

## The Mycoflora of Adult Worker Honeybees, *Apis mellifera*: Effects of 2,4,5-T and Caging of Bee Colonies<sup>1</sup>

MARTHA GILLIAM AND HOWARD L. MORTON

*Agricultural Research Service, U.S. Department of Agriculture, 2000 East Allen Road, Tucson, Arizona 85719*

DOROTHY B. PREST

*Biology Department, Keuka College, Keuka Park, New York 14478*

AND

RICHARD D. MARTIN AND L. J. WICKERHAM

*Agricultural Research Service, U.S. Department of Agriculture, 2000 East Allen Road, Tucson, Arizona 85719*

Received November 3, 1976

The guts of 195 adult worker honeybees, *Apis mellifera*, from free-flying control colonies fed sucrose, from caged control colonies fed sucrose, from free-flying colonies fed 2,4,5-T, and from caged colonies fed 2,4,5-T were examined for yeasts and molds. Only 25% of the bee guts contained fungi. *Torulopsis magnoliae*, *T. glabrata*, *Hansenula anomala*, *Penicillium cyclopium*, and *P. cyclopium* var. *echinulatum* were the most frequent isolates. Molds were most prevalent in bees from free-flying control colonies fed sucrose, and yeasts were found most often in bees from caged colonies fed 2,4,5-T.

### INTRODUCTION

Recently we reported that feeding the herbicide (2,4-dichlorophenoxy)acetic acid (2,4-D) to colonies of honeybees, *Apis mellifera*, increased the number of adult worker bees containing intestinal yeasts (Gilliam et al., 1974b). In fact, the intestinal yeast flora in worker bees in Arizona appears to develop under conditions of stress as caging of colonies, feeding deficient diets, or herbicide treatment. However, the situation was the opposite for molds. We isolated nearly as many molds from bees fed 2,4-D as from control bees (Gilliam et al., 1974a). Thus fungal populations were not reduced seriously by 1000 ppm of the herbicide.

<sup>1</sup> This paper reports the results of research only. Mention of a pesticide does not constitute recommendation for use by the U.S. Department of Agriculture nor does it imply registration under FIFRA as amended.

The present experiments were designed to assess the effects of another herbicide, (2,4,5-trichlorophenoxy)acetic acid (2,4,5-T), on the mycoflora of worker bees. In addition, we wished to examine the effects on the mycoflora of caging bee colonies and removing brood. Morton and Moffett (1972) found that eggs from colonies fed high levels of either 2,4-D or 2,4,5-T did not hatch. Also, larvae died when they were transferred into colonies receiving either of these herbicides though brood development resumed once the herbicides were removed. Therefore, bees in herbicide-treated colonies were "old" since brood development was inhibited.

### MATERIALS AND METHODS

On March 7, 1974, 12 colonies of honeybees were established and maintained as previously described (Gilliam and Morton, 1974), six at one apiary and six at another

distant apiary. Before the colonies were fed experimental diets, three adult worker bees from each colony were examined for yeasts and molds. All 12 colonies received pollen patties and fresh water, but six colonies at one apiary were fed the triethylamine salt of 2,4,5-T at a concentration of 1000 ppm active ingredient by weight in 60% sucrose-water solution (Morton and Moffett, 1972). The other six colonies at the other apiary received only 60% sucrose. Also, three colonies at each apiary were each placed in a 12 × 12 × 9-ft Saran-mesh cage. Brood was removed regularly from control colonies fed sucrose either by replacing frames containing brood with drawn empty combs or by using a hive tool to scrape out small amounts of sealed brood. Therefore, all colonies contained bees of approximately the same age.

Once the test feeding was begun, three adult worker bees from each colony were examined for yeasts and molds at 3-week intervals. The intestinal tracts were aseptically removed and individually homogenized in 2.5 ml of sterile distilled water as previously described (Gilliam and Prest,

TABLE 1

## YEASTS AND MOLDS ISOLATED FROM HONEYBEES

Organism	Number of bee guts containing the organism
<i>Torulopsis magnoliae</i>	15
<i>Hansenula anomala</i>	11
<i>Penicillium cyclopium</i>	5
<i>Penicillium cyclopium</i> var. <i>echinulatum</i>	4
<i>Torulopsis glabrata</i>	4
<i>Aspergillus fumigatus</i>	3
<i>Cladosporium cladosporioides</i>	2
<i>Pichia ohmeri</i>	2
<i>Penicillium</i> sp.	2
<i>Aureobasidium pullulans</i>	1
<i>Aureobasidium</i> sp.	1
<i>Aspergillus flavus</i> var. <i>columnaris</i>	1
<i>Aspergillus repens</i>	1
<i>Cephalosporium acremonium</i> var. <i>juniculosum</i>	1
<i>Chaetomium sprale</i>	1
<i>Cryptococcus albidus</i>	1
<i>Debaryomyces hansenii</i>	1
<i>Paecilomyces lilacinus</i>	1
<i>Penicillium brevis-compactum</i>	1
<i>Rhizopus stolonifer</i>	1
<i>Saccharomyces chevalieri</i>	1
<i>Saccharomyces rosei</i>	1
<i>Torulopsis versatilis</i>	1

TABLE 2

## YEASTS ISOLATED FROM HONEYBEES

Organism	Treatment*	Date isolated	Number of bee guts containing the organism
<i>Torulopsis versatilis</i>	from	S,F 3/7/74	1
<i>Pichia ohmeri</i>	same bee	S,F 3/7/74	1
<i>Debaryomyces hansenii</i>		S,C 3/7/74	1
<i>Saccharomyces chevalieri</i>	from	S,C 3/7/74	1
<i>Torulopsis magnoliae</i>	same bee	S,C 3/7/74	1
<i>T. magnoliae</i>		H,C 3/7/74	1
<i>P. ohmeri</i>		H,F 3/7/74	1
<i>Saccharomyces rosei</i>		H,F 3/28/74	1
<i>T. magnoliae</i>		H,C 3/28/74	2
<i>T. magnoliae</i>		H,F 4/18/74	1
<i>T. magnoliae</i>		H,C 4/18/74	3
<i>Hansenula anomala</i>		S,C 4/18/74	1
<i>H. anomala</i>		S,F 5/9/74	3
<i>H. anomala</i>		S,C 5/9/74	4
<i>T. magnoliae</i>	1 of each	H,C 5/9/74	3
<i>Torulopsis glabrata</i>	from same bee	H,C 5/9/74	2
<i>H. anomala</i>		S,F 5/30/74	3
<i>T. magnoliae</i>	1 of each	H,C 5/30/74	3
<i>T. glabrata</i>	from same bee	H,C 5/30/74	1
<i>T. glabrata</i>		H,C 6/20/74	1
<i>T. magnoliae</i>		H,C 6/20/74	1
<i>Cryptococcus albidus</i>		H,F 7/9/74	1

\* S = sucrose; H = 2,4,5-T; C = caged; F = free-flying.

1972). Then a loopful of the homogenate from each bee was streaked in duplicate on malt extract-yeast extract agar plates with 1% glucose (YM-1) and on malt extract-yeast extract agar plates containing 5% glucose (YM-5) (Wickerham, 1951). All plates were incubated under aerobic conditions at 25°C for 14 days. Selected yeast colonies were restreaked for purity on YM-1 agar plates and were maintained on slants of this medium. Molds were maintained on slants of one of the following Difco media: Czapek solution agar, malt extract agar, or potato dextrose agar.

Morphological and physiological tests used for the identification of yeasts were conducted according to Wickerham (1951), and isolates were identified according to Lodder (1970). Molds were identified according to Ames (1961), DeVries (1952), Neergaard (1945), Samson (1974), Sukapure and Thirumalachar (1966), Raper and Fennell (1965), and Raper and Thom (1949).

Also, a Barber-Coleman Model 5005 gas

chromatograph equipped with a radium-226 electron capture detector was used to analyze 0.5-g samples of honeybees for 2,4,5-T. The procedures were those of Morton et al. (1974). The bees were collected for analyses on four dates during the experiment.

Bees were sampled from March 7, 1974, through July 9, 1974. By May 30, two of the caged control colonies and one of the free-flying control colonies had died. By June 20, another caged control and another free-flying control colony had died. On July 9, all except one of the herbicide-treated colonies were dead. One free-flying herbicide-treated colony had 50 adult worker bees, no queen, and no eggs.

## RESULTS

Of the 195 bee guts examined, only 48 (25%) contained yeasts or molds. Organisms belonging to 23 species were found (Table 1). Isolations were made on both YM-1 and YM-5 media.

Tables 2 and 3 show the dates, treatment

groups of bees, and molds and yeasts isolated. *Torulopsis magnoliae*, *Hansenula anomala*, and *T. glabrata* were the yeasts isolated most frequently. *Penicillium cyclopium* and *P. cyclopium* var. *echinulatum* were the most frequently encountered molds. Yeasts were found in more bees from caged colonies fed 2,4,5-T than from other treatment groups, and molds were more prevalent in bees from free-flying control colonies receiving sucrose.

The levels of 2,4,5-T found in 0.5-g samples of honeybees are reported in Table 4. Bees from caged colonies fed 2,4,5-T contained higher levels of the herbicide than bees from treated colonies that were free-flying. Therefore, bees are probably able to eliminate the herbicide in cleansing flights when the colonies are not caged. Also, they would dilute the herbicide with food from natural sources.

## DISCUSSION

Sixteen percent of the guts of bees from free-flying colonies and 15% of the guts of

TABLE 3  
MOLDS ISOLATED FROM HONEYBEES

Organism	Treatment*	Date isolated	Number of bee guts containing the organism
<i>Aspergillus fumigatus</i>	S,F	3/7/74	2
<i>Rhizopus stolonifer</i>	S,F	3/7/74	1
<i>Aureobasidium pullulans</i>	S,F	3/7/74	1
<i>Penicillium</i> sp.	S,F	3/7/74	1
<i>Penicillium cyclopium</i>	H,F	3/7/74	1
<i>Aureobasidium</i> sp.	H,F	3/7/74	1
<i>Penicillium brevi-compactum</i>	H,C	3/7/74	1
<i>A. fumigatus</i>	H,C	3/7/74	1
<i>Penicillium</i> sp.	S,F	3/28/74	1
<i>P. cyclopium</i>	S,F	3/28/74	1
<i>P. cyclopium</i> var. <i>echinulatum</i>	S,F	3/28/74	1
<i>Aspergillus repens</i>	S,F	3/28/74	1
<i>P. cyclopium</i> var. <i>echinulatum</i>	S,C	3/28/74	1
<i>P. cyclopium</i>	S,F	4/18/74	1
<i>P. cyclopium</i> var. <i>echinulatum</i>	S,F	4/18/74	1
<i>P. cyclopium</i>	S,C	4/18/74	1
<i>Cladosporium cladosporioides</i>	H,F	4/18/74	1
<i>Aspergillus flavus</i> var. <i>columnaris</i>	S,F	5/9/74	1
<i>P. cyclopium</i>	H,C	5/9/74	1
<i>P. cyclopium</i> var. <i>echinulatum</i>	H,C	5/9/74	1
<i>C. cladosporioides</i>	H,C	5/9/74	1
<i>Cephalosporium acremonium</i> var. <i>funiculosum</i>	S,F	5/30/74	1
<i>Chaetomium spirale</i>	H,F	5/30/74	1
<i>Paecilomyces lilacinus</i>	H,F	5/30/74	1

\* S = sucrose; H = 2,4,5-T; C = caged; F = free-flying.

TABLE 4

LEVELS OF 2,4,5-T IN 0.5-g SAMPLES OF HONEYBEES

Colony	Treatment <sup>a</sup>	Date collected	2,4,5-T (ppm)
1	S,F	5/30/74	0
6	S,C	5/30/74	0
7	H,F	5/30/74	12
8	H,F	5/30/74	8
9	H,F	5/30/74	13
10	H,C	5/30/74	440
11	H,C	5/30/74	500
12	H,C	5/30/74	460
1	S,F	6/14/74	0
7	H,F	6/14/74	19
8	H,F	6/14/74	16
9	H,F	6/14/74	14
10	H,C	6/14/74	460
11	H,C	6/14/74	160
12	H,C	6/14/74	280
1	S,F	6/20/74	0
7	H,F	6/20/74	18
8	H,F	6/20/74	25
9	H,F	6/20/74	19
10	H,C	6/20/74	260
11	H,C	6/20/74	120
12	H,C	6/20/74	180
9	H,F	7/14/74	21

<sup>a</sup> S = sucrose; H = 2,4,5-T; C = caged; F = free-flying.

bees from caged control colonies contained yeasts (Table 5). Therefore, caging of colonies does not appear to be a factor in yeast buildup. However, 28% of the bee guts from caged colonies fed 2,4,5-T contained yeasts whereas only 7% of the bee guts from free-flying colonies fed 2,4,5-T contained yeasts. Thus, the combination of herbicide treatment and caging increased the number that contained yeasts. Also, bees from caged colonies fed 2,4,5-T contained higher levels of the herbicide. In previous work (Gilliam et al., 1974b), a total of 77% of the bees examined from caged colonies fed 2,4-D contained yeasts; 32% of those from caged control colonies contained yeasts.

Twenty percent of the bees from free-flying control colonies contained molds. The other treatment groups (caged control, herbicide-treated free-flying, and herbicide-

treated caged) contained fewer molds. We previously reported that molds occur most frequently in bees from untreated, free-flying colonies (Gilliam and Prest, 1972; Gilliam et al., 1974a). Also, the numbers of guts of bees from caged colonies fed 2,4-D and from caged control colonies that contained molds were about the same (Gilliam et al., 1974a). Thus, mold growth may be depressed in caged bees by limiting the sources of fresh inocula from pollen and nectar.

*Aspergillus flavus* var. *columaris*, *Cephalosporium acremonium* var. *funiculosum*, *Chaetomium spirale*, *Paecilomyces lilacinus*, *Penicillium brevi-compactum*, *Rhizopus stolonifer*, and *Saccharomyces rosei* are new records of fungi associated with honeybees. However, *Saccharomyces rosei* has been reported from stored pollen (Pain and Maugenet, 1966). The other organisms we isolated have been found in honeybees previously (Batra et al., 1973; Foote, 1966; Gilliam et al., 1974a,b; Thom, 1930).

*Torulopsis magnoliae*, *T. glabrata*, and *H. anomala* were the yeasts isolated most frequently, and *P. cyclopium* and *P. cyclopium* var. *echinulatum* were the molds most frequently isolated. These organisms, with the exception of *P. cyclopium* var. *echinulatum*, were also frequent isolates from bees in our previous work involving 2,4-D and antibiotics (Gilliam et al., 1974a,b).

Removal of brood from control colonies caused them to die earlier than colonies treated with 2,4,5-T. However, colonies

TABLE 5

BEE GUTS CONTAINING YEASTS AND MOLDS

Treatment	Bee guts containing yeasts	Bee guts containing molds
Control, free-flying	7/45 (16%)	9/45 (20%)
Control, caged	6/39 (15%)	2/39 (5%)
2,4,5-T, free-flying	4/57 (7%)	5/57 (9%)
2,4,5-T, caged	15/54 (28%)	3/54 (6%)

treated with 2,4,5-T contained a small amount of capped brood beginning 2 weeks after the initial feeding. Moffett and Morton (1975) found that feeding 2,4,5-T at a concentration of 500 ppm completely inhibited brood rearing. Eggs were laid, but they did not hatch. In the present experiment, the free-flying colonies fed 2,4,5-T contained only eggs and very young larvae within 6 weeks after the initial feeding. After 9 weeks of treatment, all but one of the colonies treated with 2,4,5-T contained only eggs. This one caged treated colony had a small amount of larvae and sealed brood in addition to eggs. All colonies gradually weakened from the lack of replacement bees and eventually died.

#### ACKNOWLEDGMENTS

We thank Dr. Joseph O. Moffett and Mr. Charles W. Shipman, U.S. Department of Agriculture Bee Research Laboratory, Tucson, for assistance with the bees.

#### REFERENCES

- AMES, L. M. 1961. "A Monograph of Chaetomiaceae," No. 2, U.S. Army Development Series.
- BATRA, L. R., BATRA, S. W. T., AND BOHART, G. E. 1973. The mycoflora of domesticated and wild bees (Apoidea). *Mycopathol. Mycol. Appl.*, 49, 13-44.
- DEVRIES, G. A. 1952. "Contribution to the Knowledge of the Genus *Cladosporium* Line ex Fr." Centraalbureau voor Schimmelcultures, Baarn, Netherlands.
- FOOTE, H. L. 1966. The mystery of the disappearing bees. *Glean. Bee Cult.*, 94, 152-153; 182.
- GILLIAM, M., AND MORTON, H. L. 1974. Enterobacteriaceae isolated from honey bees, *Apis mellifera*, treated with 2,4-D and antibiotics. *J. Invertebr. Pathol.*, 23, 42-45.
- GILLIAM, M. AND PREST, D. B. 1972. Fungi isolated from the intestinal contents of foraging worker honey bees, *Apis mellifera*. *J. Invertebr. Pathol.*, 20, 101-103.
- GILLIAM, M., PREST, D. B., AND MORTON, H. L. 1974a. Fungi isolated from honey bees, *Apis mellifera*, fed 2,4-D and antibiotics. *J. Invertebr. Pathol.*, 24, 213-217.
- GILLIAM, M., WICKERHAM, L. J. MORTON, H. L., AND MARTIN, R. D. 1974b. Yeasts isolated from honey bees, *Apis mellifera*, fed 2,4-D and antibiotics. *J. Invertebr. Pathol.*, 24, 349-356.
- LODDER, J., (ed.) 1970. "The Yeasts: A Taxonomic Study." North-Holland, Amsterdam.
- MOFFETT, J. O., AND MORTON, H. L. 1975. How herbicides affect honey bees. *Amer. Bee J.*, 115, 178-179; 200.
- MORTON, H. L., AND MOFFETT, J. O. 1972. Ovicidal and larvicidal effects of certain herbicides on honey bees. *Environ. Entomol.*, 1, 611-614.
- MORTON, H. L., MOFFETT, J. O., AND MARTIN, R. D. 1974. Influence of water treated artificially with herbicides on honey bee colonies. *Environ. Entomol.*, 3, 808-812.
- NEERGAARD, P. 1945. "Danish Species of *Alternaria* and *Stemphylium*. Taxonomy, Parasitism and Economic Significance." Einar Munksgaard, Copenhagen.
- PAIN, J. and MAUGENET, J. 1966. Recherches biochimiques et physiologiques sur le pollen emmagasiné par les abeilles. *Ann. Abeille*, 9, 209-236.
- RAPER, K. B., AND FENNELL, D. I. 1965. "The Genus *Aspergillus*." Williams and Wilkins, Baltimore.
- RAPER, K. B., AND THOM, C. 1949. "A Manual of the Penicillia." Williams and Wilkins, Baltimore.
- SAMSON, R. A. 1974. "*Paecilomyces* and Some Allied Hyphomycetes." Studies in Mycology, No. 6. Centraalbureau voor Schimmelcultures, Baarn, Netherlands.
- SUKAPURE, R. S., AND THIRUMALACHAR, M. J. 1966. Conspectus of species of *Cephalosporium* with particular reference to Indian species. *Mycologia*, 58, 351-361.
- THOM, C. 1930. "The Penicillia." Williams and Wilkins, Baltimore.
- WICKERHAM, L. J. 1951. Taxonomy of yeasts. *U.S. Dept. Agr. Tech. Bull.*, No. 1029, 1-56.