Reprinted from the
ENVIRONMENTAL ENTOMOLOGY

Influence of Water Treated Artificially With Herbicides on Honey Bee Colonies[1,2,3]

HOWARD L. MORTON, JOSEPH O. MOFFETT AND RICHARD D. MARTIN[4]
Agric. Res. Serv., USDA, 2000 E. Allen Rd., Tucson, AZ 85719

ABSTRACT

Apis mellifera L. colonies were placed in isolated desert apiaries where their only source of water contained paraquat (concentration of 1000 parts per million active ingredient by weight (ppmw)). Large numbers of bees exposed to paraquat died immediately, and all were dead before the end of the 5th week. When colonies were similarly exposed to like amounts of 2,4,5-T, large numbers of bees drowned in the water because of the lower surface tension of the water, and production of brood was reduced below that of check colonies during the period the treated water was used and for 3 months thereafter; however, in the subsequent 9 months, production returned to normal.

Concentrations of 2,4,5-T in honey bees from colonies using water containing 2,4,5-T were as high as 148 ppmw, but this level dropped to about 5 ppmw as soon as the bees began using untreated water. Likewise, honey from colonies using water containing 2,4,5-T contained concentrations of 2,4,5-T as high as 50 ppmw; however, the concentration dropped to about 5.0 ppmw within 1 week after the bees began using untreated water. The last day when any 2,4,5-T was detected in honey bees and honey from treated colonies was 480 days after the experiment began. Wax from colonies using the treated water contained detectable amounts of 2,4,5-T 650 days after the study was initiated.

It has been assumed for many years that honey is free of pesticides. This assumption is based on the fact that analyses of honey generally fail to detect pesticides. Many observations of colonies severely affected by pesticides have revealed that honey bees, *Apis mellifera* L., contacting a pesticide are killed before they return to the colony or that their behavior is so thoroughly disrupted that they cease to gather nectar or fail to process it (Schricker and Stephen 1970). On the other hand, Nasarov (1969) demonstrated that honey bees could carry sugar syrup containing insecticides at concentrations well above the LD_{50} with no apparent ill effects.

A number of herbicides are relatively low in toxicity to adult honey bees (Morton et al. 1972), and contact with many of them would not prevent honey bees from returning to the colony with the pesticide. Most investigations concerning pesticides, honey bees, and honey have dealt with foliage sprays or soil-applied systemic pesticides (Glynne-Jones and Thomas 1953, Jaycox 1964, and Johansen et al. 1957). It is possible that under the proper conditions pesticides could be present in the water used by honey bees, either because of accidental spills, improper disposal of unused materials, or runoff water on treated watersheds. We wanted to determine whether the herbicides paraquat [1,1'-dimethyl-4,4'-bipyridinium dichloride] and 2,4,5-T [(2,4,5-trichlorophenoxy)acetic acid] would influence the behavior of the bees or would be found in honey if it was present in water used by honey bee colonies.

[1] Hymenoptera: Apidae.
[2] In cooperation with the Arizona Agric. Exp. Stn., Tucson. Received for publication 23 Apr. 1974.
[3] This paper reports the results of research only. Mention of a commercial or proprietary product does not constitute endorsement by the USDA.
[4] Plant Physiologist, Res. Entomologist, and Biological Lab. Technician, respectively.

Materials and Methods

On May 20, 1971, we placed 15 colonies of honey bees on the Santa Rita Experimental Range at 3 apiaries which were located at least 2 miles from nearest water and at least 3 miles from areas treated with pesticides. The 5 colonies placed at the Muhlenburgia Station had water available that was untreated (checks). The 5-colony apiaries established at Kinney and Hughes Tanks had water available that contained triethylamine salt of 2,4,5-T and paraquat, respectively, each at a concentration of 1000 ppmw (parts per million active ingredient by weight). It was possible to limit the source of water for the colonies because the earthen tanks were dry until ca. July 20, when the summer rains began. Thus the only source of water until the summer rains at each location was three 5-qt plastic buckets. Small pieces of food were placed in each of the buckets to provide easy access to the water. Water was changed at each location every 2nd day.

Sampling Procedures

The dead bees in water buckets were counted, and they were removed each time water was changed until the herbicides were removed during the 12th wk. Dead bee traps were placed on each colony, and the bees were counted and removed every 2nd day for 12 wk. Colonies were examined at frequent intervals during the test period, and square inches of brood were measured at about monthly intervals during 1971 and at less frequent intervals during 1972. Adult worker bees, honey, and wax were removed at intervals over a 24-month period from check colonies and from colonies using water treated with 2,4,5-T as follows: ca. 15 adult worker bees were placed in 10-dr brown bottles, taken to the laboratory, and stored at −10°C until analyzed for

2,4,5-T. Liquid honey was pipetted into preweighed vials; however, when no liquid honey was available, crystalline honey was removed from cells of brood frames with stainless steel spatulas and placed in preweighed vials. Honey was obtained from at least 4 sites on each frame and in most instances from at least 2 frames. Unless otherwise indicated, honey from a colony was composited for analysis.

To determine how uniformly honey containing 2,4,5-T was distributed within a frame we removed 1 frame on Sept. 3, 1971, from each of 4 colonies that had used water containing 2,4,5-T and from 4 colonies that had used untreated water. Five honey samples were removed from each comb, 1 sample from the center and 1 from each of the 4 corners. Each sample was analyzed for 2,4,5-T. On Sept. 11, 1972, 3 colonies that had used water containing 2,4,5-T in May, June, and July, 1971, were sampled for honey and wax as outlined in Fig. 2. These colonies were resampled on Jan. 24 and Mar. 1, 1973.

Analytical Procedures

A 0.5-g sample of frozen adult honey bees was homogenized for 3 min at 45,000 rpm in a 5-ml microhomogenizer cup with 4 ml acidified acetone (0.2 ml conc HCl in 100 ml acetone). The resulting homogenate was suction-filtered through No. 2 Whatman filter paper, the homogenizer cup and filter funnel were rinsed 2 times with acetone, and the rinsing fluids were combined with the filtrate. After evaporating the acetone to dryness on a steam bath, 4 ml of boron trifluoride reagent (10% boron trifluoride in methanol) was added, and the sample again heated until fuming began. The cooled sample was transferred to a 125-ml separatory funnel with 10 ml each of distilled water and hexane. The hexane and water were thoroughly mixed and allowed to separate, and the aqueous portion was discarded. The amount of methylated 2,4,5-T in the hexane was determined by gas chromatography.

A 1-g sample of honey was dissolved in 10 ml of distilled water. After we added 2 drops of 1 N HCl, the sample and water were thoroughly mixed and transferred to a 125-ml separatory funnel with two 10-ml rinses of distilled water. The acidified aqueous sample was extracted 3 times with 10 ml of diethyl ether. If an emulsion developed, it was broken by adding a few drops of absolute ethyl alcohol. The aqueous portion was discarded, and the ether was evaporated to dryness on a steam bath. Methylation procedure was the same as that for the adult honey bees.

Before analyzing for 2,4,5-T we first washed the wax with distilled water to remove the honey. A 1-g sample of the washed wax was then placed in a 5-ml microhomogenized cup. The rest of the procedure was identical to that followed for analysis of adult honey bees.

A Barber Coleman® Model 10 gas chromatograph equipped with a radium 226 electron capture detector was used for the analyses. Purified nitrogen was used as the carrier gas at a flow rate of 65 ml/min. The U-shaped glass column was 1.8 m long and packed with 80–100 mesh acid-washed Chromosorb-W® coated with 1.5% SE-30® oil. The injector, detector, and column temperatures were 250, 250, and 200°C, respectively. One microliter of the methylated sample was used for each analysis.

Table 1.—Number of dead bees in traps of colonies using water treated with paraquat or 2,4,5-T (concentration of 1000 ppmw). May 20–Aug. 12, 1971.

Week	No. dead bees[a] in traps on colonies using water treated with:[b] No additive	2,4,5-T	Paraquat[c]
1st	2,031 a	2,235 a	16,867 b
2nd	895 a	1,142 a	5,818 b
3rd	809 a	406 a	8,190 b
4th	856 a	272 a	4,977 b
5th	208 a	194 a	659 a
6th	288 a	202 a	
7th	191 a	221 a	
8th	367 a	159 a	
9th	66 a	118 a	
10th	48 a	102 a	
11th	36 a	130 a	
12th	15 a	77 a	
Totals	5,810	5,258	38,511

[a] Total for 5 colonies.
[b] Means in the same row followed by same letter are not significantly different at the 5% level of probability.
[c] Most of the loss occurred on the 3rd and 4th days, and subsequently most of the bees were newly emerged young bees. All colonies in the paraquat test died during the 5th wk.

Experimental Results

Mortality of Adult Honey Bees

The numbers of dead bees in traps attached to colonies using untreated water (checks) and water treated with 2,4,5-T were relatively high but not significantly different during the 1st wk (Table 1). During the 2nd and subsequent weeks numbers of dead bees were much lower. There were ca. 8 times as many dead bees in traps attached to colonies using water treated with paraquat as in those attached to check colonies, and many bees died in the colonies which were not removed to the traps. Large numbers of bees which consumed water containing paraquat crawled from the colonies before dying, some as far as 100 ft. Most of the loss occurred on the 3rd and 4th days after initiation of the experiment. Since most of the adult bees died during the 1st wk, the only bees left in the colonies were a few older adult workers and emerging workers. The newly emerged bees contacted water containing paraquat shortly after emergence and died. No living bees were left after the 5th wk in colonies using water treated with paraquat. This high toxicity of paraquat to honey bees confirms the results of previous studies in which paraquat was toxic to honey bees when fed in 60% sucrose

Table 2.—Number of bees drowning in three 5-qt buckets of water containing paraquat or 2,4,5-T (concentration of 1000 ppmw). May 20–Aug. 12, 1971.

Week	No. dead bees[a] in water containing:[b]		
	No additive	2,4,5-T[c]	Paraquat[d]
1st	34 a	1,282 c	312 b
2nd	23 a	1,075 c	363 b
3rd	9 a	729 c	236 b
4th	7 a	1,036 b	152 a
5th	9 a	1,059 b	18 a
6th	6 a	1,824 b	
7th	4 a	1,788 b	
8th	2 a	2,233 b	
9th	2 a	598 b	
10th	3 a	18 a	
11th	3 a	5 a	
12th	3 a	0 a	
Totals	105	11,647	1,081

[a] Total for 5 colonies.
[b] Means in the same row followed by same letter are not significantly different at the 5% level of probability.
[c] Rainy season started during 9th wk. The bees preferred water in previously dry earthen tank to water in plastic buckets containing 2,4,5-T.
[d] All colonies in the paraquat test died during the 5th wk.

solution (Morton et al. 1972) or when sprayed on honey bees as a water solution (Moffett et al. 1972).

We found large numbers of honey bees that had downed in the water buckets treated with 2,4,5-T (Table 2). This high mortality rate continued until the 9th wk when the summer rainy season began and the bees no longer visited the water buckets. The drowning of adult honey bees apparently occurred because of the reduced surface tension of the water (Moffett and Morton 1973), 37 dynes/cm for 2,4,5-T solution vs. 74 dynes/cm for untreated water. The water treated with paraquat also had a lower surface tension, 43 dynes/cm, but the high mortality in colonies using this water reduced the number of bees collecting water and therefore the number drowning.

Effects on Brood Development

We found less capped brood in the colonies using water treated with 2,4,5-T than in the check colonies on June 16, and 4 of the 5 colonies had no sealed brood (Table 3). There was little brood production in any of these 5 colonies on July 8. On Aug. 19, 1971, ca. 1 month after the rainy season started, brood production increased; however, brood production was not as great in 2,4,5-T colonies as in the checks until late September when brood production was marginal in all colonies. Brood production ceased in late October. A colony was destroyed by flooding at the Kinney Tank on Aug. 27, and a 2nd colony died in early October. Measurements of brood production during 1972 showed no consistent reduction in brood in colonies that had used the water treated with 2,4,5-T. All 3 remaining colonies survived until the experiment was terminated in June, 1973.

Concentration of 2,4,5-T in Honey Bees, Honey, and Wax

No 2,4,5-T was detected in honey bees, honey, or wax removed from the check colonies at any sampling date. The concentration of 2,4,5-T in honey bees using water treated with 2,4,5-T increased rapidly during the first 4 days of the experiment (Fig. 1), but dropped rapidly during the next 10 days and remained low for an additional 6 days. This drop may have been caused by dilution by nectar collected from mesquite or from rain water from local showers. The concentration of 2,4,5-T returned to high levels for the remainder of the period that honey bees collected treated water. Concentration of 2,4,5-T in adult honey bees and honey dropped dramatically as soon as the rainy season started and honey bees obtained water from the adjacent earthen tank rather than the treated water in the buckets. However, 2,4,5-T was found in honey bees during the remainder of calendar year 1971 and most of calendar year 1972. Although the level in bees had dropped to ca. 5 ppmw 111 days after initiation of the study, it did not decrease appreciably from this level during the next 109 days.

The concentration of 2,4,5-T was relatively low in honey during the first 30 days (probably because of the large amount of nectar gathered from mesquite during the first 25 days of the study). However, it increased to ca. 50 ppmw 70 days after initiation of the study. The concentration of 2,4,5-T in honey from colonies provided with water treated with 2,4,5-T dropped after the start of the rainy season; however, the reduction in concentration in honey was less rapid than in the adult honey bees.

Table 3.—Square inches of capped brood per colony for colonies using water treated with 2,4,5-T (1000 ppmw). May 20 to ca. July 20, 1971.

Date	Amount (sq. in.) of brood in colonies using water treated with:[a]	
	No additive (check)	2,4,5-T
June 16,1971	410 b	281 a
July 8, 1971	97 b	4 a
July 29, 1971	183 b	61 a
Aug. 19, 1971	553 b	336 a
Sept. 8, 1971[b]	712 b	303 a
Sept. 28, 1971	23 a	146 a
Oct. 22, 1971[c]	0 a	10 a
Nov. 12, 1971	0 a	0 a
Dec. 23, 1971	0 a	0 a
Mar. 10, 1972	212 a	233 a
April 20, 1972[d,e]	486 a	429 a
June 1, 1972[f]	112 a	202 a

[a] Means in the same row followed by same letter are not significantly different at the 5% level of probability.
[b] Flooding destroyed one 2,4,5-T colony Aug. 27.
[c] One 2,4,5-T colony died in early October.
[d] One check colony destroyed and one check colony damaged by vandals.
[e] One colony in each treatment queenless.
[f] One 2,4,5-T colony queenless.

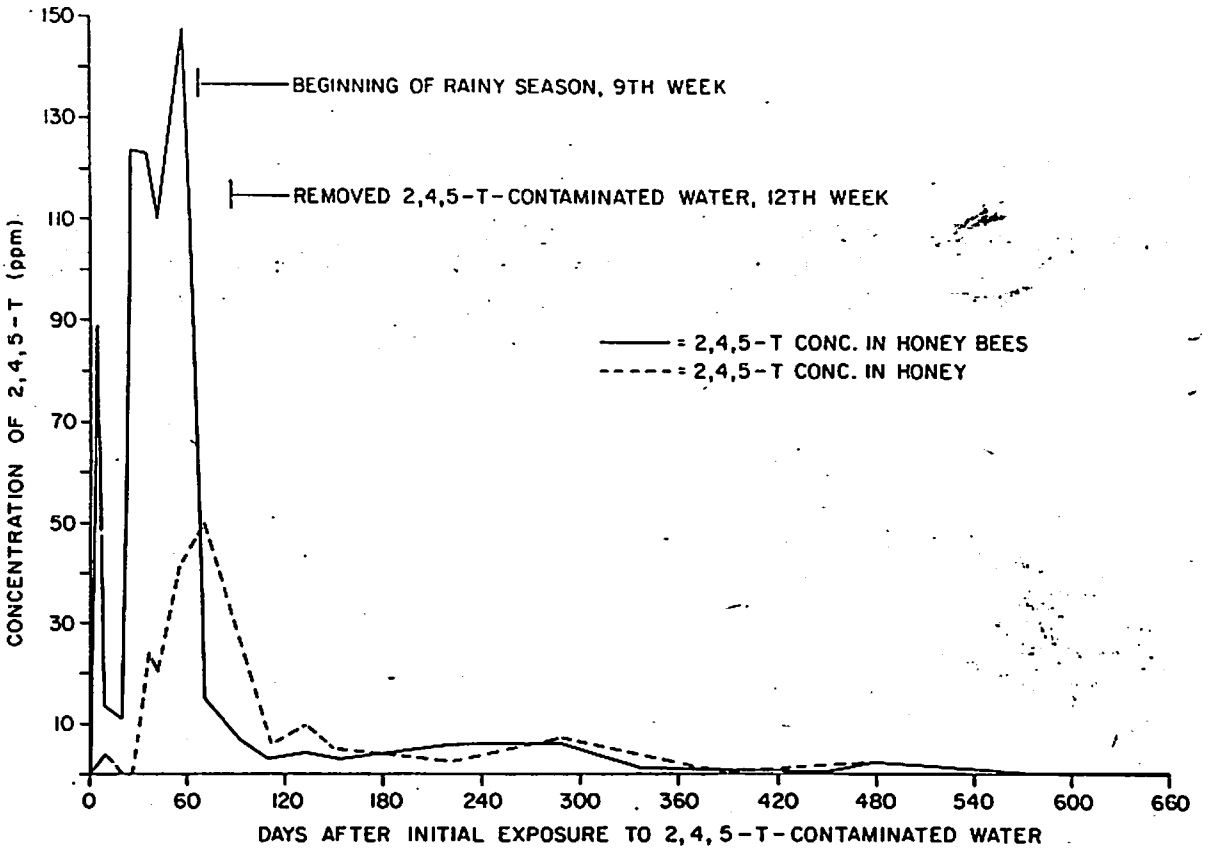

FIG. 1.—Concentration of 2,4,5-T (ppmw) in adult worker honey bees and honey from colonies using water treated with triethylamine salt of 2,4,5-T from May 20–July 20, 1971.

We continued to sample and analyze the honey bees and honey for 2,4,5-T for 650 days after initiation of the experiment. We last detected 2,4,5-T in the honey 480 days after initiation of the experiment.

Fig. 2 indicates that a colony can contain 2,4,5-T, but it will not be detected if the sampling is not thorough. Many of the combs we sampled did not contain honey with 2,4,5-T because the combs had been added during periods of honey flow after the treated water was removed. We removed honey from the colonies in November 1972 and April 1973. After honey was extracted, the frames were returned to the colonies from which they were removed. This is the reason some of the frames contained wax with detectable levels of 2,4,5-T though the honey was free of 2,4,5-T.

We detected 2,4,5-T in the wax of 1 colony 650 days after the colony was first exposed to water containing 2,4,5-T. Of the 9 wax samples collected from this colony, 6 contained detectable quantities of 2,4,5-T, with concentrations ranging from 0.06–3.30 ppmw. The wax samples from the other 2 colonies did not contain detectable quantities of 2,4,5-T on the 650th day. Concentration of 2,4,5-T detected in colony 9 were usually higher than those detected in the other 2 colonies throughout the experiment.

Table 4.—Concentration of 2,4,5-T in honey removed from 5 sites in frames from colonies exposed to water treated with triethylamine salt of 2,4,5-T. May 20–July 20, 1971.*

Frame from colony number	Concentration (ppmw) of 2,4,5-T in honey sample located in frame as indicated				
	Upper left	Upper right	Center	Lower left	Lower right
2	9.5	8.1	4.2	5.8	3.2
6	2.4	2.4	1.7	3.9	2.9
9	1.5	6.6	2.3	11.4	7.6
19	5.1	5.8	6.0	7.2	1.7

* Frames removed from colonies on Sept. 3, 1971.

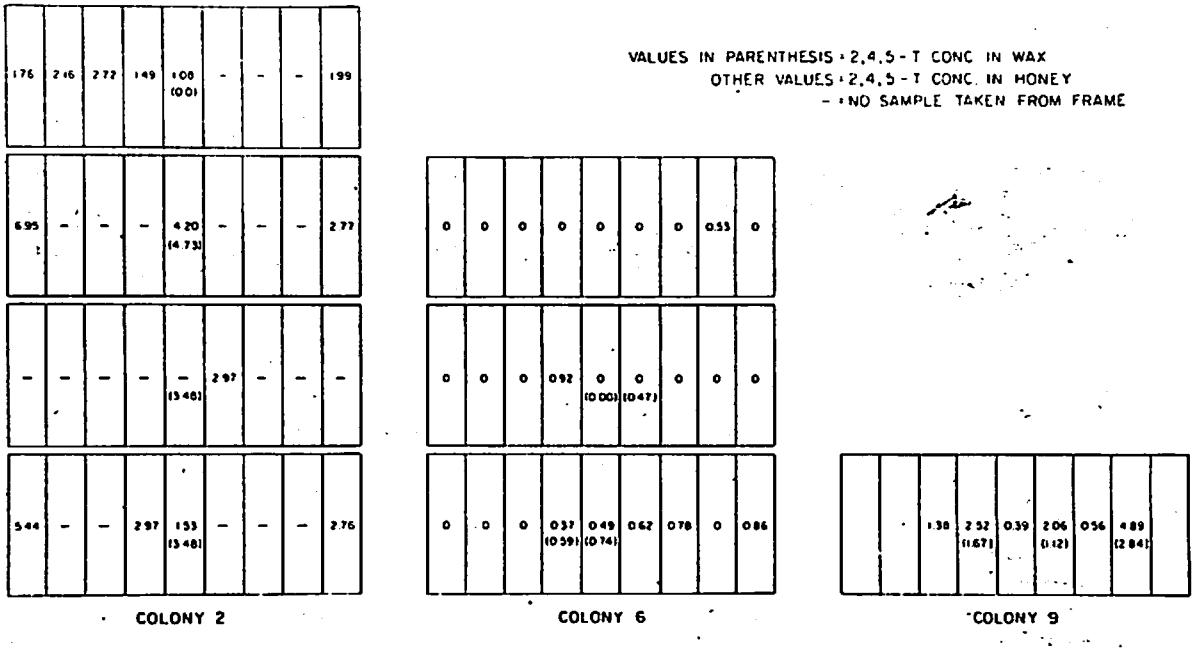

FIG. 2.—Concentration of 2,4,5-T (ppmw) Sept. 11, 1972 in honey and wax from frames in honey bee colonies exposed to water treated with triethylamine salt of 2,4,5-T from May 20–July 20, 1971.

Discussion and Conclusions

The exposure of honey bee colonies to pesticides through water used by the colonies is possible as we have demonstrated in this study. The probability of this type of exposure occurring is not great because honey bees seemed to prefer untreated water from earthen ponds to the water in plastic buckets. What would actually happen if water in earthen ponds or streams contained pesticides is not known; however, the pesticide could be transferred to colonies and honey, and the bees could be poisoned. To prevent exposure of honey bees and honey to pesticides, beekeepers should insure that their colonies have available water of good quality at all times, and users of pesticides should dispose of unused pesticides in such a manner that they will not be visited by foraging honey bees or in other ways reach the colonies.

The affinity of organic pesticides for beeswax was discussed by Johansen (1969). While the triethylamine salt of 2,4,5-T is not lipid-soluble, honey and the honey stomachs of honey bees are acidic (Root 1962), Hoskins and Harrison 1934), and acidic 2,4,5-T is relatively lipid-soluble. Our data suggest that once wax in a colony contains 2,4,5-T, it will remain for an indefinite period.

REFERENCES CITED

Glynne-Jones, G. D., and W. D. E. Thomas. 1953. Experiments on the possible contamination of honey with schradan. Ann. Appl. Biol. 40: 546–55.

Hoskins, W. M., and A. S. Harrison 1934. The buffering power of the contents of the ventriculus of the honeybee and its effect upon the toxicity of arsenic. J. Econ. Entomol. 27: 924–42.

Jaycox, E. R. 1964. Effect on honey bees of nectar from systemic insecticide-treated plants. Ibid., 57: 31–5.

Johansen, C. A. 1969. The bee poisoning hazard from pesticides. Wash. Agric. Exp. Stn. Bull. 709. 8 pp.

Johansen, C. A., M. D. Coffey, and J. A. Quist. 1957. Effect of insecticide treatments to alfalfa on honey bees, including insecticidal residue and honey flavor analyses. J. Econ. Entomol. 50: 721–3.

Moffett, J. O., and H. L. Morton. 1973. Surfactants in water drown honey bees. Environ. Entomol. 2: 227–31.

Moffett, J. O., H. L. Morton, and R. H. MacDonald. 1972. Toxicity of some herbicidal sprays to honey bees. J. Econ. Entomol. 65: 32–6.

Morton, H. L., J. O. Moffett, and R. H. MacDonald. 1972. Toxicity of herbicides to newly emerged honey bees. Environ. Entomol. 1: 102–4.

Nasarov, S. S. 1969. Possibilité pour les abeilles butineuses d'apporter dans la ruche avec la nourritus des insecticides organiques à forte concentration. Bull. Apic. 12: 159–61.

Root, A. I. 1962. The abc and xyz of bee culture. 32nd ed. The A. I. Root Co., Medina, Ohio. 712 pp.

Schricker, B., and W. P. Stephen. 1970. The effect of sublethal doses of parathion on honey bee behavior. J. Apic. Res. 9: 141–53.