JOURNAL OF INVERTEBRATE PATHOLOGY 29, 344–346 (1977)

Effect of *Bacillus sphaericus* Strain SSII-1 on Honey Bees, *Apis mellifera*[1]

ELIZABETH WEST DAVIDSON,* HOWARD L. MORTON,† JOSEPH O. MOFFETT,† AND SAMUEL SINGER‡

** Department of Zoology, Arizona State University, Tempe, Arizona 85281; † Agricultural Research Service, U.S. Department of Agriculture, 2000 East Allen Road, Tucson, Arizona 85719; and ‡ Department of Biological Sciences, Western Illinois University, Macomb, Illinois 61455*

Received May 12, 1976

When mosquito-larvicidal *B. sphaericus* strain SSII-1 cultures were fed to newly emerged adult honey bees and to bee colonies, no effect was found on the longevity of newly emerged bees nor on the brood production of colonies.

Bacillus sphaericus strain SSII-1 is insecticidal for mosquito larvae and will be field-tested as a microbial control agent. We report results of research to determine the effects of this bacterium on the longevity and reproduction of an important nontarget insect, the honey bee, *Apis mellifera*.

MATERIALS AND METHODS

Bacterial cultures. *Bacillus sphaericus* strain SSII-1 and noninsecticidal strain ATCC 7054, both var. *fusiformis*, were cultured in synthetic liquid medium to produce synchronously growing final whole cultures (FWC) at 18–24 hr after inoculation (Singer et al., 1966; Singer, 1974). Final whole cultures were diluted with 20% sucrose for feeding to bees. Insecticidal activity and bacterial viability were preserved when FWC were diluted with 20% sucrose solution, but not when diluted with 60% sucrose.

Longevity studies. Five 2 × 6 × 6-in. cages, each containing 10 g of newly emerged bees, were used for each bacterial culture at each concentration [1 part FWC/100 parts 20% sucrose (10^{-2}) and 1 part FWC/10,000 parts 20% sucrose (10^{-4})] and for 20 and 60% sucrose control solutions containing no bacterial culture. Five grams of maintenance diet, consisting of 11 parts pollen, 10 parts sucrose, 9 parts Drivert (92% sucrose, 8% invert sugar; California and Hawaii Sugar Co.), was added at the beginning of the test. Distilled water and diluted bacterial culture or sucrose solution were made available to bees in 5-dram plastic vials and were replenished daily. Dead bees were removed daily, counted, and frozen. These methods have been used previously in herbicide studies (Morton et al., 1972) and in evaluating the effects of alfalfa looper nuclear polyhedrosis virus on honey bees (Morton et al., 1975). Longevity studies took place in Tucson, Arizona, between March 4 and May 4, 1975. Colonies in reproduction studies were held in flight cages in Tucson from March 28 to May 2, 1975, when they were moved to a desert apiary where they were observed until July 10, 1975.

Reproduction studies. Honey bee colonies were initiated from 3 lb of bees and a laying queen. Following a free-flight period of 4 days, each colony was moved into a 12 × 12 × 10-ft Saran-mesh flight cage. Each bacterial culture was fed at 10^{-2} concentration to three caged colonies, as was the 20% sucrose control. Solutions were fed from 1-pint jars placed directly above the frame containing the brood and were re-

[1] Mention of a proprietary product or company name does not constitute an endorsement of this product by the U.S. Department of Agriculture.

ISSN 0022-2011

plenished daily. After 28 days of confinement within flight cages, colonies were moved to an apiary where they were allowed to free-fly and collect pollen and nectar, though bacterial culture was still replenished weekly for 4 weeks. At weekly intervals, brood development was observed, and samples of larvae and pupae were removed and frozen. The area of the capped brood was measured 21, 28, and 56 days after bacterial cultures were first introduced. These methods are similar to those described by Morton and Moffett (1972).

Reisolation of B. sphaericus. Dead frozen adult bees from longevity tests and larvae and pupae from reproduction tests were surface-sterilized by being dipped into 70% ethanol for 30 sec. They were then rinsed in sterile water and crushed in 25 ml of synthetic liquid bacterial medium in 125-ml Erlenmeyer flasks. The flasks were incubated at 28°C for 24 hr in an orbital waterbath shaker at 100 rpm. Resulting cultures were diluted and plated on brain-heart infusion agar plates (Difco, Detroit, Michigan) and were incubated for 24 hr at 32°C. Bacterial colonies were then selected on the basis of similarity of colony appearance to stock cultures of *B. sphaericus*. Colonies were confirmed as *B. sphaericus* by the presence of round terminal or subterminal spores swelling the sporangium (Gordon et al., 1973) in smears stained with malachite green and safranin (Conn et al., 1957). After restreaking to establish purity, *B. sphaericus* colonies were inoculated into liquid synthetic medium, and cultures were brought to synchrony. FWC, 18 to 24 hr old, were bioassayed against second-instar larvae of the mosquito *Culex pipiens quinquefasciatus*. Values for LD_{50}, based on dilution of FWC (Singer, 1974), were compared with stock laboratory cultures of SSII-1 and ATCC 7054.

RESULTS AND DISCUSSION

Bacillus sphaericus was isolated from adult bees fed SSII-1 and ATCC 7054 in longevity tests. When bioassayed, *B. sphaericus* cultures isolated from bees fed SSII-1 gave LD_{50} values of approximately 1 part culture/100,000 parts water (10^{-5}), similar to values found with stock cultures of SSII-1. *Bacillus sphaericus* cultures isolated from bees fed ATCC 7054 were not pathogenic to mosquito larvae. No *B. sphaericus* was isolated from bees fed 60% sucrose; however, mosquito-larvicidal *B. sphaericus*, presumed to be strain SSII-1, was isolated from controls fed 20% sucrose, indicating contamination of the 20% sucrose control. No *B. sphaericus* was isolated from larvae or pupae from bee colonies in the reproduction tests.

In longevity tests, the half-life was defined as the number of days required for one-half of the bees in a cage to die. Half-lives of treated groups did not differ significantly from the 60% control except for those fed ATCC 7054 at 10^{-2} dilution at the 5% level of probability, as calculated by Duncan's multiple-range test (Table 1).

In reproduction tests, eggs were observed in all but one of the colonies when they were moved inside the flight cages and first fed the bacterial cultures or sucrose solution.

TABLE 1

HALF-LIFE OF NEWLY EMERGED ADULT HONEY BEES FED BACTERIAL CULTURES IN 20% SUCROSE AND 20 AND 60% SUCROSE CONTROLS[a]

Bacterial culture	Concentration in 20% sucrose	Half-life[b]
B. sphaericus/SSII-1	10^{-4}	53 ab
B. sphaericus/SSII-1	10^{-2}	48 ab
B. sphaericus/ATCC 7054	10^{-4}	53 ab
B. sphaericus/ATCC 7054	10^{-2}	46 b
20% Sucrose control[c]	0	52 ab
60% Sucrose control	0	57 a

[a] Half-life is number of days required for one-half of the bees in a cage to die.

[b] Means followed by same letter(s) do not differ significantly at the 5% level of probability as calculated by Duncan's multiple-range test.

[c] Apparently contaminated with *Bacillus sphaericus*/SSII-1.

TABLE 2

AREA OF SEALED BROOD IN HONEY BEE COLONIES FED BACTERIAL CULTURES AT A CONCENTRATION OF 1 PART CULTURE TO 100 PARTS 20% SUCROSE (10^{-2})

Bacterial culture	Days after feeding started		
	21	28[a]	56[b]
	Area (in.^{2})[c]		
B. sphaericus/SSII-1	79 a	104 a	254 a
B. sphaericus/ATCC 7054	94 a	94 a	157 a
20% Sucrose control	81 a	131 a	229 a

[a] Measured immediately prior to removal from flight cages.

[b] Measured after bees had been free-flying for 4 weeks.

[c] Means in each column followed by the same letter do not differ significantly at the 5% level of probability as calculated by Duncan's multiple-range test.

The colony without eggs was queenless, and therefore, a new queen was introduced. Subsequently, this colony produced brood. The amount of brood produced in all colonies was relatively low and was probably due to the "cage effect". After the colonies were moved from the cages to an outside apiary, brood production returned to normal levels in both the check and treated colonies. No significant differences in brood production were observed or measured in the colonies receiving the bacterial solutions and the 20% sucrose controls (Table 2).

The results of this study demonstrate that *B. sphaericus* strain SSII-1 affects neither the longevity of newly emerged adult bees, nor the brood production of colonies fed cultures of the bacterium.

ACKNOWLEDGMENTS

The authors thank Sue Martynowski for her technical assistance. We thank the Division of Agriculture and the College of Engineering, Arizona State University, for the use of laboratory space and equipment. This research was supported in part by National Science Foundation Grant No. BMS 72-01954 A01, and in part by a grant from Abbott Laboratories, North Chicago, Illinois.

REFERENCES

CONN, H. J., BARTHOLOMEW, J. W., AND JENNISON, M. W. 1957. Staining methods. *In* "Manual of Microbiological Methods" (H. J. Conn, ed.), pp. 10–36. McGraw–Hill, New York.

GORDON, R. E., HAYNES, W. C., AND PANG, C. H-N. 1973. "The Genus *Bacillus*." USDA Handbook 427, pp. 1–283.

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 MACDONALD, R. H. 1972. Toxicity of herbicides to newly emerged honey bees. *Environ. Entomol.*, 1, 102–104.

MORTON, H. L., MOFFETT, J. O., AND STEWART, F. D. 1975. Effect of alfalfa looper nuclear polyhedrosis virus on honeybees. *J Invertebr. Pathol.*, 26, 139–140.

SINGER, S., GOODMAN, N. S., AND ROGOFF, M. H. 1966. Defined media for the study of *Bacilli* pathogenic for insects. *Ann. N. Y. Acad. Sci.*, 139, 16–23.

SINGER, S. 1974. Entomogenous bacilli against mosquito larvae. *In* "Developments in Industrial Microbiology," Vol. 15, pp. 187–194. Plenum Press, New York.