# Note brève

## COMPARISON OF ELECTROPHOREGRAMS FROM *BURSAPHELENCHUS* spp. (APHELENCHOIDIDAE)

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In a recent review of the genus *Bursaphelenchus*, Tarjan and Aragon (1982) synonymized several species based solely on published descriptions of spicule shape and morphometrics. Their analysis failed to account for natural morphometric variations within or between populations of a species due to different hosts, nematode age or environmental parameters. This kind of variation can be important, e.g., Pillai and Taylor (1968) found that changing the fungal host of five species of nematodes affected population size and generation time, and caused substantial morphometric variability. Chemotaxonomy has been used to classify nematodes parasitic on plants and animal where morphological differences were obscure (Hansen & Beucher, 1970 ; Hussey, 1979 ; Platzer, 1981).

Comparisons of nematode protein profiles of aphelenchoidids is feasible because large numbers of nematodes can be generated in culture. Further, species in this group often have wide fungal host ranges, thus increasing the likelihood of obtaining a food source common to those being compared. The purpose of this investigation is to compare the electrophoretic mobilities of proteins of different insect-associated *Bursaphelenchus* species.

*Bursaphelenchus* n. sp. (close to *B. ratzeburgii* (Rühm, 1956) Goodey, 1960) was isolated from a squashed scolytid beetle, *Scolytus multistriatus* (Marsham), on potato dextrose agar (PDA), from Sacramento, Sacramento Co., California. *B. kevini* Giblin, Swan & Kaya, 1984, was isolated from the lateral oviducts of the female sweat bees, *Halictus farinosus* Smith, from Davis and Newberg, Yamhill Co., Oregon and *H. ligatus* Say from Parma, Payette Co., Idaho. *B. seani* Giblin & Kaya, 1983 was isolated from the median oviducts of *Anthrophora bomboides stanfordiana* Cockerell from Bodega Head State Park, Sonoma Co. and Cambria Pines, San Luis Obispo Co., California. *B. xylophilus* (Steiner) was isolated from wood chips from galleries in *Pinus taeda* L. from Baton Rouge, East Baton Rouge Parish, Louisiana.

All of the above mentioned *Bursaphelenchus* spp. are obligate parasites and reproduce well on a common host fungus, *Monilinia fructicola* (Wint.) (Giblin & Kaya, 1983). Nematodes were subcultured to one-week-old fungal mats of *M. fructicola* on PDA supplemented with glycerol (115 mg/g hydrated PDA) at three to four week intervals. All cultures were maintained at 25° and harvested for electrophoresis at two weeks on a Baermann funnel. Nematodes were washed three times with sterile distilled water, transferred to a 0.45 µm Millipore® filter, and then excess water was removed. The nematodes were placed in a reducing buffer (Laemmli, 1970) (1 mg wet weight nematodes/10 µl buffer), boiled 3 min., sonicated for 3 min., and reboiled for 3 min. Slight turbidity indicated some membranes and cuticular structures were not completely solubilized by this treatment. Ten to 20 µl of sample were loaded on a 1.25 mm thick sodium dodecyl sulfate-polyacrylamide (SDS-PAGE) vertical slab gel (Laemmli, 1970). A 4% stacking gel and a 7-12% gradient separating gel was utilized. Samples were electrophoresed at 15 V for about 18 h. Proteins used as molecular weight standards were : phosphorylase B (92 500 daltons), bovine serum albumin (66 200 daltons, ovalbumin (45 000 daltons), carbonic anhydrase (31 000 daltons), soybean trypsin inhibitor (21 500 daltons) and lysozyme (14 400 daltons). Gels were fixed and stained in 50% methanol : 10% glacial acetic acid with 1% Coomassie brilliant blue and destained in 25% methanol : 10% glacial acetic acid.

The electrophoregram of each of the four species of nematodes examined is unique and consistent under the conditions of this investigation (Fig. 1). The SDS-PAGE electrophoregrams of the three geographically separate populations of *B. kevini* were identical, as were the two isolates of *B. seani*. This is not unexpected since, in many studies with nematodes, intraspecific differences have not been detected between proteins in electrophoregrams (Dickson, Sasser & Huisingh, 1970 ; Trudgill & Carpenter, 1971). Thus, as suggested by Ferguson

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(1980), intra-specific comparisons should be corroborated by staining for specific enzymes.

We conclude that SDS-PAGE electrophoregram comparisons are useful in supporting taxonomic decisions at the species level for *Bursaphelenchus*. The use of a single fungal host, the same fungal growth media and controlled light and temperature further accentuate real differences between species. Future work should focus on reducing variability by use of specific developmental stages and use of zymograms for intra-specific comparisons.

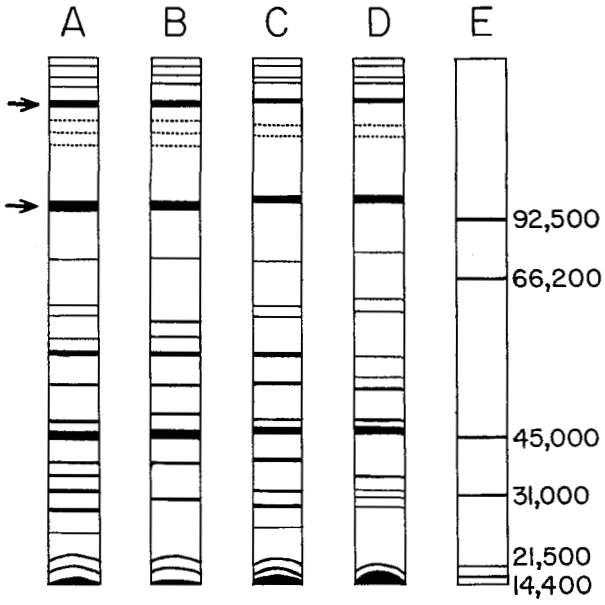


Fig. 1. Composite diagram of the observed protein profile visualized by staining with Coomassie brilliant blue after electrophoresis on a 7-12% gradient polyacrylamide gel. A : *Bursaphelenchus xylophilus ;* B : *Bursaphelenchus* n. sp. *(close to B. ratzeburgii ) ;* C : *B. seani ;* D : *B. kevini ;* E : standard proteins molecular weight in daltons).

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