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A NEW MICROSPORIDIUM IN ALFALFA WEEVIL POPULATIONS: DISTRIBUTION AND CHARACTERIZATION

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ABSTRACT

A microsporidium species, not previously reported, was found infecting field populations of the alfalfa weevil, *Hypera postica*, in Illinois. The pathogen is widely distributed thoughout the state. Percent infection ranged from 1% to 50% at different collection locations. Characteristics of the microsporidium and possible modes of transmission are presented.

Prior to this report, three different microsporidia were recovered from the alfalfa weevil, *Hypera postica* (Gyllenhal). A microsporidium, thought to be a *Nosema* species, was isolated from a single dead alfalfa weevil larva collected in Edwards County, Illinois (Maddox and Luckman 1966). Although we have Giemsa-stained slides of this microsporidium, viable spores are not presently available. Drea et al. (1969) reported a microsporidium (*Nosema* sp.) infecting a laboratory colony of alfalfa weevils in Moorestown, New Jersey. We have both Giemsa-stained slides and viable spores of this microsporidium. A third microsporidium was recovered from a laboratory colony of alfalfa weevils at Utah State University and described as *Perezia hyperae* (Youssef 1974). Later, Sprague (1977) placed *P. hyperae* in the genus *Nosema*. The microsporidium species isolated by Maddox and Luckmann (1966) is distinctly different from the other two species based on comparisons of Giemsa-stained slides.

In 1974 alfalfa weevil larvae from the Illinois counties of Washington and Mason were examined for pathogens (Roberts et al., 1977). Three of the 715 larvae examined were infected with a microsporidium considered to be conspecific with the *Nosema* species recovered in 1966. Because this *Nosema* species has been observed in alfalfa weevil populations only two times, and in both cases the prevalence was very low (<1%), we believe it is possible that the alfalfa weevil is not the primary host for this microsporidium. The *Nosema* species reported by Drea et al. (1969) and Youssef (1974) have never been reported from field collections of alfalfa weevils.

We conducted a statewide survey for alfalfa weevil pathogens in April of 1990. A microsporidium, different from the descriptions of the previously reported species, was found. This organism was recovered from alfalfa fields in 22 of 31 counties surveyed from 1990 through 1992 (Figure 1). It was also found in a colony of field-collected alfalfa weevils at Ohio State University, in a field sample of alfalfa weevils collected on burr clover and black medic near Lutcher, Louisiana, and in field samples of alfalfa from Prince Georges Co., Maryland and Lincoln Co., Missouri. Field-collections in Illinois have shown

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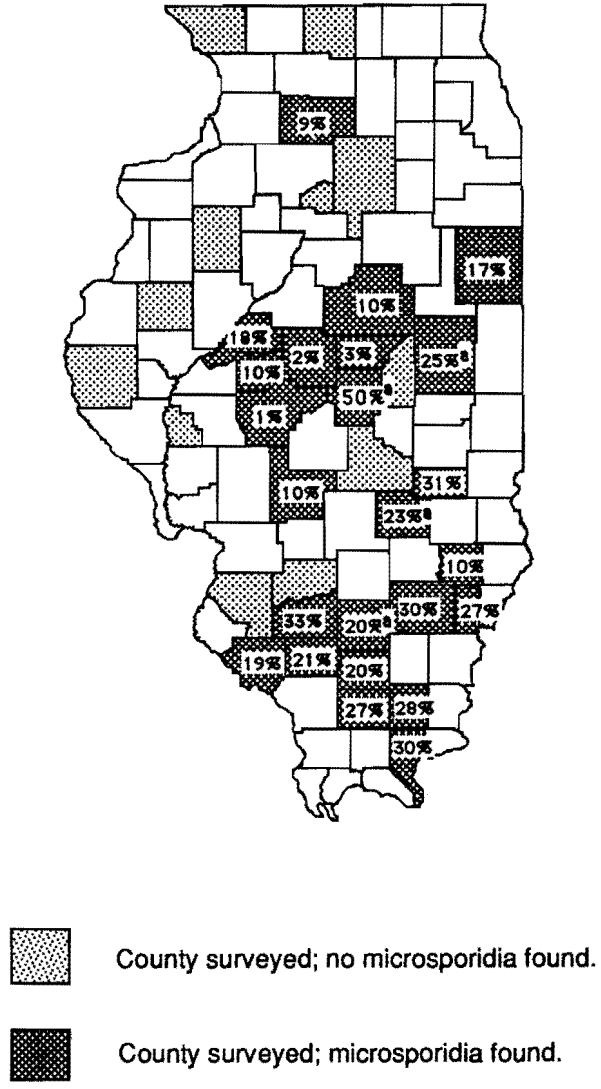

Figure 1. Prevalence of a new microsporidium species in Illinois. [a]Prevalence reported is the highest recorded between two collection years.

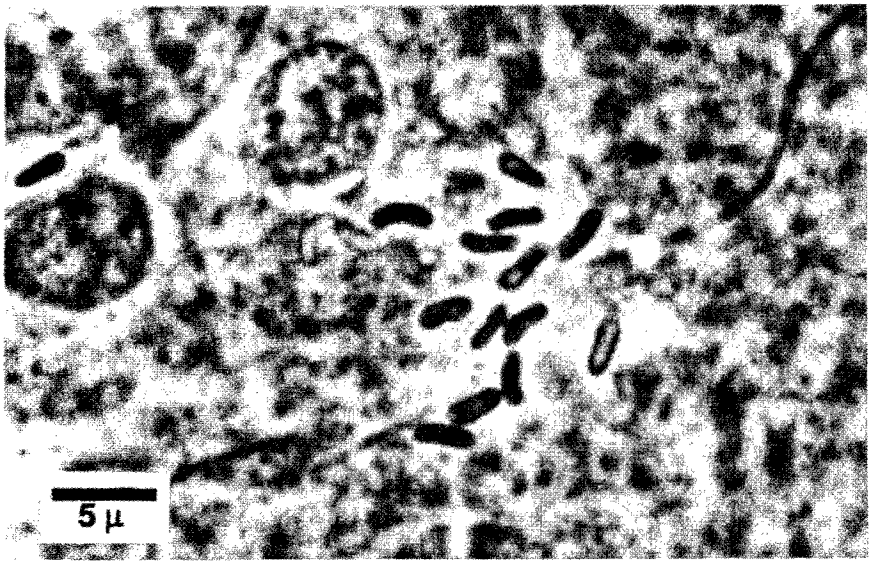

Figure 2. Spores of a new microsporidium species in the midgut tissue of the alfalfa weevil.

the prevalence of the microsporidium to be from 1% to 50% (Figure 1) in counties where it was recovered. We believe that the pathogen is relatively new to Illinois alfalfa weevil populations because it was not found in the extensive pathogen surveys in 1966 and 1974.

The spores (Figure 2) and vegetative forms of the microsporidium were found in the midgut, hindgut, fat body, and Malpighian tubules of alfalfa weevil larvae and adults, and also in salivary gland tissue in the larvae. The spores ranged in length from 2.3–3.1 μm and in width from 0.8 to 1.2 μm. The average spore length was 2.7 $\pm$ 0.2 μm and the average width was 1.0 $\pm$ 0.1 μm (Table 1).

We were able to produce horizontal transmission of the microsporidium when first-instar alfalfa weevils were fed alfalfa leaves coated with spores at a concentration of 1×10^4 spores/μl. Infection was found in 11 of 17 larvae (65%) that reached the fourth stadium. Control larvae were free of infection. Additionally, we immersed 32 first- and second-instars for 15 seconds in a spore solution at a concentration of 8.8×10^6 spores/μl. Nineteen larvae survived, and seven (37%) developed infection.

The microsporidium also appeared to be transovarially transmitted. Eggs collected from isolated alfalfa weevil females were surface-sterilized by immersion in 10% formaldehyde for 10 minutes and rinsed 3 times in distilled water. The eggs from each female were held in separate rearing dishes from those of other females and, after oviposition, the females were dissected and squash

Table 1. Comparison of microsporidia recovered from the alfalfa weevil.

Species recovered	Average length (μm)	Average width (μm)	Shape
Nosema hyperae[a]	3.1	1.7	spheroidal
Nosema sp.[b]	4.9	2.5	spheroidal
Nosema sp.[c]	6.0	4.5	ovoid
Undescribed sp.	2.7	1.0	rod

[a]Youssef (1974), revised by Sprague (1977).
[b]Maddox and Luckmann (1966).
[c]Drea et al. (1969).

preparations of midgut tissues were examined for infection. Eighteen of 21 larvae from 3 infected females were infected; 22 larvae produced by 6 uninfected females were not infected.

Additionally, we surface-sterilized 100 eggs collected at random from a heavily infected laboratory colony. Fifty larvae were retrieved and all were infected. Several noneclosed eggs were immersed in a 10% bleach solution in order to dissolve the chorions and to destroy any spores which may have been present on the surface of the eggs. Squash preparations were made of the embryos and were examined microscopically for infection. The embryonic tissue was infected with the microsporidium.

Studies on the complete life cycle and the ultrastructural characteristics of this new microsporidium are being conducted. Until these studies are completed, the generic identity of the new microsporidium cannot be determined. Likewise, the importance of the microsporidium as a biological control agent cannot be fully evaluated until studies on the host range and effect of the disease on the alfalfa weevil are completed.

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