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## Microorganisms Found in Field Specimens Of Diseased Corn Borer Larvae

By EARLE S. RAUN, WILLIAM R. LOCKHART, and RUSSELL J. BEERS

*Abstract.* Two hundred and eighty-six abnormal, field collected larvae of the European corn borer were examined for the presence of microorganisms which could have caused the abnormality or death. The most common microorganism found was a microsporidian, *Perezia pyraustae* Pailot. Next most numerous was a group of enterobacteria. Spore forming rods apparently of the genus *Bacillus*, and fungi belonging to the genera *Beauvaria* and *Metarrhizium* were also isolated. An examination of 100 apparently normal pupae revealed that 82 were hosts to *Perezia pyraustae* and two contained bacteria.

In nature many factors tend to keep an insect population from becoming too large. Among these factors are microorganisms which cause disease.

Insect control with insecticides has become an everyday practice, but the use of microorganisms to reduce damaging insect populations is relatively unfamiliar. It was not until the late nineteenth century that such workers as Elie Metchnikoff, J. L. LeConte, and Louis Pasteur suggested the use of microorganisms in insect control (Steinhaus, 1956).

In Iowa, the European corn borer, *Pyrausta nubilalis* Hübner, has caused an annual loss averaging about 70 million dollars since it was reported in the state in 1943 (Harris and Brindley). Since the continued widespread use of insecticides to control this pest could constitute a hazard to wildlife and man, a survey of the microorganisms causing disease in corn borer larvae in the field was begun in the fall of 1958.

### PROCEDURES

As state and federal workers at the Iowa State College and U.S.D.A. cooperative Corn Borer Laboratory made dissection of corn stalks in order to obtain larvae of the corn borer for routine rearing procedures, abnormal larvae were observed. These abnormal fifth instar larvae were placed in sterile vials, stoppered with cotton, and taken to the Insect Pathology Laboratory at Iowa State College, where they were stored under refrigeration for periods up to 6 weeks before being examined for the presence of microorganisms.

The skin of each larva was examined for signs of fungus growth. If such was present the entire larva was used to streak a potato agar plate which was incubated at 33 degrees C.

Each larva was then placed in 70 percent alcohol for 10 minutes

to free the outer surfaces from microorganisms, removed from the alcohol, and the malpighian tubules were removed aseptically for a microscopic examination for protozoan parasites.

The remainder of the larva was placed in sterile water in a sterile Waring Blendor jar and homogenized. A sample of the homogenate was streaked on a nutrient agar plate. Also a loopful of the material was used to inoculate a tube of nutrient broth. Both the plate and the tube were incubated at 33 degrees C.

Pure cultures were then established by picking isolated colonies of fungi and/or bacteria from the growth obtained on the original streak plates.

RESULTS

Tentative groupings of the microorganisms were made from examination of stained microscope slides, pending the outcome of biochemical and morphological tests being performed with the pure cultures obtained by the previous procedures.

Of the 286 fifth instar larvae examined to date, 259 were found to be hosts to microorganisms of one type or another, and 27 were negative in this regard. Of the several kinds of microorganisms isolated from the larvae, strains of fungi of the genera *Beauveria* and *Metarrhizium* were isolated in 8 cases (3 percent), bacteria in 90 instances (31.4 percent) and microsporidia (protozoa) in 177 instances (62 percent). In a number of cases at least two types of microorganisms occurred together.

The number and percentage of larvae that were hosts to the various types and combinations of microorganisms are shown in Table 1.

Table 1  
Types and Combinations of Microorganisms Found in 286 Field Collected European Corn Borer Larvae

Type of Microorganism	Number of Cases	Approximate Percentage Total
<i>Perezia pyraustae</i> (Total)	177	62
Enterobacteria (Total)	72	25
<i>Bacillus</i> spp. (Total)	18	6
<i>P. pyraustae</i> only	165	58
Enterobacteria only	52	18
<i>Bacillus</i> spp. only	6	2
<i>P. pyraustae</i> and enterobacteria	9	3
<i>P. pyraustae</i> and <i>Bacillus</i>	1	.4
<i>P. pyraustae</i> , <i>Bacillus</i> and enterobacteria	2	.6
<i>Bacillus</i> and enterobacteria	9	3
<i>Beauveria</i> spp. only	5	2
<i>Metarrhizium</i> spp. only	3	1
Other fungi	7	2.5
Negatives	27	9.5

A group of 100 apparently normal pupae of the European corn borer were obtained through the cooperation of workers at the Ankeny Corn Borer Laboratory. These pupae had been collected as larvae, and allowed to pupate at the laboratory under standard rearing procedures. In the Insect Pathology Laboratory these supposedly normal pupae were studied, using the same procedures as for larvae.

Of the 100 pupae which were examined for microorganisms, 82 were found to be hosts of the microorganisms *Perezia pyraustae* Paillot. Two pupae were hosts to organisms which appeared to be enterobacteria. One of the two strains was found in a pupa which also contained *P. pyraustae*, whereas the other was found alone.

#### DISCUSSION

It should be pointed out that in this study the strains of microorganisms isolated from the diseased larvae are not as yet all positively identified, nor are they certain pathogens. Pathogenicity tests are yet to be performed on the strains of bacteria and fungi.

There are several fungi known to be pathogenic to lepidopterous larvae. Among these is *Beauveria bassiana* (Balsamo) Vuill., which was reported to be pathogenic to silkworms in a communication delivered before a commission of the Faculties of Medicine and Philosophy of the University of Pavia by Agostino Bassi in 1834. (Bassi did not know this fungus by its present name. The specific name was given the organism by Balsamo about 1835, and Vuillemin made this fungus the type species of the genus *Beauveria* in 1912.)

Lefebvre (1931) and Baker *et al.* (1949) report considerable mortality from *Beauveria bassiana* in corn borers held in the laboratory but indicate that this fungus does not cause mortality in the field. However, Beall *et al.*, (1939) indicated 60 to 70 percent control of corn borer larvae when they applied spores of the fungus to corn plants infested with the young borer larvae.

Another genus of fungi pathogenic to insects is *Metarrhizium*. This was reported in 1929 by Wallengren and Johansson as being extremely pathogenic to corn borer larvae.

The only protozoan found infecting the larvae was the microsporidian *Perezia pyraustae* Paillot. Paillot (1928) did not believe the parasite to be of much importance in the natural destruction of the corn borer. He did believe, however, that the parasite reduced vitality and favored the action of other destructive factors. On the other hand, Zimmack (1957) showed that infestation with *P. pyraustae* reduced the growth rate, oviposition, and larval survival of the European corn borer.

Of the 90 strains of bacteria isolated from diseased corn borer larvae, 18 were spore forming rods probably of the genus *Bacillus*, and the remainder appeared to be enterobacteria. Although biochemical and morphological studies of the latter isolates are not yet completed, they seem to be aberrant types exhibiting rather unique characteristics (Beers, *et al.*, 1959). The large number of these strains found may indicate an important group of organisms for use in biological control of the corn borer.

Several species of the genus *Bacillus* are known to be pathogenic to insects. *Bacillus thuringiensis* Berlinger was reported as early as 1928 (Husz) as being pathogenic to corn borer larvae.

Steinhaus (1952) reported on examination of 274 larvae of the European corn borer received from Iowa and Ohio. The larvae had been held in the laboratory for varying lengths of time before any abnormalities had been noted. It is interesting to compare the infection with various microorganisms as reported by Steinhaus with those reported in this study. He found that 50 percent of the larvae showed infection with *Beauveria*, 24.4 percent with *Perezia pyraustae*, and 32.8 percent with various bacteria. Twelve percent of the specimens he examined were negative for microorganisms.

In the larval examinations reported here 3 percent were infected with *Bauveria*, or *Metarrhizium*, 62 percent with *Perezia pyraustae*, and 31.4 percent with various bacteria. Nine and one-half percent of the larvae examined showed no evidence of the presence of microorganisms.

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