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Fungi Isolated from Honey Bees, *Apis mellifera*, Fed 2,4-D and Antibiotics[1,2]

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Eighteen species of fungi were isolated and identified from the intestines of 388 honey bees, *Apis mellifera*. Bees fed a combination of oxytetracycline and fumagillin contained fewer fungi than control bees or bees fed 2,4-D. New records of fungi associated with honey bees include *Alternaria tenuissima*, *Cladosporium cladosporoides*, *Bipolaris* sp., *Curvularia brachyospora*, *Penicillium ochro-chloron*, *Penicillium urticae*, and *Rhizopus arrhizus*.

INTRODUCTION

Recently we reported the isolation of fungi from the intestinal contents of healthy foraging worker honey bees, *Apis mellifera* (Gilliam and Prest, 1972). *Penicillium frequentans*, *P. notatum*, *Aspergillus amstelodami*, and *Cladosporium* sp. were identified. We also noted the paucity of reports of nonpathogenic fungi isolated from honey bees. Subsequently, Batra et al. (1973) provided an important list of fungi associated with Apoidea and confirmed our earlier observation (Gilliam and Prest, 1972) that the few existing records of saprophytic fungi associated with honey bees dealt primarily with fungi occurring within the beehive (on combs and on pollen) and on dead bees.

[1] In cooperation with the Arizona Agricultural Experiment Station.

[2] Mention of a proprietary product or company name does not constitute an endorsement of this product by the USDA.

The purpose of the present study was to examine the fungal flora (excluding the yeasts) from the intestinal contents of adult worker honey bees from control colonies, from colonies fed the herbicide (2,4-dichlorophenoxy)acetic acid (2,4-D), and from colonies fed a combination of oxytetracycline (TM-25[§]) and fumagillin (Fumidil B®). We previously examined Enterobacteriaceae from these bees (Gilliam and Morton, 1974).

MATERIALS AND METHODS

Colonies were established, caged, maintained, and fed as previously described (Gilliam and Morton, 1974). In the first test, 2,4-D and the antibiotics were fed initially on July 14, 1971. In the second test, they were fed initially on September 17, 1971. Altogether we examined bees from 12 colonies: 4 control colonies, 4 colonies fed 2,4-D, and 4 colonies fed a combination of TM-25 and Fumidil B.

In the first test, 3 adult worker bees from each colony were examined weekly. The intestinal tracts were aseptically removed and individually homogenized in 2.5 ml of sterile 0.85% NaCl as previously described (Gilliam and Prest, 1972). A loopful of the homogenate from each bee was streaked in duplicate on each of the following solid media in Petri dishes: trypticase soy agar (BBL®), Czapek solution agar (Difco®), and Lindegren yeast agar (Difco). All plates were incubated under aerobic conditions at 37°C for 14 days.

In the second test, every 3 weeks the intestines of two bees from each colony were individually homogenized in 2.5 ml of sterile thioglycollate 135C medium (BBL). Each homogenate was streaked in duplicate on trypticase soy agar, eugonagar (BBL), and Czapek solution agar. One plate was incubated at 25°C and one at 37° under aerobic conditions for 14 days. All fungal colonies which developed were maintained on slants of Czapek solution agar or malt extract agar. Cultures were identified according to Barnett (1969), DeVries (1952), Ellis (1966), Neergaard (1945), Raper and Fennell (1965), and Raper and Thom (1949).

The first bees were sampled from July, 1971, to January, 1972, when the herbicide-fed and antibiotic-fed colonies died. Bees in the second test were sampled from September, 1971, to August, 1972, though all herbicide-fed colonies had died by April, 1972, one of the antibiotic-fed colonies died in June, 1972, and a control colony died in May, 1972.

TABLE 1

SUMMARY OF FUNGI ISOLATED FROM CONTROL BEES, FROM BEES FED 2,4-D, AND FROM BEES FED OXYTETRACYCLINE AND FUMAGILLIN

Organism	Number of bee guts containing the organism
Penicillium cyclopium	10
Aspergillus flavus	7
Alternaria tenuissima	6
Cladosporium cladosporoides	4
Aspergillus sydowi	2
Bipolaris sp.	2
Mycelia sterilia	2
Penicillium chrysogenum	2
Penicillium frequentans	2
Aspergillus niger	1
Aspergillus terreus	1
Curvularia brachyospora	1
Penicillium citrinum	1
Penicillium corylophilum	1
Penicillium notatum	1
Penicillium ochro-chloron	1
Penicillium urticae	1
Rhizopus arrhizus	1

RESULTS

Forty-six fungi belonging to 18 different species were isolated and identified from the 388 bees that we examined. Isolations were made on all media used and after incubation at both 25°C and 37°C. Table 1 shows the organisms isolated and the frequency of occurrence. *Penicillium cyclopium*, *Aspergillus flavus*, *Alternaria tenuissima*, and *Cladosporium cladosporoides* were found most frequently.

Table 2 lists the organisms isolated in the first test, the dates of isolation, and the treatment of the bees. Thirteen isolates were identified from control bees, 4 from bees fed 2,4-D, and 3 from bees fed antibiotics. Therefore, in this experiment, bees fed 2,4-D or antibiotics had fewer fungi in their intestines. All organisms, including the fungi, had been eliminated from the intestines of bees treated with antibiotics approximately 3 months after the start of the feeding of the antibiotics.

Table 3 lists the organisms isolated in the second test. Eleven isolates were from bees fed 2,4-D; 10 from control bees; and 5 from bees fed antibiotics. *Penicillium cyclopium* was the most frequent isolate in the second test and *Aspergillus flavus* occurred most frequently in bees sampled in the first test; however, *P. cyclopium* was the second most frequent isolate in the second test.

TABLE 2
FUNGI ISOLATED FROM HONEY BEES: FIRST TEST

Organism	Treatment[a]	Date isolated
Aspergillus flavus	C	Aug. 23, 1971
Aspergillus flavus	C	Aug. 23, 1971
Bipolaris sp.	H	Aug. 30, 1971
Penicillium cyclopium	C	Sept. 7, 1971
Bipolaris sp.	A	Sept. 13, 1971
Penicillium frequentans	H	Sept. 13, 1971
Penicillium frequentans	C	Sept. 13, 1971
Alternaria tenuissima	A	Sept. 20, 1971
Cladosporium cladosporoides	C	Sept. 27, 1971
Penicillium cyclopium	C	Oct. 12, 1971
Penicillium cyclopium	C	Oct. 12, 1971
Aspergillus flavus	C	Oct. 12, 1971
Aspergillus flavus	C	Oct. 12, 1971
Aspergillus flavus	C	Oct. 12, 1971
Mycelia sterilia	C	Oct. 12, 1971
Aspergillus terreus	A	Oct. 12, 1971
Aspergillus flavus	C	Nov. 22, 1971
Alternaria tenuissima	H	Nov. 29, 1971
Aspergillus flavus	C	Nov. 29, 1971
Penicillium cyclopium	H	Nov. 29, 1971

[a] C = control; H = 2,4-D; A = antibiotics.

Aspergillus sydowi, *P. corylophilum*, *Rhizopus arrhizus*, *P. chrysogenum*, *P. ochro-chloron*, *Aspergillus niger*, *Curvularia brachyospora*, *P. notatum*, *P. citrinum*, and *P. urticae* were isolated in the second test but not in the first. *Aspergillus flavus*, *P. frequentans*, *Bipolaris* sp., and *Aspergillus terreus* were isolated in the first test but not in the second.

DISCUSSION

From the results of this study, fungi, excluding yeasts, do not appear to be prevalent in the intestines of caged honey bees. They were more prevalent in the intestines of free-flying bees (Gilliam and Prest, 1972). Also, we found fewer Enterobacteriaceae in caged bees than in free-flying bees (Gilliam and Valentine, 1974; Gilliam and Morton, 1974). Caging bees may, therefore, decrease the numbers of some types microorganisms in the intestine. However, the yeast flora increases in caged bees (Gilliam, 1973).

Twenty-three isolations were obtained from control bees, 15 from herbicide-fed bees, and 8 from antibiotic-fed bees. Therefore, the antibiotics appeared to depress the fungal flora. In the first test, herbicide treatment decreased the fungal flora, but in the second test it did not.

Organisms belonging to the genera *Penicillium* and *Aspergillus* were found most frequently. This agrees with our data from free-flying bees (Gilliam and Prest, 1972).

Obviously, none of the fungi isolated are symbiotes in the intestines of bees since many bees contained no fungi in their guts. This is also true of the Enterobacteriaceae that have been found in the intestines of bees (Gilliam and Morton, 1974). Therefore, the intestines of adult worker bees are contaminated by microorganisms which are

TABLE 3
FUNGI ISOLATED FROM HONEY BEES: SECOND TEST

Organism	Treatment[a]	Date isolated
Penicillium notatum	A	Oct. 19, 1971
Penicillium citrinum	A	Oct. 19, 1971
Curvularia brachyospora	H	Nov. 30, 1971
Penicillium chrysogenum	H	Nov. 30, 1971
Penicillium cyclopium	A	Dec. 21, 1971
Mycelia sterilia	C	Dec. 21, 1971
Alternaria tenuissima	C	Jan. 11, 1972
Alternaria tenuissima	C	Jan. 11, 1972
Aspergillus niger	H	Feb. 1, 1972
Penicillium cyclopium	H	Feb. 23, 1972
Cladosporium cladosporoides	H	Feb. 23, 1972
Alternaria tenuissima	H	March 14, 1972
Alternaria tenuissima	C	March 14, 1972
Cladosporium cladosporoides	C	March 14, 1972
Cladosporium cladosporoides	H	March 14, 1972
Penicillium chrysogenum	H	March 14, 1972
Penicillium cyclopium	H	March 14, 1972
Penicillium ochro-chloron	H	March 14, 1972
Penicillium cyclopium	C	April 4, 1972
Penicillium uticae	A	April 4, 1972
Aspergillus sydowi	H	April 4, 1972
Penicillium cyclopium	C	April 25, 1972
Rhizopus arrhizus	C	April 25, 1972
Penicillium corylophilum	C	May 16, 1972
Penicillium cyclopium	A	June 6, 1972
Aspergillus sydowi	C	June 28, 1972

[a] C = control; H = 2,4-D; A = antibiotics.

probably ingested since the guts of worker larvae, pupae, and newly emerged adults are sterile (Gilliam, 1971).

Penicillium cyclopium, *Aspergillus sydowi*, *P. chrysogenum*, and *P. corylophilum* have been isolated from dead bees and combs (Burnside, 1927). The following organisms have been found in hives: *P. frequentans*, *P. citrinum* and *Aspergillus terreus*. *Aspergillus flavus* has been found in larvae with "stone brood" disease, on combs, and in the honey stomach of adult worker bees, and *Aspergillus niger* in adults and mummified larvae (Burnside, 1930; Batra et al., 1973). Mitroiu et al. (1966) found *Aspergillus flavus*, *A. niger*, and *A. terreus* in the intestines of adult bees. *Penicillium notatum* and *P. frequentans* were found in the intestines of foraging worker bees (Gilliam and Prest, 1972). Therefore, *Alternaria tenuissima*, *Cladosporium cladosporoides*, *Bipolaris* sp., *Curvularia brachyospora*, *P. ochro-chloron*, *P. urticae*, and *Rhizopus arrhizus* are new records of fungi associated with honey bees.

In the present test, only *Penicillium cyclopium* and *Alternaria tenuissima* were isolated from control, antibiotic-fed, and herbicide-fed bees. *Aspergillus flavus*, *Rhizopus arrhizus*, and *P. corylophilum* were isolated only from control bees. Perhaps 2.4-D and the antibiotics had a detrimental effect on the growth and survival of these organisms. Organisms isolated from both control and herbicide-fed bees were *P. frequentans*. *Cladosporium cladosporoides*, and *Aspergillus sydowi*. This suggests that the antibiotics, but not 2,4-D, might have affected these organisms. *Curvularia brachyospora*, *Aspergillus niger*, *P. chrysogenum*, and *P. ochro-chloron* were isolated only from bees fed 2,4-D. Therefore, herbicides might favor the growth of these organisms, or, perhaps, these fungi can use 2,4-D as a substrate. *Aspergillus terreus*, *P. notatum*, *P. citrinum*, and *P. urticae* were isolated only from bees fed antibiotics. Thus, the growth of these fungi might be stimulated by antibiotic treatment. *Bipolaris* sp. was isolated from bees fed antibiotics and from bees fed herbicide.

The effects of 2,4-D on fungi in soil are believed to be minimal (Jensen, 1963); however, concentrations of 2,4-D in soil are usually much lower than those we used. The fact that we isolated nearly as many fungi from bees fed 2,4-D as from control bees suggests that even at this higher concentration (1000 ppm), 2,4-D does not seriously reduce fungal populations. Then it is possible that the species isolated from control bees and bees fed antibiotics but not from bees fed 2,4-D are seriously affected by 2,4-D. A determination of the effects of 2,4-D on individual species of fungi was beyond the scope of this study.

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