INFECTIVITY OF *ZOOPHTHORA RADICANS* (ZYGOMYCETES: ENTOMOPHTHORALES) TOWARDS *TRIALEURODES VAPORARIORUM* (HOMOPTERA: ALEYRODIDAE) NYMPHS

SERGIO R. SANCHEZ-PEÑA
Departamento de Parasitología Agrícola
Universidad Autónoma Agraria Antonio Narro, Saltillo, Coahuila, C.P. 25315, México

Current address: School of Biological Sciences, 1.22 Painter Hall
The University of Texas at Austin, Austin, TX 78712

Whiteflies (Homoptera: Aleyrodidae) are primary pests in a wide variety of agroecosystems worldwide. Fungal pathogens of the immature and/or adult stages of Aleyrodidae include the Hyphomycetes *Aschersonia* spp., *Beauveria bassiana* (Balsamo) Vuillemin, *Paecilomyces farinosus* (Holm ex. S. F. Gray), *Paecilomyces fumosoroseus* (Wize) Brown & Smith, *Verticillium lecanii* (Zimmerman) Viégas, and *Metarhizium anisopliae* var. *anisopliae* (Metschnikoff) Sorokin (Osborne & Landa 1992, Lacey et al. 1996, Sánchez-Peña & Vázquez-Jaime 1996, Sánchez-Peña 1997). Natural infections caused by fungi classified in the Entomophthorales have rarely been observed in whiteflies (Lacey et al. 1995). *Zoophthora* (= *Erynia*) *radicans* (Brefeld) Batko was reported to attack *Bemisia tabaci* (Gennadius) in Israel (Ben-Ze'ev et al. 1988) and Chad (Silvie & Papierok 1991). Steinkraus et al. (1998) described a new fungal pathogen *Orthomyces aleyrodis* Streinkraus, Humber & Oliver (Entomophthorales: Entomophthoraceae) from adult *Trialeurodes abutilonea* (Haldeman) whiteflies in Alabama.

Zoophthora radicans, as a morphological species, has a broad host range and, in addition to the Aleyrodidae, has been reported to attack insects in the families Curculionidae (Coleoptera), Anthomyiidae (Diptera), Diprionidae (Hymenoptera), Geometridae, Noctuidae, Pieridae, Plutellidae, Pyralidae, and Tortricidae (Lepidoptera), Miridae (Hemiptera), and Aphidae, Cercopidae, Cicadellidae, Delphacidae, and Psyllidae (Homoptera) (Humber 1992). In the present work, induced infection by *Z. radicans* in greenhouse whitefly, *Trialeurodes vaporariorum* Westwood, nymphs is reported.

A *Z. radicans* strain was isolated from a field-collected cabbage looper, *Trichoplusia ni* Hübner, larva collected on cabbage, *Brassica oleracea* L., 15 October 1998, at

the Universidad Agraria Antonio Narro Campus in Saltillo, Coahuila, Mexico. The fungus was isolated on Sabouraud maltose agar (Bioxon, Mexico) plus boiled, blended egg yolk (one egg yolk/200 ml) and has been deposited in the USDA-ARS (Plant Protection Research Unit, Ithaca, NY) collection as ARSEF 6003. First-transfer pure cultures on agar obtained from the field-collected larva were used for inoculation of whiteflies. Using a spatula, mycelia were gently scraped from the surface of the actively growing cultures on agar. Three small, moist mycelial masses (approximately 2 mm diam) were each adhered, by natural stickiness, to the inside of the lid of individual petri dishes (100 mm. diam × 20 mm deep). Pinto bean, *Phaseolus vulgaris* L., leaves infested with *T. vaporariorum* 2nd and 3rd instars (>50 nymphs/cm^2) were collected from a greenhouse. Leaves (nymphs side up) were placed on the bottom of petri dishes with the mycelia adhered to their covers. A small piece of moistened cotton was placed inside the dishes to maintain high humidity. Dishes were incubated at 22-25°C in the darkness. Under these conditions mycelia sporulated heavily within 12 h. Conidiophores of *Zoophthora* spp. forcibly discharge primary conidia; in each dish nymphs were showered with primary conidia produced by the mycelial fragment above. Previous to exposure to the fungus, an area of 50-100 mm^2 was cut and removed from the leaves directly below the mycelia and a slide was placed below the leaf under this hole. After allowing conidia to fall directly on the leaves, nymphs, and exposed area of the slides for 76 h, these slides were examined with the microscope to determine the number of conidia deposited/mm^2. Nymphs were removed from the leaves, placed in a drop of lactophenol/cotton blue mounting media between slide and coverslip, and examined with a compound microscope. Alternatively, leaves with exposed nymphs were dried for 96 h; nymphs were then removed and examined microscopically. Those showing *Zoophthora* mycelial development (Fig. 2) were placed on a sterile slide in a moist chamber to observe if the fungus in these dead insects would produce conidia.

Fungal sporulation from mycelia resulted in a mean of 374 primary conidia/mm^2 on the slides below. My observations showed that *Z. radicans* conidia invaded the *T. vaporariorum* nymphs and proliferated, causing a lethal infection. *Zoophthora radicans* resting spores (Fig. 1), mycelia (Fig. 2) or both were observed to fill the body cavity of the mycosed nymphs (n = 57). The fungus infected 71% (n = 80) of the exposed nymphs. No *Z. radicans* developmental stages were observed in non-exposed nymphs (n = 45) incubated under similar conditions. My observations revealed that the fungus completed its asexual life cycle (from conidia to conidia) in the infected nymphs. Also, it sporulated after the insects were dessicated for 96 h. Primary conidia were produced and ejected from the nymphs, and these conidia in turn germinated, producing adhesive capillary conidia typical of *Z. radicans*.

In other Entomophthorales, the production of resting spores in insects topically inoculated with primary conidia has been reported. Lin & Harper (1984) inoculated *Pseudoplusia includens* (Walker) with *Pandora gammae* (Weiser), and Steinkraus et al. (1993) inoculated *Heliothis virescens* (F.) with *Furia virescens* (Thaxter) Humber. Following topical application of primary conidia, the fungi killed both hosts and produced resting spores inside them, rather than growing externally as conidiophores and conidia. Fungal development was similar in the present work: resting spores were also produced inside nymphs which had been invaded by germ tubes from conidia.

Darkness is mentioned as one possible factor inducing resting spore formation, rather than conidiophores and conidia, in Entomophthorales pathogenic to Lepidoptera (Li & Humber 1984). Lin & Harper (1984) maintained their inoculated larvae under unspecified laboratory conditions, and 90-100% RH. Steinkraus et al. (1993)

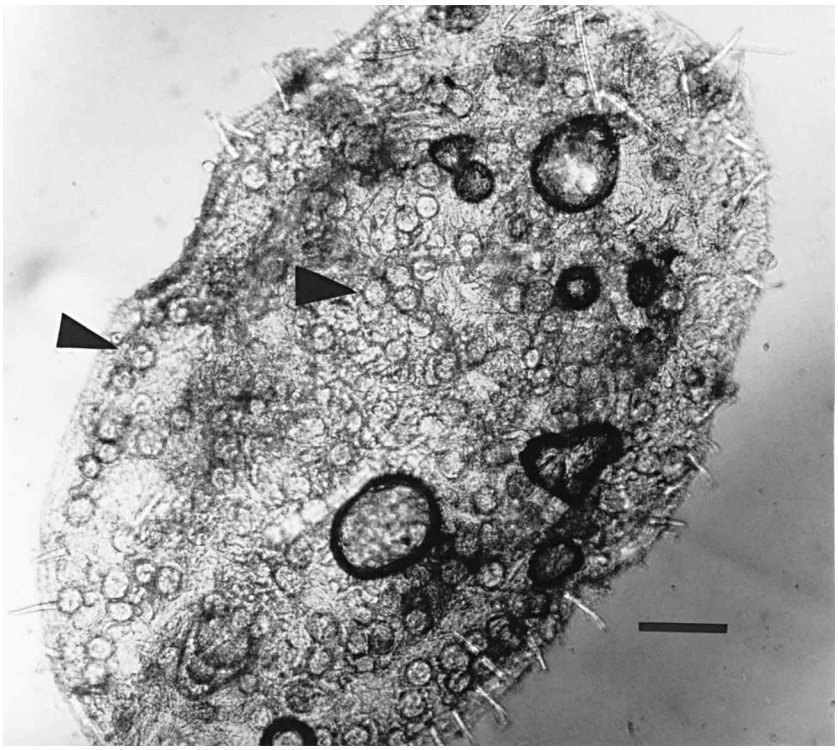

Fig. 1. Third-instar *T. vaporarioum*. The body cavity is filled with spherical, immature *Zoophthora radicans* resting spores (arrowheads). Bar = 70 μm.

kept their larvae at 25°C with a photoperiod of 12:12 (LD) and unspecified high humidity (enough to induce sporulation). In the present work, nymphs showing *Z. radicans* resting spores had been previously maintained at 22-25°C in the darkness and 100% RH during fungal exposure and development for 96 h.

The use of *Z. radicans* as a biological control agent of whiteflies would be a complicated task considering the relatively fastidious procedures required to produce and apply conidia of this fungus compared to some entomopathogenic Deuteromycetes. However, further research in this area is warranted especially considering the inherently high virulence of fungi of the Entomophthorales (Carruthers et al. 1995). *Zoophthora radicans* has been manipulated to induce epizootics in another homopteran pest, potato leafhopper, *Empoasca fabae* (Harris) (Wraight et al. 1986) using as inoculum dry mycelia. The strain used in the current work was collected in a Chihuahuan desert locality with an annual precipitation of <0.4 m, so it is adapted to xeric conditions. Epizootics of *Z. radicans* in *T. ni* larvae on cabbage occur in this zone (unpublished data). Unlike other fungal pathogens of whiteflies, *Z. radicans* and *P. fumosoroseus* are capable of causing epizootics in foliage-inhabiting insects in arid and semiarid climates (Lacey et al. 1995, unpublished data). This too suggests that further studies of the potential of *Z. radicans* as a whitefly biocontrol agent are warranted.

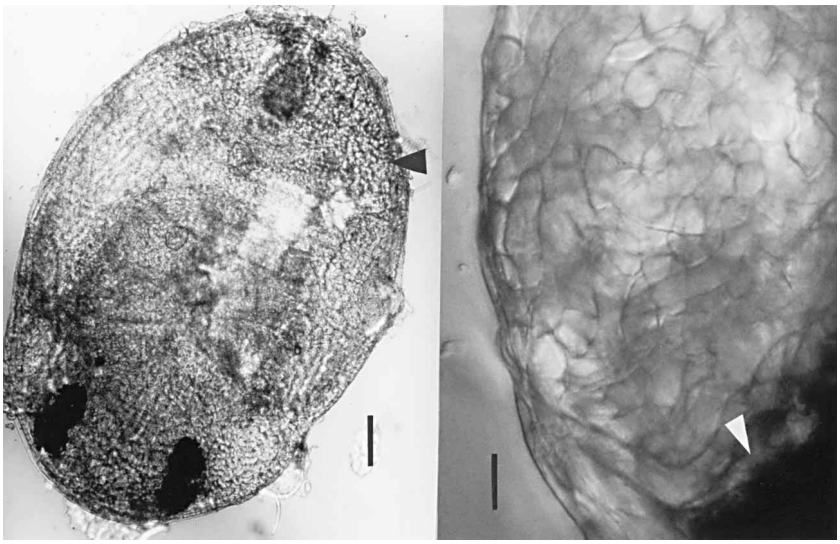

Fig. 2. Third-instar *T. vaporariorum*. (A) the body cavity is full of *Zoophthora radicans* mycelia (arrowheads). Bar = 65 μm. (B) higher magnification of *T. vaporariorum* nymph showing *Zoophthora radicans* mycelia. Arrowhead indicates an eye. Bar = 10 μm.

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Summary

Infection of *Trialeurodes vaporariorum* nymphs after showering them with *Zoophthora radicans* primary conidia from *in vitro* cultures is reported. The fungus completed its asexual life cycle in the infected nymphs.

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