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First Report of *Pasteuria* sp. Attacking *Heterodera glycines* in North America[1]

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Abstract: Endospores of a *Pasteuria* sp. were observed on *Heterodera glycines* second-stage juveniles and males recovered from soil in microplots in which nematode populations had been declining for several years. Conventional scanning electron microscopy was used to observe and measure endospores on second-stage juveniles (J2) of both a race 3 and a race 4 population. Endospores were elipsoidal; those attached to J2 of race 3 measured ($\bar{X}$) 4.2 × 3.7 μm with a height of 2.0 μm, and those on race 4 were 4.3 × 3.7 μm with a height of 2.3 μm. Measurements taken under light microscopy indicated that endospores attached to J2 of race 3 were 5.0 × 4.8 μm with a height of 2.2 μm. The velutinous exosporium of the *H. glycines*-infecting *P. nishizawae* from Japan was not visible in the Illinois isolate. Differences in endospore morphology and the apparent inability of the Illinois isolate to complete its life cycle in females indicate that the Japanese and Illinois isolates are distinct species of *Pasteuria*.

Key words: biological control, *Heterodera glycines*, nematode, *Pasteuria* sp., soybean cyst nematode.

Nishizawa (4) reported that population densities of *Heterodera elachista* Oshima increased for 4 years in experimental plots of monocultured upland rice established on newly cleared forest land, but began declining the fifth year. In 1984, a similar decline in numbers of *H. glycines* Ichinohe was observed in a field where soybean had been grown in monoculture (5). The organism responsible for these two declines was identified as *Pasteuria penetrans* sensu lato (9) and subsequently described as *P. nishizawae* Sayre, Wergin, Schmidt, & Starr (8).

Infestations of *H. glycines* were established in research plots on the USDA nematology farm at Urbana, Illinois, in 1982. After several years, population densities of *H. glycines* began to decline. Endospores of a *Pasteuria* sp. were observed on second-stage juveniles (J2) and males. In this report, we describe the first natural occurrence other than in Japan of *Pasteuria* on *H. glycines* and our preliminary observations on the morphology and life cycle of this isolate.

MATERIALS AND METHODS

Soil samples were obtained from microplots on the USDA nematology farm and processed in July 1993. Cysts of *H. glycines* races 3 and 4 were extracted from 250 cm^3 soil by gravity sieving (1). Centrifugal flotation (3) was used to extract *H. glycines* males and J2 and other vermiform nematodes from the suspension from which cysts were removed. Temporary mounts in 2% formalin were prepared for light microscopic observation of endospores attached to vermiform nematodes and to observe sporangia freed from crushed cysts. Endospores attached to J2 of race 3 were measured with the aid of a camera lucida (13). Measurements were made at ×2,000 on the drawing surface. For measurements of endospore diameters n = 17 and for height n = 5. Several J2 of race 3 and race 4 were selected individually and processed (6) for conventional scanning electron microscopy (SEM) with an ISI DS 130 scanning electron microscope (Topcon, Pleasanton, CA 64556) operated at 10 kV. Measurements of endospores were made from micrographs with the scale provided by the instrument. For race 3, n for diameter and height was 17 and 5, respectively, and for race 4, n was 5 and 3 for diameter and height, respectively.

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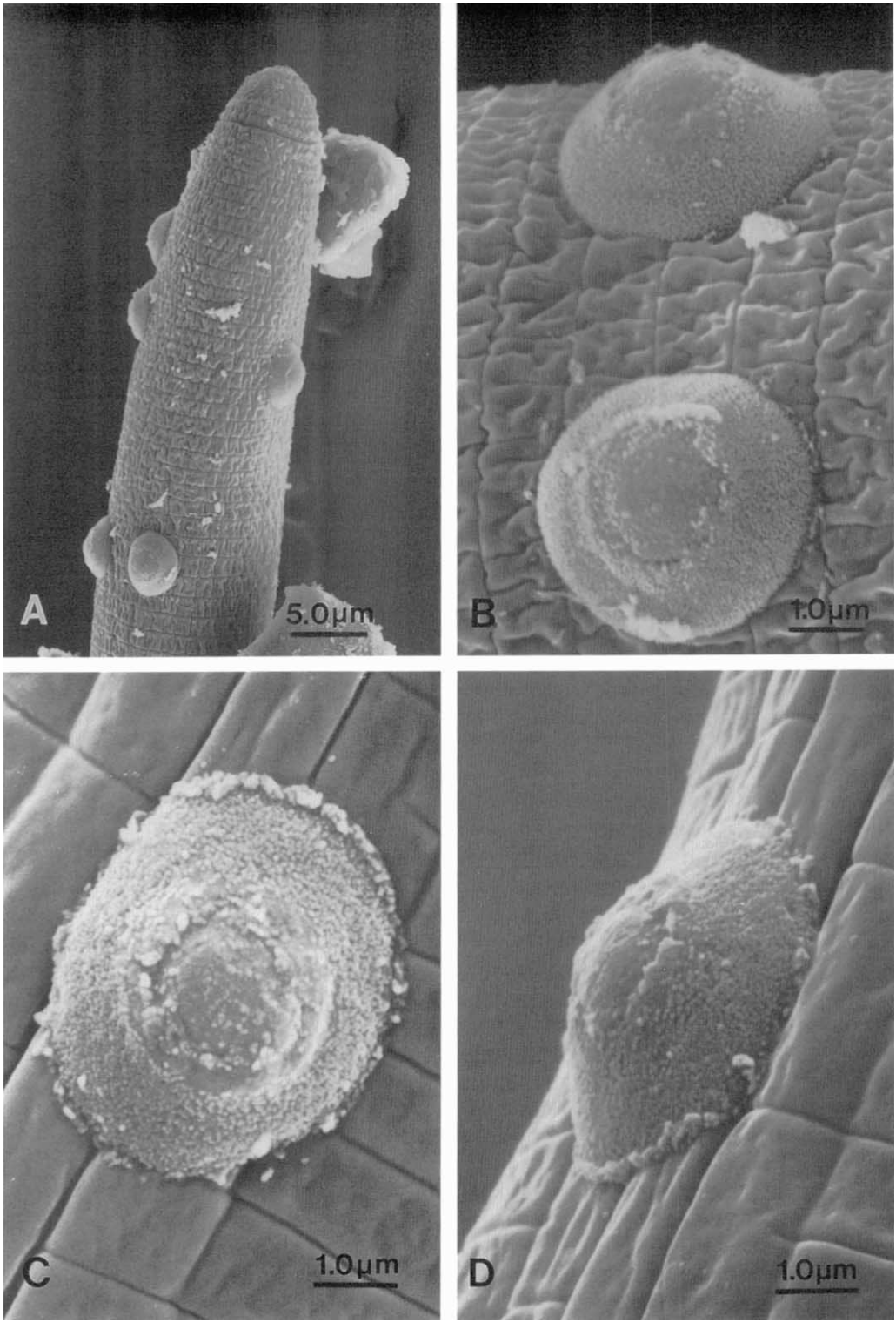

FIG. 1. *Heterodera glycines* second-stage juveniles encumbered with endospores of a *Pasteuria* isolate from Illinois. A) Anterior of a race 3 juvenile. B) Higher magnification of two endospores attached to the juvenile in A. C,D) Top and lateral view of an endospore attached to a race 4 juvenile.

Results

Endospores attached to J2 and males were observed readily at ×400 on temporary mounts. Of 21 race 3 J2 examined by light microscopy, 19 were encumbered with one to several endospores. Both of the race 3 males recovered were encumbered with endospores. Endospores attached to J2 of race 3 were ($\bar{X} \pm s_{\bar{x}}$) 5.0 ± 0.1 μm × 4.8 ± 0.1 μm in diameter with a height of 2.2 ± 0.1 μm. Light microscopy was not used to measure endospores attached to males of race 3 or J2 of race 4. Other genera, including *Aphelenchus, Helicotylenchus,* and *Pratylenchus,* were not encumbered with endospores.

Many partially fragmented cysts devoid of eggs were found in the sample of soil infested with race 3. These did not contain sporangia of *Pasteuria,* nor did 20 white females and light tan to brown cysts extracted from soil. Many of the white females and cysts were infected with fungi.

Endospores attached to J2 and visualized with conventional SEM were ellipsoid, measuring ($\bar{X} \pm s_{\bar{x}}$) 4.2 ± 0.2 μm × 3.7 ± 0.3 μm with a height of 2.0 ± 0.3 μm for race 3 and measuring 4.3 ± 0.4 μm × 3.7 ± 0.3 μm with a height of 2.3 ± 0.3 μm for race 4 (Fig. 1). The exosporium was not observed on any of the 30 endospores examined with SEM. The central endosporal body and peripheral fibers were visible.

Discussion

The source of the Illinois *Pasteuria* infestation is not known. The field in which microplots were established had no history of soybean cultivation, and *H. glycines* was not recovered from the field prior to microplot establishment. Soil for the microplots was transported to Urbana, Illinois, from a farm approximately 100 km distant. That soil was examined, and no cyst-forming nematode species were found; however, *Catenaria auxiliaris* was found, indicating that a cyst nematode may have infested the soil (10). The *Pasteuria* may have infected the southern Illinois populations of *H. glycines* used to infest the microplots. The host specificity described for other isolates of *Pasteuria* (11) and lack of infection of other genera preclude other nematodes in the field as the source of the Illinois isolate.

Differences in morphology, absence of an exosporium on endospores attached to J2 and males, and the inability to recover sporangia from cysts indicate that the *Pasteuria* associated with *H. glycines* in Illinois is not *P. nishizawae.* Although J2 infected with the Illinois isolate have not been excised from roots, the life cycle of the Illinois isolate may be the same as the *Pasteuria* that infects *H. avenae* (2) and *H. goettingiana* (12). Light and SEM measurements of endospores of the *H. goettingiana* parasite are similar to those of the *Pasteuria* isolate that infects *H. glycines* in Illinois. The morphological difference between endospores attached to the race 3 and race 4 J2 are believed to be due to effects of fixation for SEM. Dehydrated endospores observed by SEM were smaller than endospores on J2 mounted in 2% formalin and observed by light microscopy (7).

Additional morphological studies, description of the life cycle, species identification, determination of host specificity, and proof of pathogenicity will elucidate the biology and biological control potential of the *H. glycines*-infecting *Pasteuria* from Illinois.

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