

Association of *Bursaphelenchus* sp. (Nematoda : Aphelenchoididae) with nitidulid beetles (Coleoptera : Nitidulidae)

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SUMMARY

Dauer juveniles (JIII) of an undescribed *Bursaphelenchus* sp. were recovered from the median oviduct and ovipositor sac of adult females and the internal sac of adult males of the pineapple beetle, *Urophorus humeralis*. This nematode was reared on cultures of the fungi *Monilinia fructicola* or *Penicillium* sp. In experimental studies, dauers (dispersal stage) of *Bursaphelenchus* sp. could be recovered internally from adult beetles only and four species of nitidulid beetles that occurred sympatrically with *U. humeralis* were infestable. *Carpophilus hemipterus*, *C. mutilatus*, and *U. humeralis* were consistently associated with more dauers than *Haptonchus luteolus* or *Stelidota geminata*. Male *C. hemipterus* venereally transmitted dauers of *Bursaphelenchus* sp. to females in 33% of experimental crosses (n = 18). Female to male transmission was not observed. *Bursaphelenchus* sp. dauers freed themselves from dead male or female *C. hemipterus* hosts to propagate on *M. fructicola*. Live females of *C. hemipterus* contaminated fungal plates with nematodes. The transgenerational and horizontal transmission of *Bursaphelenchus* sp. by nitidulid beetles is discussed.

RÉSUMÉ

Association de *Bursaphelenchus* sp. (Nematoda : Aphelenchoididae)
avec des Nitidulides (Coleoptera : Nitidulidae)

Les « dauer Larven » (J III) d'un *Bursaphelenchus* non décrit ont été trouvées dans la partie médiane de l'oviducte et dans l'ovipositeur de femelles adultes d'un Nitidulide parasite de l'ananas, *Urophorus humeralis*, ainsi que dans le sac interne des mâles de cet insecte. Ce nématode a été élevé sur cultures des champignons *Monilinia fructicola* ou *Penicillium* sp. Les études expérimentales ont montré que les J III (stade de dispersion) de *Bursaphelenchus* sp. ne peuvent être obtenus que des parties internes des insectes adultes, et que quatre espèces de Nitidulides sympatriques de *U. humeralis* peuvent être infestées. A *Carpophilus hemipterus*, *C. mutilatus* et *U. humeralis* sont constamment associés de plus grands nombres de J III qu'à *Haptonchus luteolus* ou *Stelidota geminata*. Le mâle de *C. hemipterus* a transmis les J III de *Bursaphelenchus* sp. à la femelle, lors de la fécondation, dans 33 % des croisements expérimentaux (n = 18). La transmission de femelle à mâle n'a pas été observée. Les J III de *Bursaphelenchus* sp. se libèrent d'eux-mêmes des cadavres, mâles et femelles, de *C. hemipterus* pour se développer sur *M. fructicola*. Les femelles vivantes de *C. hemipterus* infestaient d'autre part les cultures de champignons avec les nématodes. Les transmissions généalogique et spatiale de *Bursaphelenchus* sp. par les Nitidulides sont discutées.

Nitidulid beetles are economically important pests of dried fruits in California and are commonly found in rotting fruit or vegetables. These beetles also attack live fruit and can vector brown rot (*Monilinia fructicola*) or *Ceratocystis* canker (Okumura & Savage, 1974). Giblin, Powers and Platzer (1984) reported that the dauer juvenile (= dispersal stage) of an undescribed *Bursaphelenchus* sp. (Aphelenchoididae) occurs in the reproductive tracts of adults of the pineapple beetle, *Urophorus humeralis* (Nitidulidae). This *Bursaphelenchus* sp. is mycophagous and reproduces on *Monilinia fructicola* or *Penicillium* sp. isolated from the beetle environment. Although *Bursaphelenchus* spp. have been reported from scolytid and cerambycid beetles (Poinar, 1975), and anthophorid and halictid bees (Giblin & Kaya, 1983 a;

Giblin, Swan & Kaya, 1984) this is the first known report of *Bursaphelenchus* being associated with nitidulid beetles. This *Bursaphelenchus* sp. occurs in the reproductive tracts of its nitidulid host and behaves similarly to the bee associated *Bursaphelenchus* spp. which are carried in the reproductive tracts of their hosts (Giblin & Kaya, 1983b; Giblin, Swan & Kaya, 1984). Conversely, all other beetle associated *Bursaphelenchus* spp. are carried under the elytra, in intersegmental folds of the abdomen, or in the tracheae of their hosts (Rühm, 1956; Mamiya & Enda, 1972).

Nitidulids are easily reared in the laboratory (Lindgren & Okumura, 1973) and *Bursaphelenchus* sp. is easily cultured on fungus. Accordingly, this association was chosen as a model system to examine venereal

transmission of the nematode by host beetles, host specificity of the nematode, transgenerational transfer of the nematode by its host, and stages of the beetle associated with the nematodes.

Materials and methods

A culture of *Bursaphelenchus* sp. (isolate BNUH 1) was initiated from a single fertilized female from an original culture (see Giblin, Powers & Platzer, 1984) onto *M. fructicola* on potato dextrose agar (P.D.A.). All nematodes for the research presented in this paper and for the forthcoming species description (Giblin, in prep.) were derived from subcultures from culture (BNUH 1). *Bursaphelenchus* sp. cultures were inoculated onto one or two week-old cultures of *M. fructicola* on P.D.A. and kept at room temperature at least two weeks between subculturing. Dauer juveniles (J III) of *Bursaphelenchus* sp. began to appear in abundance in older cultures (more than four weeks old) and were especially abundant in cultures supplemented with glycerol (100 ml/1.1 l hydrated P.D.A.) or Tween 40[®] (Poloxyethylene sorbitan monopalmitate) (10 g/1 l). We used more than four week-old cultures of *Bursaphelenchus* sp. grown on *M. fructicola* on P.D.A. supplemented with glycerol or Tween 40 as a source of nematode inoculum in the beetle infestation experiments and procedures.

U. humeralis was isolated from the nematode's type locality: the intersection of Jackson St. and Victoria Ave., Riverside, Riverside Co., California, in a grapefruit orchard and cultured on dried fig cultures as described by Lindegren and Okumura (1973). Nematode-free beetle cultures were established from carefully washed beetle pupae.

A nematode-free culture of *Carpophilus hemipterus* was obtained from Dr. J. Lindegren and maintained as above. Adult nitidulid species and a staphylinid species that occurred sympatrically with *U. humeralis* and *C. hemipterus* at the nematode's type locality were collected, and used immediately in host specificity experiments.

Larvae, pupae, and adults of *U. humeralis* were placed in cultures containing dauers of *Bursaphelenchus* sp. for 48 h and rinsed with distilled water, dissected, and examined internally for nematodes.

Host specificity experiments were conducted with *U. humeralis* and *C. hemipterus* from nematode-free cultures and field collected *Carpophilus mutilatus*, *Haptonchus luteolus*, *Stelidota geminata*, and an unidentified staphylinid. Beetles were placed in conspecific groups in cultures containing dauers of *Bursaphelenchus* sp. for more than 24 h at room temperature and the reproductive tracts were examined for the presence and number of nematodes. Any nematodes found internally in the field collected beetles were cultured on *M. fructicola* to confirm that they were *Bursaphelenchus* sp. Two

to five adult *C. hemipterus* beetles were placed in each of the host specificity trials for comparison with the species of beetle being tested.

Mating experiments were conducted by individually confining pupae of *C. hemipterus* from a nematode free culture in 13 ml plastic vials with a small piece of *Calymyrra* fig. Adult beetles were infested with *Bursaphelenchus* sp. by placing a 2 × 2 mm square of agar culture containing dauer nematodes in the vial for 24-48 h. Infested males or females were then paired with uninfested beetles of the opposite sex. If matings were observed immediately after pairing, the pair was washed externally, dissected and examined internally. Otherwise, the pairs were left for 48 h before both beetles were dissected and examined as above.

Adult male and female *C. hemipterus* were exposed to *Bursaphelenchus* sp. for 48 h, rinsed three times in distilled water, and the head capsule was crushed before individual beetles were transferred to a *M. fructicola* culture. There were four trials for each sex. Culture plates for each trial were checked for nematode growth after two weeks. In addition, two trials each for alive adult males and females were done as above.

Light photomicrographs of excised male or female reproductive tracts, stained and fixed in hot acid fuchsin-lactophenol for 1-3 mn (Southey, 1970), were taken with a Zeiss photomicroscope III. For scanning electron photomicrographs, adult females of *U. humeralis* and *C. hemipterus* were infested as above. The ovipositor was extended by squeezing the abdomen with a pair of forceps and the entire beetle was frozen on dry ice. The beetles were then lyophilized, sputter coated with gold, and viewed on a JEOL SEM microscope at 15 kV.

Results

Dauers of *Bursaphelenchus* sp. were not associated internally with larvae ($n = 10$) or pupae ($n = 10$) of *U. humeralis*. All *U. humeralis* adults ($n = 10$) tested were infested by *Bursaphelenchus* dauers. This is similar to other reported *Bursaphelenchus* sp.-insect associations. *C. hemipterus*, *U. humeralis*, *C. mutilatus*, *H. luteolus*, and *S. geminata* were found to occur sympatrically in rotting grapefruit at the type locality. Adult males and females of these nitidulids could be infested with *Bursaphelenchus* sp. in the laboratory (Table 1). *Bursaphelenchus* sp. dauers were always found in the ovipositor sac and median oviducts in females and internal sac of the aedeagus of male beetles. *C. hemipterus*, *U. humeralis*, and *C. mutilatus* were consistently associated with more dauer nematodes per host than *H. luteolus* or *S. geminata* (Table 1). *C. hemipterus* placed in with other species of nitidulids during host specificity trials were infested to the same degree as *C. hemipterus* alone. Sympatrically occurring staphylinid beetles were not infestable with *Bursaphelenchus* sp. dauers.

Table 1

Experimental association of *Bursaphelenchus* sp. dauer juveniles with sympatrically occurring adult nitidulid beetles.

Beetles species	Female beetle	Male beetle
	Nematodes*	Nematodes*
<i>Urophorus humeralis</i>	(n = 10) : 36 20 (10-79)	(n = 7) : 3 4 (0-12)
<i>Carpophilus hemipterus</i>	(n = 29) : 47 40 (0-220)	(n = 28) : 12 11 (0-51)
<i>Carpophilus mutilatus</i>	(n = 4) : 35 18 (11-55)	(n = 6) : 12 9 (0-25)
<i>Haptonchus luteolus</i>	(n = 16) : 5 4 (0-14)	(n = 11) : 1 1 (0-3)
<i>Stelidota geminata</i>	(n = 10) : 2 2 (0-6)	(n = 4) : 1 1 (0-3)

* Mean number of internally associated nematodes followed by the standard deviation and range.

Thirty-three percent of the *Bursaphelenchus* sp. infested male *C. hemipterus* successfully transferred dauer nematodes to uninfested female beetles in mating experiments (n = 18). An average of 8.5 ± 8.2 (range = 3-22) dauers were transferred per successful cross. In one case the beetles mated immediately after confinement. Mating took ca 8 mn and successful transfer was documented. No successful female to male transfers of nematodes were observed (n = 12 crosses). *Bursaphelenchus* sp. dauers established themselves on fungus cultures within two weeks from individually confined and killed adult *C. hemipterus* males (n = 4) and females (n = 4). Propagating *Bursaphelenchus* sp. were recovered from fungal cultures inoculated with live adult females of *C. hemipterus* (n = 2). However, nematodes were not recovered from cultures inoculated with live male beetles (n = 2).

Light and SEM photomicrographs of dauer juveniles of *Bursaphelenchus* sp. infesting the ovipositor sac of female *U. humeralis* (Fig. 1 A, 2 B) and *C. hemipterus* (Fig. 1 B, 2 A) are shown. *Bursaphelenchus* sp. dauers are also shown infesting the internal sac of males of both these nitidulid species (Fig. 1 C, D).

Discussion

The *Bursaphelenchus* sp.-nitidulid association is depicted in figure 3. The development of *Bursaphelenchus* sp. is probably similar to *B. seani* and *B. kevinci* (Giblin & Kaya, 1983a; Giblin, Swan & Kaya, 1984). *Bursaphelenchus* sp. can continually cycle through successive generations on fresh fungus as a food source. Dauer juvenile formation probably occurs as described for *Caenorhabditis elegans* (Golden & Riddle, 1982) which is dependent upon declining titers of a « food factor » and increasing titers of a population density indicator pheromone. Numbers of dauer juveniles of *Bursaphe-*

lenchus sp. were increased in culture by the presence of glycerol or Tween 40. As for *B. seani* (Giblin & Kaya, 1984 b), these media additives in a monoxenic culture tell us little about the chemical cues required for initiation of dauer juvenile formation. Dauers become more numerous as the quality of the environment declines and they infest adult male or female nitidulids. Successful transgenerational transmission of the nematodes to a new breeding site is accomplished by both males and females and is enhanced by wide phoretic host ranges and by venereal transmission. The most common mode of transgenerational transmission for *Bursaphelenchus* sp. dauers appears to be oviposition by females into a new environment (Fig. 2).

Venereal transmission of nematodes between phoretic hosts has been implicated but has never been experimentally demonstrated (Poinar, 1971; Giblin & Kaya, 1983 b; 1984 a). Venereal transmission would be adaptive when males would otherwise be dead-end hosts. This is the case for the bee-nematode associations where the male bees do not enter the brooding environment and their only contact with the next generation is during mating. However, the brooding environment for nitidulid beetles is a source of food and shelter for both adult males and females. Adult male beetles must be considered as potential transgenerational vectors of dauers of *Bursaphelenchus* sp. because they can disperse nematodes by moving to and then dying in a new brooding environment. In addition, nitidulid males can venereally transmit the nematodes to females.

Dauers of *Bursaphelenchus* sp. infested the different sympatrically occurring genera and species of nitidulids from the nematode type locality. This is not surprising because some species of *Bursaphelenchus* are phoretically associated with more than one species of host (Mamiya & Enda, 1972; Massey, 1974; Giblin & Kaya, 1984 a). The quantitative preference demonstrated by

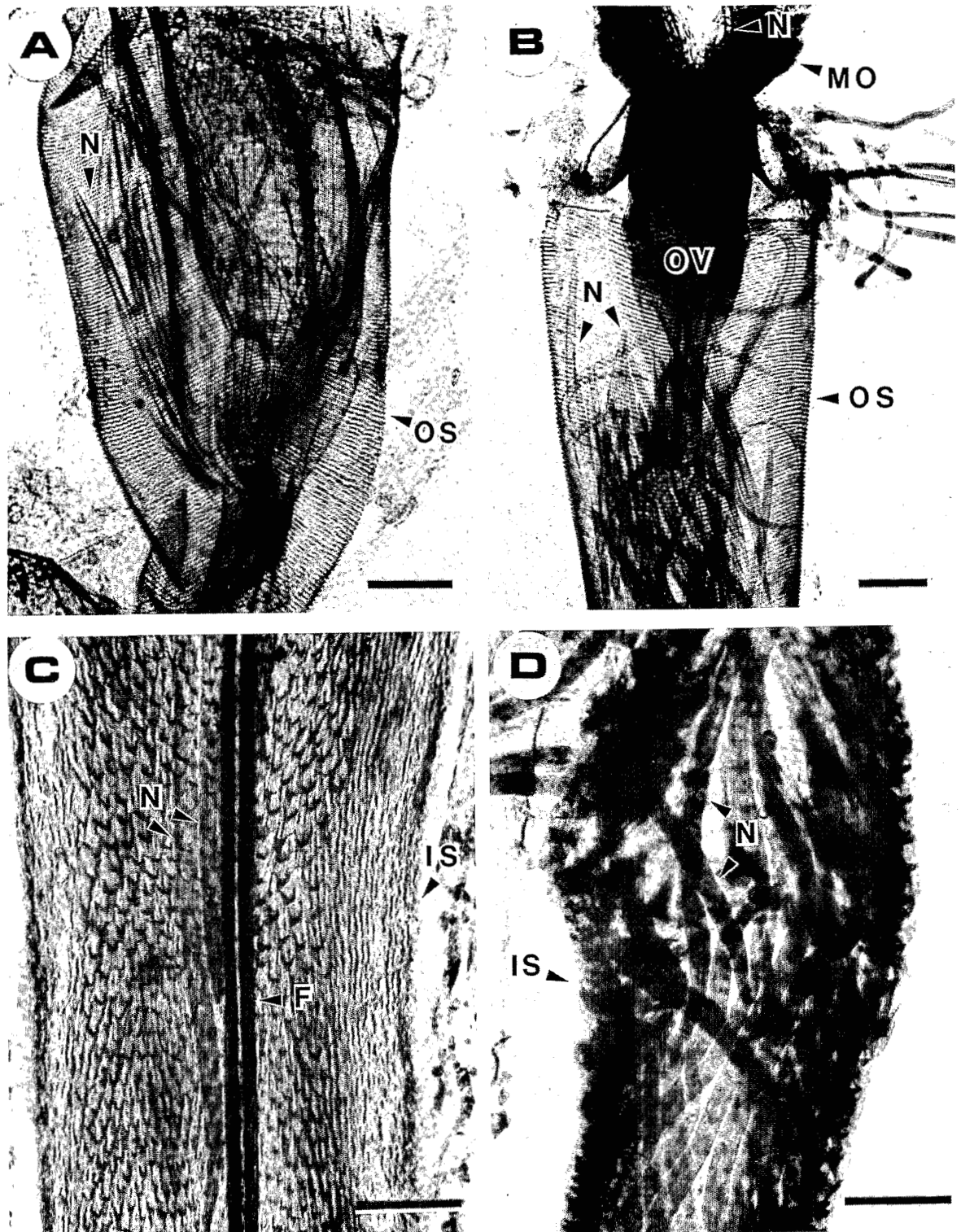


Fig. 1. Light photomicrographs. A : *Urophorus humeralis* female, excised ovipositor sac internally infested with *Bursaphelenchus* sp. dauers; B : *Carpophilus hemipterus* female, excised ovipositor sac and median oviduct internally infested with *Bursaphelenchus* sp. dauers; C : *U. humeralis* male, excised aedeagus internally infested with *Bursaphelenchus* sp. dauers; D : *C. hemipterus* male, excised aedeagus internally infested with *Bursaphelenchus* sp. dauers. (E.O.S. = everted ovipositor sac, F = flagellum, I.S. = internal sac, M.O. = median oviduct, N = nematodes, O.S. = ovipositor sac, and O.V. = ovipositor. A, B : bar = μm ; C, D : bar = 50 μm).

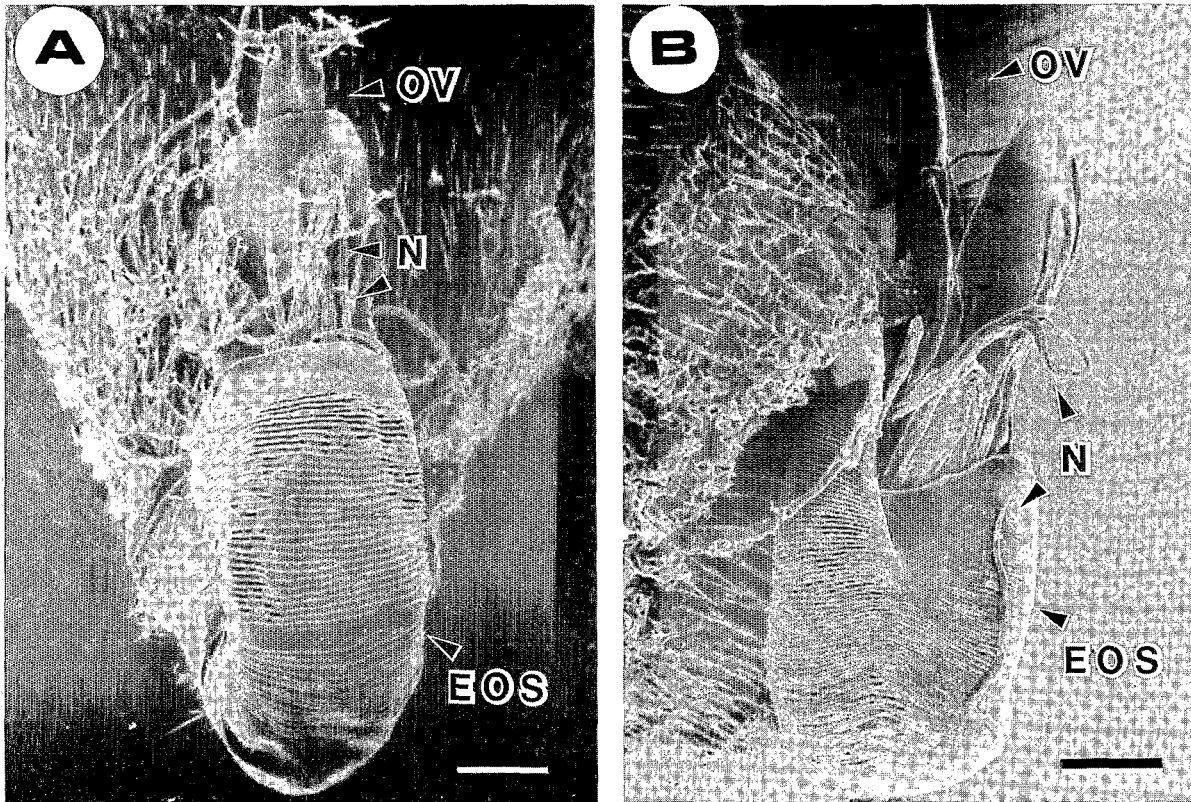


Fig. 2. Scanning electron photomicrographs. A : *Carpophilus hemipterus* female with ovipositor sac everted and *Bursaphelenchus* sp. dauers exposed as might occur during oviposition by an infested female; B : *Urophorus humeralis* female with ovipositor sac everted and *Bursaphelenchus* sp. dauers exposed. (See Fig 1 for legend. Bar = 100 µm.)

Bursaphelenchus sp. dauers for different nitidulids as phoretic hosts may indicate slight differences in the physical and/or chemical suitability of a host. Certainly, the strategy of dispersing with a wide range of host species that will be locating and colonizing similar types of breeding habitats has adaptive significance for these nematodes.

Most *Bursaphelenchus*-insect associations are characterized as being phoretic; the nematode benefits from the increased power of dispersion and the insect is not harmed or benefited. Wilson (1980; 1983) has argued that; 1) phoretic associations may be predisposed to evolve towards mutualism, and 2) that sampling error (genetic drift) will supply the variability necessary to drive these changes with intrademic group selection (I.G.S.). In this light, many of the *Bursaphelenchus*-insect associations may be examples of « population mutualism ». For example, *B. xylophilus* benefits from its association with cerambycid beetles with increased powers of dispersion to stressed or susceptible pine trees or to cut logs, and can benefit its host by killing trees that

will be used as brooding environments for the next generation of beetles (Mamiya, 1983). Another possibility for « population mutualism » exists in the association between *B. seani* and the digger bee, *Anthophora bomboidea stanfordiana*. The nematode benefits by being dispersed to a rich but predictably unstable environment (bee brood cell) and the bee population may benefit because of *B. seani*'s wide fungal host range which may help to reduce sporulation and inoculum levels of bee pathogenic fungi in the brooding environment (Giblin & Kaya, 1984 b).

The *Bursaphelenchus* sp.-nitidulid association reported here is obviously beneficial to the nematode but as with many of the *Bursaphelenchus*-nitidulid associations it is not clear what benefits or costs are accrued by the phoretic host. Unlike many of the *Bursaphelenchus* associations, both the host (nitidulid) and the nematode can be easily reared in the laboratory. Future studies should deal with how the insect and nematodes benefit or harm each other, how I.G.S. may have worked in the association, and whether phoresy is a common starting point for « population mutualism » in nematodes.

BURSAPHELENCHUS - NITIDULIDAE ASSOCIATION

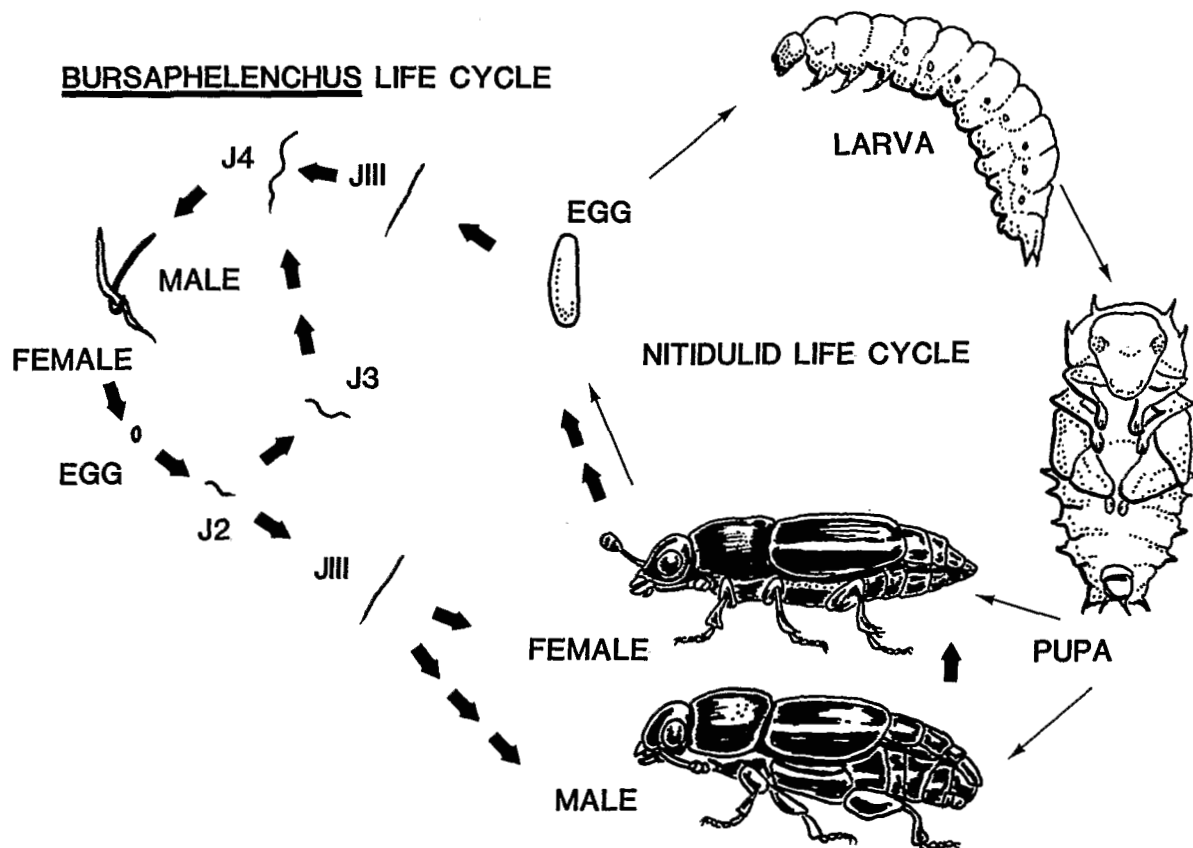


Fig. 3. *Bursaphelenchus* sp.-nitidulid beetle association. J 2, J 3, J 4 = Propagative second, third, and fourth stage juveniles, J III = dauer juvenile or third stage dispersal juvenile.

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REFERENCES

GIBLIN, R. M. & KAYA, H. K. (1983 a). *Bursaphelenchus seani* n. sp. (Nematoda : Aphelenchoididae), a phoretic associate of *Anthophora bombooides stanfordiana* Cockerell, 1904 (Hymenoptera : Anthophoridae). *Revue Nématol.*, 6 : 39-50.

GIBLIN, R. M. & KAYA, H. K. (1983 b). Field observations on the association of *Anthophora bombooides stanfordiana* (Hymenoptera : Anthophoridae) with the nematode *Bursaphelenchus seani* (Aphelenchida : Aphelenchoididae). *Ann. Entomol. Soc. Amer.*, 76 : 228-231.

GIBLIN, R. M. & KAYA, H. K. (1984 a). Associations of halictid bees with the nematodes, *Aduncospiculum halicti* (Diplogasterida : Diplogasteroididae) and *Bursaphelenchus kevinci* (Aphelenchida : Aphelenchoididae). *J. Kans. Entomol. Soc.*, 57 : 92-99.

GIBLIN, R. M. & KAYA, H. K. (1984 b). Host, temperature and media additive effects on the growth of *Bursaphelenchus seani*. *Revue Nématol.*, 7 : 13-17.

GIBLIN, R. M., POWERS, T. O. & PLATZER, E. G. (1984). *Bursaphelenchus* sp. and *Sheraphelenchus entomophagus* (Aphelenchoididae), phoretic associates of *Urophorus humeralis* (Coleoptera : Nitidulidae). *Proc. 1st Intern. Congr. Nematol., Guelph, Canada, August 5-10, 1984* : 31 [abstr.].

- GIBLIN, R. M., SWAN, J. L. & KAYA, H. K. (1984). *Bursaphelenchus kevinci* n. sp. (Aphelenchida : Aphelenchoididae), an associate of bees in the genus *Halictus* (Hymenoptera : Halictidae). *Revue Nématol.*, 7 : 177-187.
- GOLDEN, J. W. & RIDDLE, D. L. (1982). A pheromone influences larval development in the nematode *Caenorhabditis elegans*. *Science*, 218 : 578-580.
- LINDEGREN, J. E. & OKUMURA, G. T. (1973). Pathogens from economically important nitidulid beetles. *U.S. Dept. Agric., Agric. Research Serv., Western Regi., ARS W-9*, 7 p.
- MAMIYA, Y. (1983). Pathology of the pine wilt disease caused by *Bursaphelenchus xylophilus*. *Ann. Rev. Phytopathol.*, 21 : 201-220.
- MAMIYA, Y. & ENDA, N. (1972). Transmission of *Bursaphelenchus lignicolus* (Nematoda : Aphelenchoididae) by *Monochamus alternatus* (Coleoptera : Cerambycidae). *Nematologica*, 18 : 159-162.
- MASSEY, C. L. (1974). *Biology and Taxonomy of Nematode Parasites and Associates of Bark beetles in the United States*. U.S. Dept. Agric. Forest Serv., Agric. Handbook No. 446, 233 p.
- OKUMURA, G. T. & SAVAGE, I. E. (1974). Nitidulid beetles most commonly found attacking dried fruits in California. *Natl Pest Control Operators News*, 34 : 4-7.
- POINAR, G. O., Jr. (1971). *Rhabditis adenobia* sp. n. (Nematoda : Rhabditidae) from the colleterial glands of *Oryctes monoceros* L. and other tropical dynastid beetles (Coleoptera : Scarabaeidae). *Proc. helminth. Soc. Wash.*, 38 : 99-108.
- POINAR, G. O., Jr. (1975). *Entomogenous Nematodes*. Leiden, E. J. Brill, 317 p.
- RÜHM, W. (1956). Die Nematoden der Ipiden. *Parasitol. Schriftenr.*, 6 : 437 p.
- SOUTHEY, J. F. (1970). *Laboratory Methods for Work with Plant and Soil Nematodes*. London, Her Majesty's Stationary Office, 148 p.
- WILSON, D. S. (1980). *The Natural Selection of Populations and Communities*. Menlo Park, California, The Benjamin Cummings Publ. Co. Inc., 186 p.
- WILSON, D. S. (1983). The effect of population structure on the evolution of mutualism : a field test involving burying beetles and their phoretic mites. *Am. Naturalist*, 121 : 851-870.

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