TOXICITY EFFECTS AND CHALKBROOD INCIDENCE IN HONEY BEE[1] COLONIES FED CONTROLLED DOSES OF FUNGICIDES[2,3]

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ABSTRACT

Folpet and a combination with folpet, benomyl, citral, sodium propionate, and sorbic acid were fed or exposed to honey bee, *Apis mellifera* L., field colonies to: 1) determine their long-term toxic effects on the bees; and 2) to determine if chalkbrood (CB) disease, *Ascosphaera apis* (Maassen ex Claussen) Olive et Spiltor, is inhibited or controlled by the compounds.

When folpet was fed to honey bee colonies in sucrose syrup, the group fed the highest rate (1000 ppm) never differed significantly ($P > 0.05$) from the control colonies. There was an equal number of adult bees, an equal amount of sealed brood, and mortality was identical. Analysis indicated significantly ($P < 0.05$) fewer CB mummies in treatment groups fed 10 or 1000 ppm folpet, but this apparent benefit may have been due to seasonal changes of reduced CB infection that occur in late summer.

Folpet or a combination of folpet, benomyl, citral, sodium propionate, and sorbic acid (1000 ppm each, total 5000 ppm) incorporated into lipid/sucrose extender patties produced no significant ($P > 0.05$) effect of any kind on colonies to which they were applied, including CB infection. However, when the combination of five fungicides (5000 ppm) was impregnated into beeswax foundation and exposed to honey bee colonies, only a small amount of comb was drawn on the test foundation, indicating a repellent effect. Otherwise, the test foundation had no significant ($P > 0.05$) effect on the honey bees or the CB infection.

Key Words: Honey bee, toxicity, fungicides, chalkbrood.

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INTRODUCTION

Folpet [(N-trichloromethylthio) phthalimide; Phaltan®] is a fungicide in the phthalimides group (McEwen and Stephenson 1979) that is applied to a wide range of plants, often during the blooming stage, to control fungus disease. Many of these plant species are attractive to honey bees, *Apis mellifera* L. Atkins et al. (1973) list folpet as relatively nontoxic to honey bees in laboratory tests, but Stoner (1970) noted reduced longevity of laboratory-reared *Geocoris punctipes* (Say) when a 1% solution was used to retard spoilage of a fresh vegetable offered as a food supplement.

Various chemicals have been tested, with little success, for control or inhibition of chalkbrood (CB), a fungal disease caused by *Ascosphaera apis* (Maassen ex Claussen) Olive et Spiltor that attacks the brood of honey bees. These compounds include: sodium propionate and sorbic acid (Taber et al. 1975), citral and geraniol (Gochnauer et al. 1979), sodium propionate, potassium sorbate, and benomyl

[1] Hymenoptera: Apidae

[2] Approved by the Director of the Wyoming Experiment Station as Journal Article #1182.

[3] This paper reports the results of research only. Mention of a pesticide in this paper does not constitute recommendation for use by the USDA, or does it imply registration under FIFRA as amended.

(Thurber 1979), and sorbic acid and griseofulvin, citral, and a combination of sodium propionate and potassium sorbate (Menapace and Hale 1981). However, to our knowledge, no one has previously tested folpet for controlling chalkbrood. Our purpose for testing folpet and the combination of folpet, benomyl, citral, sorbic acid, and sodium propionate was two-fold: 1) determine the toxicity to field colonies of honey bees when fed or exposed in controlled doses over long periods of time, and 2) determine if CB was inhibited or controlled by these compounds.

MATERIALS AND METHODS

Colonies used in the studies (1981 and 1982) were overwintered, queenright populations, each housed in 2 or 3 10-frame deep hive bodies. In both years, colonies were assigned to treatments or equalized with the intent of giving similar amounts of sealed brood and adult bees at the beginning of the studies.

In 1981, all study colonies, including one control group, were naturally infected with CB.

In 1982, though probably unnecessary, all colonies, including those in the control group, were fed pollen cake (50/50 pollen and granulated sucrose moistened with water) containing macerated CB mummies (2 mummies/454 g patty) on June 24. No attempt to include a control group free of CB infection was considered, because it is nearly impossible to locate or maintain colonies in such a condition (Heath 1982).

In 1981, we used a completely randomized design with 4 treatments and 6 colonies per treatment, except the control group that had 12 colonies. The different levels of folpet were fed to the bees ad libitum in sucrose syrup (50% by volume) at the rate of 2 L per colony per week for 8 consecutive weeks (June 30 - August 21). Folpet was fed at levels of 0.0, 10, 100, or 1,000 ppm (by wt). Unused syrup was measured each week to calculate the mean consumption per colony. Syrup was fed to each colony by inverting a 2 L plastic bucket (2 - 3 mm diam hole in the lid) over the bee escape hole of the inner cover. The buckets were enclosed with empty deep hive bodies and top covers to prevent bees from robbing syrup from around the lids. An additional application of folpet (plain sucrose syrup for the control) was given to all colonies on July 10 by spraying all combs in each colony with the same dose required for each individual treatment. This application was made to assure that folpet had contacted all brood comb. During spraying, combs were removed one-by-one, shaken to remove adult bees, sprayed on each side with a 2-second burst (across the upper half, back across the lower half) from a compressed air sprayer (2.1 kg/sq cm), and then returned to the hive body.

In 1982, we used a completely randomized design with 6 treatments and 4 colonies per treatment. Folpet alone at rates of 0.0, 100 or 1,000 ppm (by wt) was incorporated into 454 g patties (⅓ vegetable fat and ⅔ granulated sucrose) that were placed on the top bars of the frames in the lower broodnest, constituting 3 of the treatments. A fourth treatment was a mixture of compounds that included folpet, benomyl, citral, sorbic acid, and sodium propionate at rates of 1,000 ppm each (total 5,000 ppm by wt) in 454 g patties and placed in the broodnest as above. The fifth treatment was the same mixture of compounds (by wt) at the same rates incorporated into beeswax foundation (Stoner et al. 1984). After the treated foundation was wired into frames, the frames were then placed into the

lower broodnest, 5 frames per hive spaced every other frame of a 9-frame total. In the sixth treatment, control colonies each contained 5 frames of untreated foundation and a 1-lb patty of untreated vegetable fat and granulated sucrose. The treatments were applied only once, at the beginning of the 7-week-long study (July 8 - August 24). Unused patty material was weighed at the end of the study, and the amount of drawn comb on the treated foundation was measured then also.

Hive weights were recorded in both years: in 1981, all hives were weighed on July 24, then again after syrup feeding was terminated on August 25; in 1982, all hives were weighed on June 24, then again on August 24 when the study was terminated.

In both years, each hive had a modified Todd dead trap (Atkins et al. 1970 and Stoner et al. 1979) installed over the entrance to determine the daily number of adult bees dying within the hive, and the daily number of CB mummies discarded by each colony. In 1981, weekly records were made of cm^2 of sealed brood and frames of adult bees for 8 consecutive weeks. In 1982, the same measurements were made, but only 4 times over a 7-week period that included pretreatment records. Analysis of variance and Duncan's multiple range test were performed on the data.

RESULTS AND DISCUSSION

Study performed in 1981. Supersedure of queens occurred in all treatment groups: control, 2 queens; 10 ppm folpet, 1 queen; 100 ppm folpet, 2 queens; 1000 ppm folpet, 1 queen. As with previous studies utilizing controlled doses of pesticides on field colonies (Stoner and Wilson 1983), the current study indicated that queen loss or supersedure was related to intensive weekly manipulation of the colonies, rather than pesticide toxicity.

When 2 liters of sucrose syrup was provided weekly to each colony, the mean rate of consumption was not affected by the tratment dose level, because the mean rate of syrup consumption for all treatment groups was 1.4 L per colony. Weight gain among treatment groups was similar, each colony gained a mean of 10.5 kg.

Colonies in all treatment groups showed an increase in the mean number of frames covered with adult bees (Table 1). However, analysis of the percent difference between pre- and posttreatment records were not significant ($P > 0.05$), and the treatment group fed 1000 ppm folpet did not differ ($P > 0.05$) from the control group when posttreatment records were analyzed. Folpet was not toxic to adult bees at the rates applied.

There was an increase in dead adult bees in all treatment groups when comparing pre- and posttreatment records (Table 1), but analysis of the differences was not significant ($P > 0.05$). Also, the treatment group fed 1000 ppm folpet did not differ ($P > 0.05$) from the control group when posttreatment records were analyzed. Death of adult bees in all treatments was well within the normal die-off range of up to 100 dead bees per colony per day (Johansen 1977). Again, folpet was not toxic to adult bees at the rates applied.

The amount of sealed brood in the treatment group fed 1000 ppm folpet did not differ ($P > 0.05$) from the control group (Table 1). Although the group fed 100 ppm folpet had significantly ($P < 0.05$) less sealed brood than other treatment groups or the control, it is unlikely that folpet had any effect on sealed brood.

Table 1. The effects of feeding folpet to honey bee colonies. Laramie, WY. 1981.

Treatment (folpet)*	Frames of adult bees		Dead adult bees		cm^2 sealed brood		CB mummies	
	Pre-treatment mean	Post-treatment weekly mean†	Pre-treatment mean	Post-treatment daily mean†	Pre-treatment mean	Post-treatment weekly mean†	Pre-treatment mean	Post-treatment daily mean†
0.0 ppm (Control)	11.1	14.6 b	37.1	51.9 a	4640	4233 b	6.2	25.6 a
10 ppm	14.0	17.6 a	24.8	38.3 b	5210	5192 a	9.0	17.1 b
100 ppm	9.5	11.9 c	24.8	46.6 a	3543	3670 c	17.8	19.8 ab
1,000 ppm	13.0	15.3 b	28.2	53.7 a	4196	4128 bc	10.3	8.3 c

* Six colonies per treatment, twelve colonies in the control. Folpet fed weekly for eight weeks in two liters of sucrose syrup.

† Means followed by the same letter are not significantly different at the 0.05 level according to Duncan's new multiple range test.

Colonies in all groups fed folpet had fewer CB mummies than the control group, but only the groups fed 10 or 1000 ppm had significantly ($P < 0.05$) fewer CB mummies (Table 1). Analysis of the percent difference between pre- and posttreatment revealed no significant ($P > 0.05$) differences.

Study performed in 1982. No queen mortality or supersedure was encountered. The colonies were manipulated only half as many times over a period similar to that in 1981.

The 454 g patties were fed only once, July 8. By July 30, all colonies still retained some patty material on the top bars of the lower broodnest, but by August 26, the mean amount of patty material remaining per colony in each treatment group was as follows: 0.0 ppm (control) 0.0 g; 100 ppm folpet 0.0 g; 1000 ppm folpet 74.2 g; and 5000 ppm (5 fungicides) 154.2 g. Patties containing 1000 ppm folpet or 5000 ppm of the 5 fungicides, though less palatable, were not toxic to the bees (Table 2). Weight gain among treatment groups was similar, each colony gained a mean of 38.5 kg.

Colonies in treatment groups fed folpet or 5 fungicides in patties or exposed to 5 fungicides in impregnated foundation exhibited no significant difference ($P > 0.05$) in cm^2 sealed brood, number of frames of adult bees, daily number of dead adults, or daily number of CB mummies (Table 2). The decreased mummy count between pre- and posttreatment was seasonal (Heath 1982) and unrelated to the fungicidal treatments. None of the fungicides were toxic to the bees at the levels applied. However, honey bee colonies in the treatment group exposed to 5 frames each of foundation containing 5 fungicides constructed significantly less ($P < 0.05$) comb (440 cm^2 per colony) than control colonies (6125 cm^2 per colony), indicating a repellent effect. Whether the repellency was due to one or a combination of the fungicides is unknown. However, the fungicide-impregnated foundation was not toxic to the bees, because all treatment groups maintained similar amounts of bees and sealed brood, and had similar mortality rates.

None of the fungicides appeared to be toxic to honey bees, regardless of the level we applied, whether they were fed in sucrose syrup or patties, or exposed via beeswax foundation. In both years, the number of frames of adult bees remained similar in all colonies, sealed brood was not affected, and mortality of adult bees remained within the normal die-off range of 100 or fewer bees per colony per day (Johansen 1977). Reduction of CB mummies by feeding folpet in sucrose syrup was more apparent than real. Decreased CB mummies in either year was likely due to seasonal changes that occur in late summer (Heath 1982), rather than from the application of fungicides. Because honey bee colonies were little effected by any of the fungicides at the levels we used, foragers encountering them at levels applied to crop plants would not be expected to be damaged.

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Table 2. The effects of exposing honey bee colonies to folpet and a combination of folpet, benomyl, citral, sorbic acid and sodium propionate. Laramie, WY. 1982.

	Frames of adult bees		Dead adult bees		cm^2 sealed brood		CB mummies	
Treatment*	Pre-treatment mean	Post-treatment weekly mean†	Pre-treatment mean	Post-treatment daily mean†	Pre-treatment mean	Post-treatment weekly mean†	Pre-treatment mean	Post-treatment daily mean†
0.0 ppm (Control)	13.1	15.7 a	19.4	37.3 a	5480	5194 a	44.7	4.1 a
100 ppm (Folpet patty)‡	15.3	16.4 a	24.0	30.3 a	5577	5368 a	34.1	14.2 a
1,000 ppm (Folpet patty)‡	15.3	19.1 a	24.3	40.0 a	5861	5654 a	51.9	11.6 a
5,000 ppm (Five fungicides in patty)‡	12.8	15.0 a	26.9	41.1 a	4709	4654 a	37.3	3.9 a
5,000 pm (Five fungicides in beeswax)§	13.5	15.3 a	31.9	38.9 a	5408	5087 a	38.6	3.3 a

* Four colonies per treatment.
† Means followed by the same letter are not significantly different at the 0.05 level according to Duncan's new multiple range test.
‡ Seeded 454 g patties (⅓ vegetable fat plus ⅔ granulated sucrose) applied once at beginning of 7-week study.
§ Seeded beeswax foundation (five frames/colony) applied once at beginning of 7-week study.

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