The Great Lakes Entomologist

Volume 27
Number 1 - Spring 1995 *Number 1 - Spring 1995*

Article 3

April 1995

A Laboratory Infection of Alfalfa Weevil, *Hypera Postica* (Coleoptera: Curculionidae), Larvae With the Fungal Pathogen *Zoophthora Phytonomi* (Zygomycetes: Entomophthoraceae)

S. J. Roberts
University of Illinois

J. V. Maddox
University of Illinois

E. J. Armbrust
University of Illinois

A LABORATORY INFECTION OF ALFALFA WEEVIL, *HYPERA POSTICA* (COLEOPTERA : CURCULIONIDAE), LARVAE WITH THE FUNGAL PATHOGEN *ZOOPHTHORA PHYTONOMI* (ZYGOMYCETES : ENTOMOPHTHORACEAE).

S. J. Roberts, J. V. Maddox, and E. J. Armbrust[1]

ABSTRACT

Larvae of the alfalfa weevil, *Hypera postica*, were infected by an *in vitro* colony of *Zoophthora phytonomi*. Two spore types (infective conidia, and resting spores) were produced from infection trials. The spore type produced may be influenced by the physiological state of the larvae. Trials using field collected larvae which would produce diapausing adults formed both conidia and resting spores. Trials using larvae from a nondiapausing colony, however, formed only resting spores.

Zoophthora phytonomi (Arthur), an entomopathogenic fungus of *Hypera postica* (Gyllenhal) was first reported in Illinois by Barney et al. (1979). A survey of selected counties showed that this fungus was distributed over most of Illinois. *Z. phytonomi* has been recognized as an important fungal pathogen of *H. postica*, the impact of which has been documented by Harcourt et al. (1990), Watson et al. (1981), and Nordin et al. (1983)

Reinfection using conidia from field collected *H. postica* larval cadavers was described by Watson et al. (1981). The fungus may be isolated from larval cadavers and grown on artificial media, but conidia produced on artificial media are uninfective to weevil larvae. There have been repeated attempts by other researchers to infect alfalfa weevil larvae using media isolated *Z. phytonomi*. A similar pathogen *Zoophthora radicans* (Brefeld) infects the potato leafhopper, *Empoasca fabae* (Harris). This fungal pathogen is isolated and maintained on the same artificial media used for *Z. phytonomi*. *Zoophthora radicans* will remain infective to *E. fabae* as long as nymphs are reinfected periodically, and the fungus is re-isolated back on to artificial media (McGuire et al. 1987). The ability to maintain an active, infective culture of a fungal pathogen on artificial media in the laboratory greatly facilitates laboratory studies on morphology, development, dose rates, and host range.

MATERIALS AND METHODS

Field Isolations. In order to do continuous laboratory research with *Z. phytonomi* it is necessary to be able to infect with *in vitro* material. Since 1979, *Z. phytonomi* has been detected in *H. postica* larvae during years when

[1]Illinois Natural History Survey and Agricultural Entomology, University of Illinois, Champaign, IL 61820.

there is adequate rainfall. Drought occurred in 1988 and 1989 and rearings of *H. postica* larvae produced only a limited number of infections. On April 26, 1989, cadavers of *H. postica* larvae infected with *Z. phytonomi* were found on the undersides of leaves located near the tops of alfalfa plants in Effingham Co., Illinois. Three to five hundred sweeps were taken from this field and the contents of the net along with fresh alfalfa were placed in brown paper grocery bags. The tops of the bags were rolled and stapled shut to prevent escape of living larvae. Samples were returned to the laboratory and ca. three days later larval cadavers were removed from the insides of the paper bags and from the foliage. Both light tan (type I) and black (type II) cadavers (Harcourt et al. 1974) were found. Type I larvae were placed on a small block of water agar positioned in the center of a 60 × 15mm petri dish lid. The bottom of the petri dish containing Egg Yolk Agar (EYA) was inverted over the larval cadaver on the lid. This unit, sealed with parafilm and maintained in the inverted position, was incubated at 22°C for 72 hours. After the incubation period conidia showered upward from the larval cadaver onto the EYA. At this time the lids with the cadavers were discarded and sterile lids placed on the bottom of the petri dish containing the EYA. The fungal isolate grew out concentrically on the surface of the agar until vegetative growth reached approximately 3 cm. At this time the fungus began showering conidia profusely and conditions appeared favorable to expose uninfected *H. postica* larvae.

Laboratory Inoculations. In order to force the *in vitro* fungus to shower conidia, single 4 × 4 mm squares of thinly sliced mycelial mat were placed in several 60 × 15 mm plastic petri dishes containing approximately 10 ml of water agar. These plates were incubated for 24 hours at 22°C.

Larvae were exposed to the fungus by placing 15 to 25 second instar *H. postica* larvae in each of the sterile 60 × 15 mm dish bottoms. Each petri dish bottom containing the actively showering squares of excised fungus was placed on top of a dish bottom containing the larvae which served as an inoculation chamber. The chamber was sealed by wrapping parafilm around the juncture of the dish bottoms. All chambers were then incubated at 22°C. Prior to setting up the inoculation chambers, a like number of larvae were placed in pint size paper cartons with alfalfa and moist cotton wicks as a non-treated check. All alfalfa used in the inoculation trials was grown in a greenhouse. The source of larvae for trials 1 and 2 were from field collected larvae, and those from trial 3 from a laboratory colony.

After 5 to 16 hours of inoculation time the larvae in each dish were transferred to a pint paper carton containing alfalfa and several moist cotton wicks. All cartons (treatments and checks) were incubated at 22°C for 4 days prior to examination. Wicks were moistened and fresh alfalfa was added as necessary.

After 4 days the larvae in each carton were examined. Surviving larvae were returned to their respective cartons and examined for 1 to 2 additional days before the trial was terminated.

RESULTS AND DISCUSSION

We found that most infections occurred within 4 to 5 days after inoculation. Three infection trials were successful. Both conidia and resting spores were produced when field collected larvae (predisposed to diapause) were used in the first two inoculation trials. The first trial produced conidia infections only with 12.5 percent infected. In trial 2 one replication produced 4.0 and 36.0 percent infections of conidia and resting spores, respectively, with a total infection of 40.0 percent.

Another replication in trial 2 produced only resting spores with 4.0 per-

cent infected. In the third trial, the resultant infections produced only resting spores with 15.0 percent infected. The larvae used in this trial were from an alfalfa weevil laboratory colony and were non-diapausing larvae. It is possible that conidia formation is linked to the changes in larval physiology which occur in diapausing weevils.

Several points are evident from the data. First, *H. postica* larvae can be infected with an in vitro colony of *Z. phytonomi* and is fully substantiated by trial 3, although the authors are unsure why the 1989 Effingham isolate was infective. Second, why was infectivity so low and variable? Three additional exposure experiments produced no infections. Third, these findings should help open up additional areas of research with this fungal pathogen of the alfalfa weevil.

ACKNOWLEDGMENTS

We thank Leellen F. Solter, Catherine E. Eastman, and Hassan Oloumi-Sadeghi for their critical review of this manuscript. This research was supported by the Illinois Natural History and by the College of Agriculture, University of Illinois.

LITERATURE CITED

Harcourt, D. G., J. C. Guppy, D. M. MacLeod, and D. Tyrell. 1974. The fungus *Entomophthora phytonomi* pathogenic to the alfalfa weevil, Hypera postica. Can. Entomol. 106:1295-1300.

Harcourt, D. G., J. C. Guppy, and D. Tyrell. 1990. Phenology of the fungal pathogen *Zoophthora phytonomi* in southern Ontario populations of the alfalfa weevil (Coleoptera: Curculionidae). Environ. Entomol. 19:612-617.

Los, L. M., and W. A. Allen. 1982. Visual technique for determining presence and stage of *Zoophthora phytonomi* in dead alfalfa weevil larvae. J. Econ. Entomol. 75:375-377.

McGuire, M. R., J. V. Maddox, and Edward J. Armbrust. 1987. Effect of temperature on distribution and success of introduction of an *Empoasca fabae* (Homoptera: Cicadellidae) isolate of *Erynia radicans* (Zygomycetes: Entomophthoraceae). J. Invert. Path. 50:291-301.

Nordin, G. L., G. C. Brown, and J. A. Millstein. 1983. Epizootic phenology of Erynia disease of the alfalfa weevil, *Hypera postica* (Gyllenhal) Coleoptera: Curculionidae), in central Kentucky. Environ. Entomol. 12:1350-1355.

Watson, P. L., R. J. Barney, J. V. Maddox, and E. J. Armbrust. 1981. Sporulation and mode of infection of *Entomophthora phytonomi* a pathogen of the alfalfa weevil. Environ. Entomol. 10:305-306.