Furia virescens (Thaxter) Humber (Zygomycetes: Entomophthoraceae) Infections in the Armyworm, *Pseudaletia unipuncta* (Haworth) (Lepidoptera: Noctuidae) in Arkansas with Notes on Other Natural Enemies[1]

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ABSTRACT An epizootic caused by *Furia virescens* (Thaxter) Humber (Zygomycetes: Entomophthorales) was studied in a population of armyworms, *Pseudaletia unipuncta* (Haworth), in a tall fescue, *Festuca arundinacea* Schreber (Poaceae), pasture in Arkansas. Prevalence rates in live larvae collected from the field were 21.6, 8.1 and 13.5% for *F. virescens,* the braconid parasitoid *Glyptapanteles militaris* (Walsh), and an unidentified mermithid nematode, respectively. In a laboratory trial one *Heliothis virescens* (F.) larva was infected by *F. virescens*. Previously unrecorded aspects of the morphology and life cycle of *F. virescens* are described. *Pseudaletia unipuncta* and *H. virescens* are new host records for *F. virescens.*

KEY WORDS *Glyptapanteles militaris,* mermithid, mycosis, *Heliothis virescens.*

The armyworm, *Pseudaletia unipuncta* (Haworth), is periodically a serious pest of wheat, fescue, and other grasses (Breeland 1958). In spring 1992, areas of northwest Arkansas experienced severe armyworm outbreaks in wheat and fescue. In one case in Madison County, a grower lost hundreds of hectares of a fescue seed crop due to a massive infestation. Populations collapsed in some fields and it was determined that a fungus, *Furia virescens* (Thaxter) Humber (Zygomycetes: Entomophthoraceae), was involved.

Fungi are known to infect *P. unipuncta.* Breeland (1958) reported an unidentified *Empusa* sp. infecting armyworm from Tennessee, and Steinhaus and Marsh (1962) reported an armyworm infected with an unidentified *Entomophthora* species. Williams (1931) and Walkden (1950) reported *Metarhizium anisopliae* (Metsch.) Sorokin infections in armyworm. However, *F. virescens* has not been reported previously from *P. unipuncta.*

The fungus, *F. virescens,* has been little studied and is known only from its original description from *Agrotis* (now *Actebia*) *fennica* (Tauscher) (Thaxter 1888), an unidentified caterpillar on *Brassica* sp. in England (Petch 1944), *Euxoa messoria* (Harris) and *Euxoa ochrogaster* Guenée larvae in Canada (Bucher and MacLeod 1974), and *Hadena* (now *Dargida*) *procincta* (Grote) (Li and Humber 1984).

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In addition to recording new hosts and a new locality for *F. virescens,* the objectives of this study were to examine *F. virescens* in more detail than previously and to gather preliminary information on natural enemies of *P. unipuncta* in Arkansas.

Materials and Methods

Field Study Site. The study site consisted of a 28-ha fescue hay field approximately 8 km east of Huntsville in Madison County, Arkansas. The flora was composed primarily of tall fescue, *Festuca arundinacea* Schreber (Poaceae), with some orchard grass, *Dactylis glomerata* (L.) (Poaceae), English plantain, *Plantago lanceolata* (L.) (Plantaginaceae), and an undetermined mustard, *Brassica* sp. (Brassicaceae). The tall fescue was flowering at the time of sampling and meadowlarks (*Sturnella magna* [L.]), red-winged blackbirds (*Agelaius phoeniceus* [L.]) and other icterid birds, known predators of armyworms (Luginbill 1928, Breeland 1958), were present in the field.

The field's owner contacted us with a report of dead armyworms attached to fescue stems. On 27 and 29 May 1992 we visited the field to collect larvae and to investigate an apparent fungal epizootic.

Field Survey. To estimate the number of live *P. unipuncta* larva present in the field and to collect live larvae for determining the prevalence of the fungus and other natural enemies, samples were taken by walking diagonally through the field and sampling at 20-m intervals. At each stop, the crowns of five fescue grass clumps and the litter under the crowns were searched for live *P. unipuncta* larvae. Thirty samples were taken (150 plants) on 29 May. The ground area searched around each plant was approximately 0.09 m^2. Armyworm larvae found during the search were placed individually in 30-ml clear plastic creamer cups containing 10 ml pinto bean diet (Burton 1969) and taken to the laboratory. They were kept at 20°C, 12:12 light:dark (LD), for 3 wks and observed daily for mortality caused by *F. virescens* and other natural enemies.

Field Survey for Infected Larvae on Plants. To estimate the number of armyworms killed by *F. virescens,* twenty 3.14-m^2 samples (direct visual counts) were taken every 25 m in a diagonal transect through the field on 29 May. Larval size, plant species, height on plant, position (head up or down), and color of the fungal hymenium were recorded.

Laboratory Infection Trials. To determine the susceptibility of three species of noctuids to *F. virescens, P. unipuncta* larvae infected with *F. virescens* (conidial stage) were collected from fescue stems from the study site. Each *P. unipuncta* cadaver was cut into three equal-size pieces. Laboratory-reared 3rd-stage *Spodoptera exigua* (Hübner) (n = 10), 4th-stage *Helicoverpa zea* (Boddie) (n = 20), and 4th-stage *Heliothis virescens* (F.) (n = 20) larvae were placed individually in 30-ml clear plastic creamer cups containing 10 ml pinto bean diet. They were inoculated by placing one of the actively sporulating *F. virescens*-infected *P. unipuncta* cadaver pieces into each cup. The number of conidia discharged per cup was not quantified. Larvae were held at 25°C, 12:12 LD, and examined daily for 2 wks for larval death or pupation.

Morphology of *F. virescens*. Infected larvae from the field and laboratory were examined with a dissecting microscope to describe the gross morphology of

the fungus on the host. Primary conidia were collected on glass coverslips suspended for 1 h over sporulating armyworm cadavers in humid chambers. Coverslips were mounted in a small drop of room temperature lactophenol or aceto-orcein. Secondary conidia were collected by placing slides covered with primary spores under glass coverslips in humid chambers. This allowed discharge of secondary spores onto the coverslips above. Secondary conidia were mounted in lactophenol. Resting spores, cystidia, and rhizoids were teased from armyworm cadavers with a fine needle and placed on slides in a small drop of lactophenol or aceto-orcein.

Measurements were made using a phase contrast microscope equipped with an ocular micrometer and are reported as mean ± standard deviation.

Results and Discussion

Field Survey. A total of 37 live *P. unipuncta* larvae were found in the 30 samples, a mean of 1.23 (SD = 1.68, range = 0-6) larvae/sample, or 2.65 larvae/m^2. All the larvae were 5th or 6th instars and were found curled in a typical C-shaped position on the soil surface (Fig. 1a).

Field-collected armyworm larvae fed poorly on pinto bean diet. Three natural enemies caused substantial mortality in the field-collected larvae. Natural infections producing the resting spore stage of *F. virescens* killed 21.6% of the larvae within several days after collection (Fig. 1a-d). Parasitization by the braconid wasp, *Glyptapanteles militaris* (Walsh) (Fig. 1e), killed 8.1% and parasitization by an unidentified mermithid nematode killed 13.5% (Fig. 1f). Two larvae were parasitized simultaneously by two natural enemies and both natural enemies survived. One larva had both *G. militaris* and the resting spore stage of *F. virescens* (Fig. 1e), and one contained a mermithid and resting spores of *F. virescens*. The remainder of the larvae either successfully pupated (24.3%) or died of unknown causes probably related to their inability to feed on the diet (37.8%). These percentges add up to more than 100% because of the two larvae with dual infections.

Glyptapanteles militaris has been previously noted to be an important natural enemy of the armyworm (Tower 1916, Thompson 1945, Breeland 1958). Breeland (1958) stated that *G. militaris* was the most effective parasitoid of *P. unipuncta* and was reared from 11.7% of all armyworms collected over a two-year period, a parasitism rate similar to that found in our study.

Armyworm larvae parasitized by mermithids remained alive until the mermithids exited the larvae. The mean length and width of the white-colored mermithids were 248.6 ± 30.2 mm and 0.48 ± 0.07 mm (n = 5), respectively (Fig. 1f). Only one mermithid emerged per larva. We were unable to identify the mermithid. Based on the extensive host lists of Poinar (1979) and Wouts (1984) this is the first report of *P. unipuncta* as a mermithid host. The unidentified mermithid from *P. unipuncta* resembled *Mermis nigrescens* Dujardin, a common parasite of grasshoppers, which experimentally parasitized a lepidopteran, *Hemileuca olivae* Cockerell, in a study by Capinera (1987). However, the mermithids parasitizing *P. unipuncta* were considerably larger than reported lengths (110 mm long by 0.46 mm wide) of postparasitic juveniles of *M. nigrescens* (Poinar 1979). While *M. nigrescens* is primarily a grasshopper

parasite, it is reported to have a wide host range including Lepidoptera larvae, and Poinar (1979) concluded it was not difficult to explain parasitism of other hosts that feed on plant leaves covered with eggs of *M. nigrescens*. However, Wouts (1984) considered it unlikely that *M. nigrescens* parasitizes Lepidoptera. Further work is needed to identify the mermithid from *P. unipuncta*.

Field Survey for Infected Larvae on Plants. Forty-seven *F. virescens*-infected dead larvae were collected in the 20 samples, a mean of 2.35 (SD = 1.95) infected larvae per sample (0.75 dead infected larvae/m^2). Dead larvae were producing conidia at the time of the survey (0900-1100) and presumably had climbed up the plants during the preceding night and died during the early morning hours. Healthy larvae hide beneath plant crowns during the day (Breeland 1958). Most infected larvae died on fescue stems (76.6%) and 17.0, 4.3, and 2.1% were found on stems or seedheads of English plantain, orchard grass, and mustard, respectively. The mean height of final resting sites of infected larvae on all the plant species was 67.4 ± 20.3 cm (n = 47) above the soil surface. Most larvae died head downward (78%). Larval stages were 5th-6th instars (82.9%), 3rd-4th (7.3%), and 1st-2nd (9.8%).

Laboratory Infection Trials. By 14 d after treatment, 90% of *S. exigua,* 95% of *H. zea,* and 85% of *H. virescens* had pupated. One *H. virescens* larva became infected with *F. virescens,* died 4 d after treatment, and produced the resting spore stage of *F. virescens.* The morphology of the fungal structures from the infected *H. virescens* larva was identical to those in field-collected live armyworms that died with the resting spore stage. *Heliothis virescens* is a new host record for *F. virescens,* and this is the first report of laboratory transmission of *F. virescens.* It is also the first experimental evidence of the resting spore stage of *F. virescens* and demonstrates that the conidial stages and resting spore stages found in *P. unipuncta* were both caused by *F. virescens,* although no infected larvae were found to have produced both conidia and resting spores.

Morphology of Conidial Stage of *F. virescens*. Infected armyworm cadavers were light to medium grey in color, laterally flattened, and covered with a dense fungal hymenium from which primary conidia were forcibly discharged (Fig. 2a). Cadavers were fastened to the plant by their prolegs and adherence of large numbers of conidiophores (Fig. 2b). Specialized rhizoids were not observed although small, sparse rhizoids, little differentiated from vegetative hyphae, may have been present. Cadavers were weakly attached to stems and sometimes slipped off the stem when collected or disturbed.

Primary conidia closely resembled those described by Thaxter (1888) in his original description except for being slightly smaller, and those described by Li and Humber (1984) from their examination of original material in the Thaxter collections in the Farlow Herbarium. The bitunicate, uninucleate primary conidia were 25.2 ± 1.9 μm long by 11.9 ± 0.9 μm wide (n = 20), with a length/width ratio of 2.1 (Fig. 2c). The nucleus in primary conidia was relatively large (8.4 ± 1.0 μm in diameter, n = 10) as is typical of *Furia* species (Humber 1989).

Capilliconidia were not observed, but such conidia are not known for any *Furia* species. Secondary conidia (Fig. 2d) resembled primary conidia and were produced on the end of a short germ tube (8.3 ± 1.4 μm long, 3.7 ± 0 μm wide, n = 3). Secondary conidia were 17.9 ± 1.6 μm long by 11.9 ± 1.6 μm wide (n = 10).

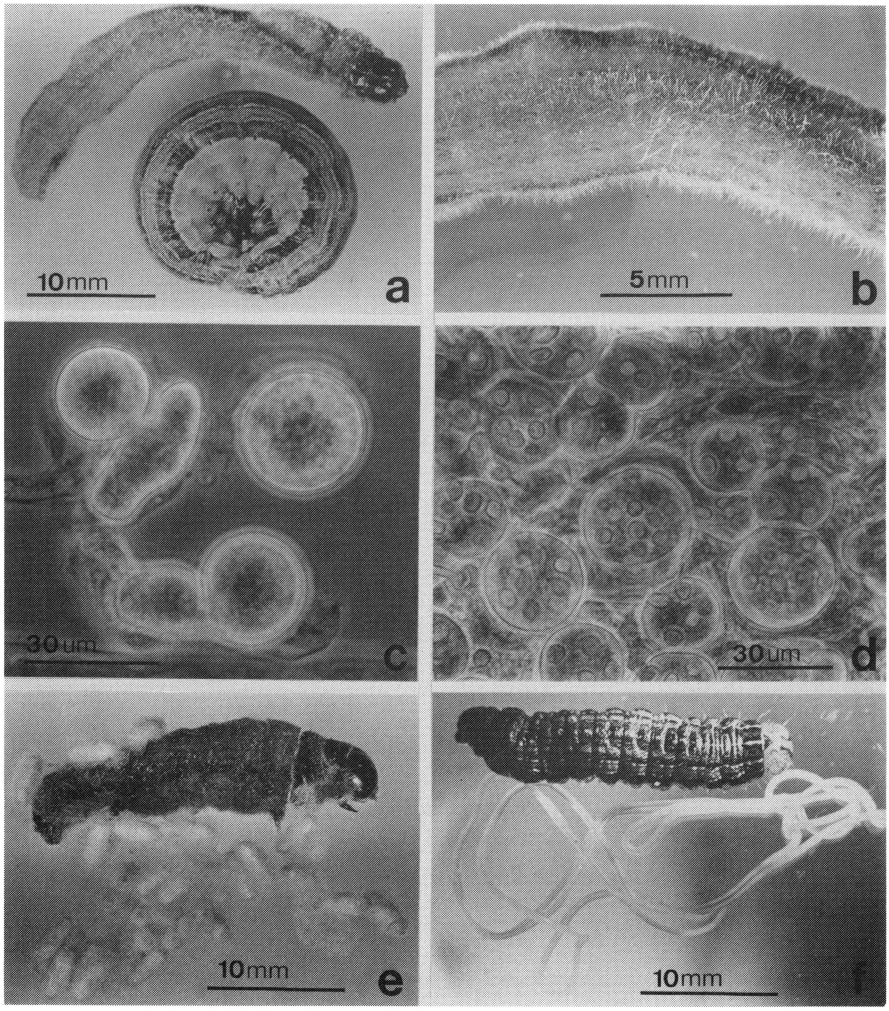

Fig. 1.

Fig. 1a. Resting spore stage of *F. virescens* in an armyworm larva (upper larva) compared to live larva in typical curled position (lower larva).

Fig. 1b. Abdomen of armyworm larva killed by resting spore stage of *F. virescens*. Note the abundant long cystidia, giving the larva a hairy appearance.

Fig. 1c. Developing resting spores (azygospores) of *F. virescens*. Note that the resting spore buds off a short neck from a hyphal body.

Fig. 1d. Multinucleate resting spores of *F. virescens* stained in aceto-orcein to show the relatively large nuclei.

Fig. 1e. Armyworm larva that was simultaneously infected with resting spore stage of *F. virescens* and parasitized by the braconid wasp, *Glyptapanteles militaris*.

Fig. 1f. Armyworm larva parasitized by unidentified mermithid nematode which has just exited the moribund larva. Larvae died soon after the mermithids exited.

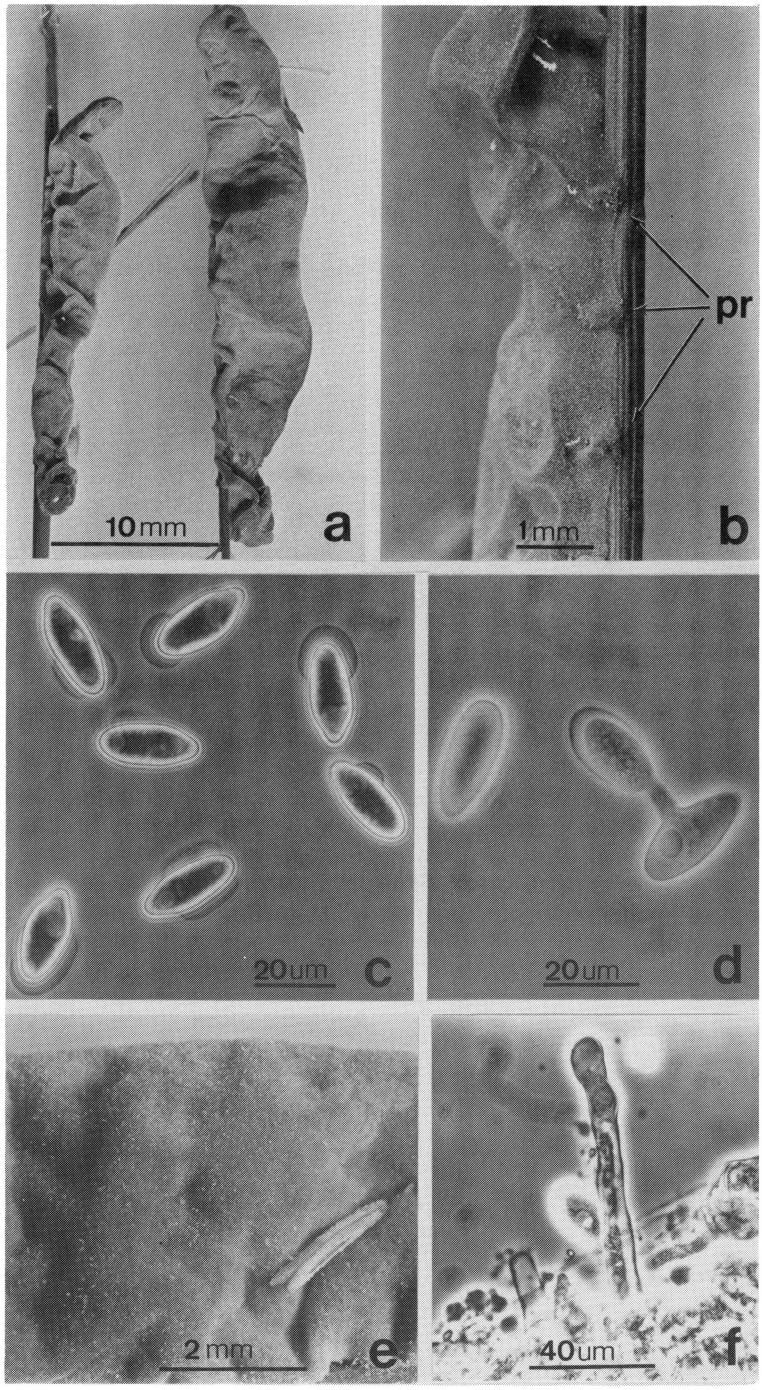

Fig. 2

Fig. 2a. Field collected *Furia virescens* infected armyworm larvae attached to stems of tall fescue. These laterally flattened larvae died of the conidial stage of the fungus and were almost entirely covered with a grey, velvety hymenial layer of conidiophores and sparse cystidia.

Fig. 2b. Armyworm larva infected with conidial stage of *F. virescens*. Note prolegs (pr) grasping fescue stem and lack of obvious rhizoids.

Fig. 2c. Primary conidia of *F. virescens*. Note bitunicate nature where the outer wall layer lifts off of the conidial surface.

Fig. 2d. Formation of an obviously bitunicate secondary conidium of *F. virescens*. Note short secondary conidiophore.

Fig. 2e. Hymenium of conidial stage of *F. virescens* on dead armyworm. Note the velvety texture. Cystidia are extremely sparse, relatively fine and difficult to see.

Fig. 2f. Cystidium of *F. virescens* from conidial stage on armyworm larvae.

Relatively small cystidia were present but sparse (Fig. 2e). Cystidial length was measured from the point where the cystidium left the hymenial layer to its tip. Cystidia were 84 ± 65.9 μm long by 8.25 ± 2.42 μm wide (n = 5) (Fig. 2f). That Thaxter (1888) did not observe cystidia or rhizoids in his original description is not surprising considering their sparseness, relatively small size and fragility, and the poor state of preservation of his material. Conidiophore and hyphal growth of *F. virescens* was determinate as shown by the smooth velvety appearance of the hymenium, unlike that of *F. pieris* (Li and Humber 1984), but resembled *Entomophthora crustosa* [= *Furia crustosa* (MacLeod & Tyrrell) Humber] (MacLeod and Tyrrell 1979).

Morphology of Resting Spore Stage of *F. virescens*. Living infected armyworm larvae collected from under plants during the field survey and the laboratory-infected *H. virescens* larva produced *F. virescens* resting spores after their deaths. Conidia were not produced, and the appearance of affected cadavers differed greatly from those producing conidia. The strong similarity of the fungus in the infected *H. virescens* larva with that of field-collected armyworms confirms that *F. virescens* was the infective agent in both the conidial and resting spore forms in armyworms. In contrast to the conidial stage, abundant long cystidia were produced on resting spore stage cadavers and gave them a hairy appearance; these hosts were also held firmly to the substrate by abundant rhizoids (Fig. 1a, b). Cystidia averaged 506 ± 258 μm long and 10.5 ± 1.4 μm wide (n = 5) and were multinucleate (with seven or more nuclei present).

Embedded within the armyworm cadavers were numerous relatively clear, globose resting spores (Figs. 1c,d). Resting spores were apparently azygospores because they were formed by budding off a short neck from a hyphal body (Fig. 1c) without any apparent sign of gametangial conjugations of paired hyphal bodies. Newly-formed resting spores were multinucleate (3.9 ± 1.1 nuclei per resting spore, n = 19), and the nuclei averaged 5.6 ± 1.1 μm in diameter (n = 10). Mean diameter of resting spores was 27.6 ± 1.5 μm (n = 20).

This is the first confirmed report of the resting spore stage of *F. virescens*. MacLeod and Müller-Kögler (1970) and Bucher and MacLeod (1974) found *Tarichium megaspermum* infecting the cutworms *E. messoria* and *E. ochrogaster* in Canada. They reported that *T. megaspermum* had "coal black" resting spores and believed that these were the resting spore stage of *F. virescens*. However, based on the experimental and field evidence from our study, it is clear that either *T. megaspermum* is not the resting spore stage of *F. virescens* or that some other factors caused resting spores in the cutworms to be "coal black."

Behavior of *F. virescens*-infected *P. unipuncta* larvae, like behavior of insects infected with some other species of Entomophthorales, was altered. Typically, *P. unipuncta* larvae hide during the day, curled on the soil surface in litter beneath the grass, emerging at night to feed (Breeland 1958, Tashiro 1987). Nocturnal feeding is an important factor in the armyworm's ability to escape predation and explains why infestations often go unnoticed until damage is severe (Breeland 1958). However, as was seen in the survey for armyworm cadavers, large numbers (ca. 7,500 *F. virescens*-killed larvae per hectare) of infected armyworms died high on plant stems in positions where the insects

were easily visible and exposed to predation, but where the forcibly discharged primary conidia of the fungus shower down over a wide area. This position increases the likelihood of healthy armyworms becoming infected. During our survey of the affected field, we flushed meadowlarks and other passerine birds whose food is primarily insects (Breeland 1958, Ehrlich et al. 1992). It is unknown whether birds find infected armyworms palatable. Entomophthoralean alteration of behavior of infected insects has been frequently noted (Evans 1989). These behavioral changes generally benefit the fungus by enhancing the dissemination of fungal spores although the fungus would also be harmed if predators consume infected insects before sporulation. The mechanisms of these fungus-mediated behavioral changes have been studied little and are poorly understood (Evans 1989).

In order to explain the production of conidia of *F. virescens* in armyworms dying attached to plants and the resting spore stage in armyworms collected from beneath plants, we speculate that early-season larvae became infected by germinated resting spores in the soil. Armyworm larval habits of residing on the moist soil surface during the day could put them in direct contact with germinating infective resting spores. Larvae infected by resting spore germ conidia may produce only the dispersive conidial stage of the fungus on infected larvae dying in elevated positions. Larvae infected by conidia as they fed on exposed foliage returned to the soil and may produce only the overwintering resting spores that infect larvae the following season. Evans (1989) suggested that resting spore formation is directly related to host age: resting spores develop only in physiologically older insects, possibly dependent on the nutritional status of the host. Evans further stated that insects producing resting spores behave differently from those producing externally-borne conidia. Infected armyworms in our study exhibited this dichotomy in behavior with those producing externally borne conidia climbing plants to die while those producing resting spores remained on the soil surface.

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