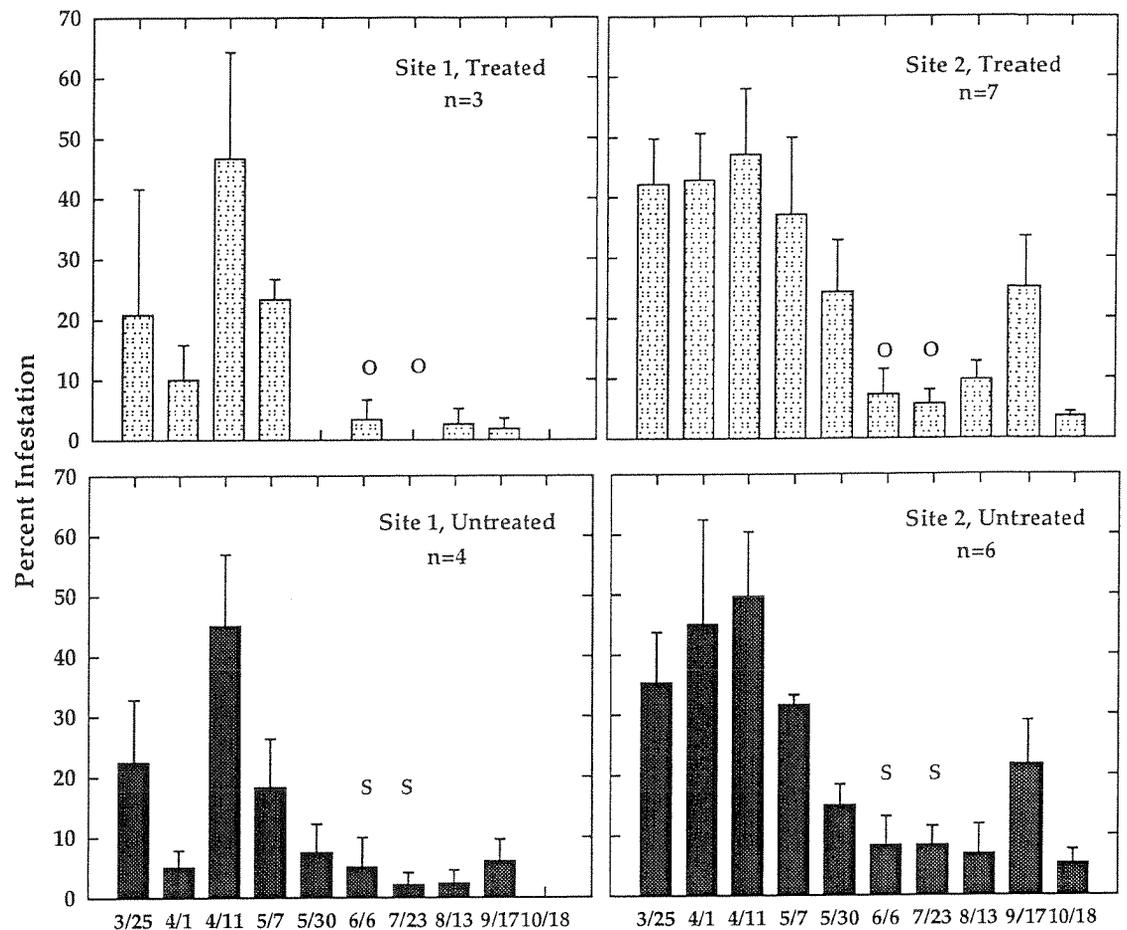


Fig. 1. Average infestation rates with standard error bars by treatment and site for 1991 study. Colonies (number of colonies in figure) treated with oil-only patties (O) had no significant decrease in mite infestation compared with those colonies treated with sugar-only (S) patties. Bee populations were at their highest levels during these months and mite populations at their lowest.

General Observations. Colony responses to mite infestations fluctuated widely, probably influenced by sampling techniques, ages of bees collected, queen supersedure, bees drifting from other colonies, or genetic resistance. It is difficult, despite using genetically similar lines, to account for all variations in a field situation and to make generalizations about colony performance in honey production, winter survival, and mite levels. However, there were some trends and noticeable changes in some colonies that are reported here.

Control colony mortality (four of six) was greatest at site 1. One colony died of starvation during the winter. Three of those four contained the highest infestation rates at that site before death (Table 1). All of the dead hives at this site were stained heavily with fecal material, and *Nosema* disease and dysentery may have been associated with these deaths. We did not observe fecal staining in the two remaining controls nor in the other treatments. In addition, 1992 was a poor

honey season in this location. Thus, colonies were more stressed than during a good honey flow, which may have contributed to the poor overwintering survivorship.

The oil-treated hives had the lowest mite populations of all treatments only after August. This may have been caused by the gradual replacement of all infested bees with younger bees protected by the oil patty.

In one Terramycin-treated colony the queen was lost, and this colony was united with another hive in May 1993. Another colony superseded its introduced queen in May 1992 and was removed from the study. Apparently, colonies treated with Terramycin at site 1 appeared healthier and were, in general, more populous by the spring of 1993 than those treated with oil-only patties. Although these results are not statistically significant, this observation warrants further study.

At site 2, low mite levels were found in two of the control colonies throughout the year (Table 1), possibly as a result of the later start-up ma-

nipulations, such as colony splitting and requeening, which were completed 2 mo later than at site 1. Two other control colonies survived the winter despite early high levels of mites (51–92%, respectively), which dropped to <10% by spring.

Fecal staining on hive bodies was not apparent in site 2, where only one control colony expired. One Terramycin colony died by spring 1993 after having >50% infestation the previous winter. Cause of death for this colony was not obvious because no common microbial diseases were evident and adequate honey stores were present. Also, most of the queens were dissected from site 2, and a total of four queens were superseded (two in the Terramycin treatment and one each in the oil and control colonies). One Terramycin-superseded queen had a light mite infestation. The tracheae of some queens (two in the control, one in the Terramycin treatments) were heavily scarred and blackened on one side indicating an old infestation. All of the other queens had no mites (one in Terramycin, three in oil, and one in control). In queens from two colonies, we found a heavy amoebae infestation (*Malpighamoeba mellifica* Prell) in the malpighian tubules.

Discussion

In our initial study, we placed oil patties in colonies during the summer to determine whether

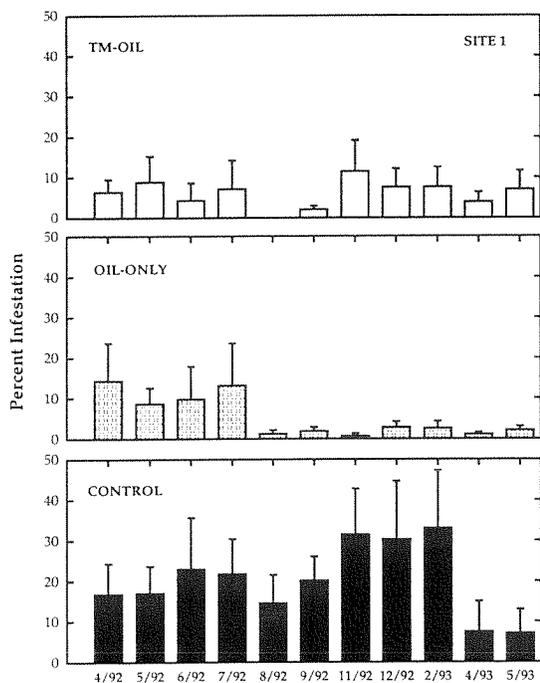


Fig. 2. Average percentage infestation rates with standard error bars, by treatment at site 1, 1992 to 1993 over 11 mo. Colonies ($n = 5$) treated with Terramycin patties (called Terramycin-oil patties) and oil-only patties ($n = 6$) had significantly lower mite infestations over the season than the control hives ($n = 6$).

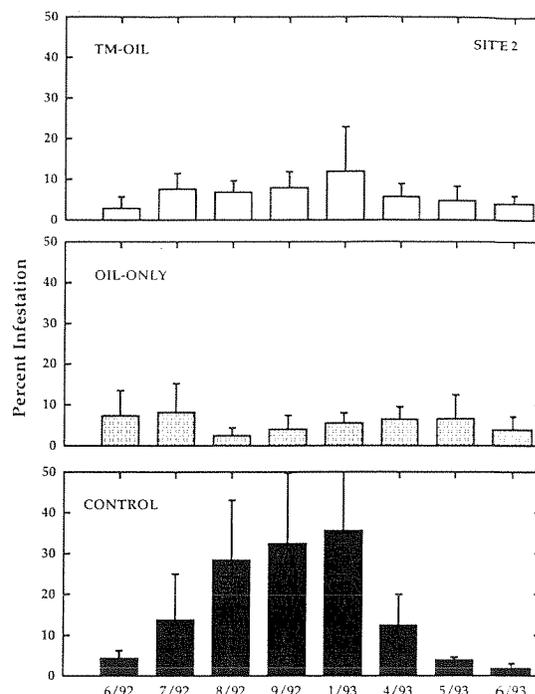


Fig. 3. Average percentage infestation rates with standard error bars, by treatment at site 2 during 8 mo. Colonies treated with Terramycin–oil patties ($n = 5$) and oil-only patties ($n = 5$) had significantly lower mite infestations over the season than the control hives ($n = 5$).

mite infestations could be reduced to nonthreatening levels by fall. Summer treatments failed to prevent mite populations from rebounding when bees clustered during inclement weather. However, mite levels were restricted by an uninterrupted application of oil, never reaching fatal populations. Although the deleterious effects of this mite are questioned by some (Bailey & Perry 1982), there is no doubt that this mite has had a significant effect on honey bee survival in some areas (Delfinado–Baker 1988, Otis 1990), especially in northern climates where bees are confined for several months. Distinguishing mite-infested colonies from mite-free ones without dissecting bees is impossible. Visible symptoms are unreliable even for highly infested bees but are reported to include bees crawling on the ground in front of the colony, *K-winged* bees (bees with hindwings held forward of forewings, making a ‘K’), and dead hives with large amounts of remaining honey stores in the spring. Some colonies are abandoned outright in midseason when infested bees crawl out, leaving behind brood and food stores (D. S., personal observation; Thoenes & Buchmann 1992). Additionally, a correlation of *Nosema* disease with mite-infested hives has been reported by Jadczyk (1990) but not found by others (Otis & Scott–Dupree (1992).

Table 1. Percentage of mite infestations on select dates spanning the length of the study

Treatment	Site 1			
	29 April 1992	14 Sept. 1992	9 Feb. 1993	14 May 1993
Terramycin	11.8	5	14.1	10
	0	0	0	0
	0	2.5	24	23.8
	15.4	0	0	0
Oil	4.8	2.5	0	1.4
	8.7	0	0	2.2
	0	1.1	11.1	1.3
	5.3	0	0	0
Control	0	5	2.2	6
	60	5	1.8	0
	8.7	0.8	5	Dead
	27.3	25	35	Dead
	7.7	30	78.6	Dead
	50	17.5	0	1.2
	0	7.5	8	13
	6.7	40	71.4	Dead
Treatment	Site 2			
	10 June 1992	14 Sept. 1992	28 Jan. 1993	2 June 1993
Terramycin	0	21.2	55.6	Dead
	0	2.1	2.3	8.9
	14.3	12.5	2	5
	0	0	0	22.2
Oil	0	4	0	0
	4.17	0	2.5	0
	0	0	2.2	0
	32	17.5	10.3	2.5
Control	0	2.4	12.5	16.7
	0	0	0	0
	9.5	2.1	4	0
	0	2	2.5	5
	8	15	36.4	Dead
	4	51	51	0
	0	91.7	84	22

Numbers are percentage of sampled bees with mites. Rows are individual colonies of bees.

The greatest challenge to controlling tracheal mites is that they virtually live their entire lives within honey bee tracheal tubes. Following development and mating, females exit the trachea in search of new hosts. They climb onto plumose setae and assume an ambush position (Morse & Nowogrodzki 1990). Within 24 h, emigrating mites attach themselves to callow bees, <4 d old. Young bees are selected by the detection of cuticular lipids not abundant in older bees (Phelan et al. 1991). Once a host is found, mites enter and lay eggs, and, after ≈ 16 d, gravid females again emerge (Bailey & Ball 1991, Pettis 1990) to continue the cycle. A single mite-laden bee can infest an entire mite-free colony within a short time.

Mite populations decline naturally as a result of several factors. An interruption in the brood cycle by swarming reduces infestation levels (Royce et al. 1991). Similar reductions are found when older field bees (Delaplane 1992b, Eischen et al. 1988) and drones are driven from the colony (Dawicke et al. 1992, Royce & Rossignol 1991).

The cause of colony death remains to be determined, but various factors, including microbial diseases vectored by mites, stress, or blocking air flow in the tracheal tubes, have been

suggested. Our data suggest that bee health, stress, and interactions between mites may contribute to colony demise. Spiroplasmas or other bacterial or viral pathogens may cause bee death when heavily infested with mites (Bailey et al. 1980, Clark 1977). The addition of an antibiotic appears to be controlling some bacteria vectored by or the result of mites. The effect of spiroplasmas and other pathogens still must be tested in a rigorous manner.

Our study shows that oil treatment interferes with one or more aspects of the mite's life cycle. The continuous presence of an oil patty with or without Terramycin helped lower tracheal mite populations and increased colony survivorship. The application of oil and Terramycin treatments, combined with conventional management practices, may significantly suppress mite populations and thereby benefit all aspects of the beekeeping industry.

Acknowledgments

We thank Pat Radloff (Ohio State Beekeepers Association), Danile Mancoba Nkhanbule, and Aaron Gallagher (The Ohio State University) for examining the

bees. Brian Burrell (The Ohio State University) assisted with the 1991 study. In addition, we thank the California State Beekeeping Association and the Ohio State and Northeast Indiana State Beekeepers Associations for financial support.

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Received for publication 6 October 1993; accepted 1 February 1994.