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 hueoelo* 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 » (JIII) 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 la 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 à *Haptonchus hueoelo* 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* infesteraient 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 anthophilid 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 (Rúthm, 1956; Mamiya & Enda, 1972).

Nitidulids are easily reared in the laboratory (Lindegren & 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)
on 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
subculturings. Dauer juveniles (J III) of Bursaphelenchus
sp. began to appear in abundance in older cultures
(more than four weeks old) and were especially abun-
dant in cultures supplemented with glycerol
(100 ml/1.1 l hydrated P.D.A.) or Tween 40® (Polysyo-
ethylene sorbitan monopalmitate) (10 g/l 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 dauer 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 un-
distinguished staphylinid. Beetles were placed in conspecific
groups in cultures containing dauer 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. fruc-
ticola 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
Calymyrna fig. Adult beetles were infested with Bursa-
phelenchus 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. Other-
wise, 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 fuch-
sin-lactophenol for 1-3 mn (Southey, 1970), were taken
with a Zeiss photomicroscope III. For scanning electron
photomicrographs, adult females of U. hemipterus and
C. mutilatus 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 dauer. This is similar
to other reported Bursaphelenchus sp.-insect associa-
tions. C. hemipterus, U. humeralis, C. mutilatus, H. luteo-
lus, 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 Bursa-
phelenchus sp. in the laboratory (Table 1). Bursaphelenchus
sp. dauer were always found in the ovipositor sac
and median oviducts in females and internal sac of the
adeagus of male beetles. C. hemipterus, U. humeralis,
and C. mutilatus were consistently associated with more
dauer nematodes per host than H. luteolus or S. gemi-
Evatata (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.
Sympatringly occurring staphylinid beetles were not
infectable with Bursaphelenchus sp. dauer.
Table 1

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

<table>
<thead>
<tr>
<th>Beetles species</th>
<th>Female beetle</th>
<th>Male beetle</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Nematodes*</td>
<td>Nematodes*</td>
</tr>
<tr>
<td>Urophorus humeralis</td>
<td>(n = 10) : 36 20 (10-75)</td>
<td>(n = 7) : 3 4 (0-12)</td>
</tr>
<tr>
<td>Carpophilus hemipterus</td>
<td>(n = 29) : 47 40 (0-220)</td>
<td>(n = 28) : 12 11 (0-51)</td>
</tr>
<tr>
<td>Carpophilus mutilatus</td>
<td>(n = 4) : 35 18 (11-55)</td>
<td>(n = 6) : 12 9 (0-25)</td>
</tr>
<tr>
<td>Haptonchus luteolus</td>
<td>(n = 16) : 5 4 (0-14)</td>
<td>(n = 11) : 1 1 (0-3)</td>
</tr>
<tr>
<td>Stelidota geminata</td>
<td>(n = 10) : 2 2 (0-6)</td>
<td>(n = 4) : 1 1 (0-3)</td>
</tr>
</tbody>
</table>

* 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 min 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. kevini (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 Bursaphelenchus 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. dauer seems 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 dauer 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

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Fig. 1. Light photomicrographs. A: *Urophorus humeralis* female, excised ovipositor sac internally infested with *Bursaphelenchus* sp. dauer; B: *Carpophilus hemipterus* female, excised ovipositor sac and median oviduct internally infested with *Bursaphelenchus* sp. dauer; C: *U. humeralis* male, excised aedeagus internally infested with *Bursaphelenchus* sp. dauer; D: *C. hemipterus* male, excised aedeagus internally infested with *Bursaphelenchus* sp. dauer. (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 = 3 μm; C, D: bar = 50 μm).
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Bursaphelenchus sp. dauer 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 bomboides 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.

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 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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