BIOLOGY OF *DELADEenus siricidicola* (Neotylenchidae)
AN ENTOMOPHAGOUS-MYCETOPOGOUS NEMATODE PARASITIC IN SIRICID WOODWASPS

BY

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*De/adenus siricidicola* has two life cycles each of which can continue indefinitely without the intervention of the other. One cycle is free-living involving a neotylenchid form of female that reproduces oviparously while feeding on the symbiotic fungus of *Sirex* woodwasps. The other cycle is parasitic, with a sphaerulariid form of female reproducing ovoviviparously within the haemocoel of four species of *Sirex* and two associated insects. Macro- and microspermatozoa are associated with the free-living and parasitic forms of female respectively. Juvenile nematodes may become adults of either cycle depending upon environmental conditions. Detailed observations have been made on the mycetophagous life cycle from plate cultures and within timber. Details of the mode and localization of penetration by infective females into *Sirex* larvae are included. The development of parasitic females particularly with regard to ovarian development has been followed. It has been shown that the time at which reproduction by the parasitic nematode commences in relation to host development may be dependant either on the species of host or the strain of nematode involved. This factor is correlated with the severity of the effect produced by parasitism on the reproductive system of the female host.

The woodwasp, *Sirex noctilio* F. is a serious pest of *Pinus radiata* D. Don. forests in Australia and New Zealand and since 1962 has been the subject of an extensive programme of research in Australia with particular emphasis on control. The important discovery (Zondag, 1962) of nematode parasitism of *S. noctilio* in New Zealand led in 1965 to the undertaking of investigations into nematodes of siricids by C.S.I.R.O. The life history of these nematodes was shown by Bedding (1967, 1972) to be of an unusual kind involving striking female dimorphism associated with free-living, mycetophagous and parasitic life cycles and the species concerned was described and named *De/adenus siricidicola* Bedding (Bedding, 1968). The present paper is an account of the general biology of this extraordinary nematode. Research is still in progress on details of biological control, insect host and fungal specificity, factors affecting infective production, distribution of parasitism and population dynamics.

**METHODS**

To obtain uncontaminated cultures of the symbiotic fungus *Amylusterum areolatum*, adult female *Sirex* were dipped in alcohol, flamed, and then dissected under sterile water in order to remove the ooidal glands. The burst glands were streaked on plates of potato dextrose agar and the growing front sub-cultured
after incubation at 24°C. Testes containing juvenile *D. siricidicola* similarly removed from male *Sirex* were placed in the centre of fungal cultures that had grown over about one third of the plate. After one day sub-cultures (about 2 cm square) of several hundred nematodes together with fungus were taken from the growing fungal edge, and used to establish a monoxenic culture. In sub-culturing a balance must be maintained between nematodes and fungus since the nematodes can breed successfully only on the growing edge of the fungus.

Several attempts were made to observe entry using the method adopted for *Tripsius sciarae* by Poinar & Doncaster (1965) but *D. siricidicola* infectives appeared unable to enter under these conditions. Using a modified technique satisfactory results were obtained: small *Sirex* larvae were left under water until immobile and placed half immersed in a thin layer of molten 1% ion agar on a coverslip. Infective nematodes were then added to the larval surface and after these had migrated between larva and agar, 1% ion agar at 35°C was poured over to fully embed larva and nematodes. After trimming, the agar block was suspended from the coverslip in a plastic cell for observation.

**Biology of the Hosts**

*D. siricidicola* has been found parasitizing the siricids *S. noctilio*, *S. juvenescens* L., *S. cyanus* F., *S. nitobei* Matsumura and *Xeris spectrum* L. and an associated beetle, *Serropalpus barbatus* (Schall.).

Female *S. noctilio* drill into the wood of living pine trees to oviposit and inject toxic mucus and a symbiotic fungus (Fig. 4 A). The mucus and fungus kill susceptible trees (Coutts, 1969) and *S. noctilio* larvae bore through the wood feeding on the fungus as it grows in the tree. Adult *S. noctilio* emerge one, two or three years after oviposition. *S. juvenescens*, *S. cyanus* and *S. nitobei* have similar life cycles to this but usually attack only dying or dead trees. All four species carry the same symbiotic fungus (*A. areolatum*). *X. spectrum* carries no symbiotic fungus but lays eggs in dead timber in which fungus, usually from other siricid species, is already established (Spradbery, pers. comm.). *S. barbatus* larvae, also wood borers, are found in dead coniferous timber attacked by a variety of fungi including *A. areolatum*.

**Observations**

*Mycetophagus* life cycle (Fig. 1).

Juvenile nematodes removed aseptically from their host and placed on young cultures of *A. areolatum* growing on potato dextrose agar migrate to the growing edge of the fungus and commence to feed. Within 5 days at 22°C adult neotylenchid-like males and females mature and mate.

Mating, which has been observed on some 50 occasions, lasts for 10 to 30 seconds and is preceded by several minutes of manoeuvring into position. Some 200-500 amoeboid spermatozoa are rapidly injected between the spicules and into the vulva, to occupy the uterus to a length of about five times the female
Eggs laid in tracheids, resin canals and host galleries

**MYCETOPHAGOUS FREE – LIVING CYCLE**

Eggs hatch

Juveniles feed on fungus and develop into adults of either cycle depending on environmental conditions

Juveniles enter host eggs and are transmitted into new habitat during host oviposition

After moulting, infectives grow considerably in host haemocoel and evaginiparously release juveniles into pupa

Amoeboid spermatozoa transferred during copulation

Microspermatozoa transferred during copulation

Fertilized infective females penetrate host larva

**PARASITIC CYCLE**

Adults feed on fungus in wood

Fig. 1. Diagram of the life history of Deladenus siricidicola.
body width. The male rapidly withdraws and inevitably a dozen or more spermatozoa are lost to the outside. Egg laying rarely occurs without prior copulation and unfertilized eggs do not develop. Single male and female *D. siricidicola* placed at opposite sides of 9 cm diameter culture plates almost invariably copulate within 24 hours. Copulation and oviposition occurs at temperatures from 3° to 30°. Both males and females feed on the fungus before and after copulation and both sexes will mate more than once.

Oviposition usually commences within a few hours of copulation and takes only a fraction of a second. Eggs are laid about one per hour initially but, after several hours, from two to three per hour at 22° under ideal conditions. Adult mycetophagous females feed on young fungus cultures almost continuously and may survive for several weeks at 22°, often laying in excess of 1,000 eggs.

The time taken for eggs to hatch is very variable but averages 4 to 5 days at 22°. Juveniles soon begin feeding on fungus and, when this is young and uncontaminated by foreign organisms, take about 7 days to mature into mycetophagous adults at 22°. This cycle can continue indefinitely in the presence of young uncontaminated fungus and some cultures have been maintained for 4 years (approximately 100 generations) without intervention of an insect host.

When females become very old they no longer oviposit but retain up to 20 eggs within the oviduct. When the parent dies these hatch and larvae eventually rupture the parent cuticle to escape.

*Nematode behaviour within timber*

In the wood juvenile nematodes emerge from host eggs within a few hours of oviposition, but in living or very wet wood, little nematode migration occurs until the wood dries out below 50% moisture content (based on oven dry weight). Cotton blue lactophenol stained sections across *Sirex* oviposition holes show that juveniles grow into adults and lay rows of eggs in the tracheids within 1 to 2 cm of host oviposition (Fig. 3c) (Akhurst & Bedding, unpublished). After sufficient drying of the wood, nematodes can be found breeding wherever there is growing fungus, this being within tracheids, between wood and bark, within the host galleries where fungal growth is often particularly dense, and also within resin canals.

*Infertile female and associated male production*

In monoxenic culture and on young fungus all larvae hatching from eggs become free-living, mycetophagous, oviparous females (Fig. 3A) or males containing large amoeboid spermatozoa. However, cultures of *D. siricidicola* that become old and brown (usually after about 1 month at 22°) produce infective females (Fig. 3A) and males with very small (1 to 2 μm diameter) round spermatozoa consisting mainly of the nucleus. The spermatozoa from these males is quite unlike the large (10 to 12 μm diameter) amoeboid spermatozoa found in males associated with mycetophagous females.
Depending on environmental conditions, the same eggs may give rise to mycetophagous females and associated males or to infective females and associated males. Males containing small sperm never attempt to mate with mycetophagous females even when males with amoeboid sperm are absent, and the converse is also true. Fertilized infective females contain an estimated 15,000 spermatozoa. Repeated sub-culturing over several years in one strain of *D. siricidicola* has led to the cessation of production of infective females in plate cultures.

In the natural habitat it is thought that infectives are usually only produced in the immediate vicinity of the host larva. The rich fungal growth around the larva in its cavity provides enough food for several free-living generations of nematode but conditions must eventually approximate to those in old culture plates and infectives will be produced. Also several species of bacteria have been identified (Madden, pers. comm.) from *Sirex* frass and, as on some culture plates, these doubtless contribute to a tendency for infectives to be formed. Elsewhere in the wood conditions are relatively sterile.

**Entry of nematode into host larvae**

Only fertilized, adult, female infective nematodes can enter siricid larvae. They move over the outside of the larva often for several hours before attempting to penetrate. The infective usually adopts a position for entry with its head held at right angles to the larval surface, then the large tubular stylet begins to thrust in and out at 60 to 100 thrusts per minute. During attempted penetration, the head remains immobile while the stylet thrusts into the cuticle in a circular patch with diameter similar to that of the nematode.

No secretions, such as occur during penetration of *Tripinus* (Poinar & Doncaster, 1965), were observed and there is no apparent difference between the oesophageal glands of pre- and post-entry nematodes.

Penetration of the cuticle of very small larvae takes from 30 seconds to 6 minutes, and entry is completed after a period varying from 4 to 60 minutes.

In most instances the head and oesophageous enters rapidly, followed by a pause of a minute or more during exploratory movements inside the host and then the nematode penetrates 20 to 100 μ at a time followed by pauses of several seconds when the infective may back out a little before penetrating further. This process is repeated until the anterior half of the nematode has entered; then the posterior half of the body enters more rapidly, the minimum time recorded being 30 seconds. The host cuticle closes behind the infective after entry and often small quantities of blood exude from the wound for a few seconds afterwards. A characteristic melanized spot develops on the host surface within a few hours of entry or attempted entry (Fig. 3B). The spots which are light orange-brown to dark brown in colour, appear to be situated in the hypodermis, are roughly circular, and vary from 0.05 mm to 0.15 mm in diameter.
Entry occurs anywhere on the host body when larvae are exposed to nematode attack under artificial conditions. However from Figs. 2A, 2B and 2C, which are compiled from examination of twenty larvae after natural attack, it can be seen that the positions of entry are not then distributed at random, there being many more entries ventrally (448) and laterally (239) than there are dorsally (66). Most entries are at the edges of segments; also the posterior end of the host is preferred. A probable explanation for this conspicuous localization of attack is that other areas of the larva are continually pressed against the abrasive tunnel walls as the larva moves.

From one to over 100 infective nematodes may enter a single host but the number is usually between five and twenty.
Parasite development

The rate of infective development after entry is rather variable even within the same host larva. No development was observed in infectives 24 hours after entry, but 3 days after penetration at 22°C, development in 60 infectives, taken from ten larvae varied from a slight to a threefold increase in breadth; at this stage the length may be slightly decreased. From 3 to 10 days after entry separation of the cuticle from underlying tissue occurs but infectives grow considerably before the cuticle finally ruptures. It is doubtful whether this is a normal moult since the stylet is unaffected.

Early development of the infective was also observed in hanging drops of blood from *S. noctilio* larvae. Prior to moulting, blood is ingested through the stylet and passes into a functional gut (contrary to Bedding, 1968, who thought no functional gut was present). Much of the early swelling is attributed to a rapid growth of the hypodermal cells. After the “moult” within the host, microvilli develop on the outside of the nematode (Riding, 1970) allowing absorption through the general body surface and much more rapid growth.

The rapidity of growth of the parasitic nematode and its final size are partially dependant on the size of host and total numbers of nematodes within one host.

Development of the gonad in parasitic females is of particular interest, for although the nematode may reach full size (3-25 mm long) within a few weeks of entering, its reproductive system remains almost unchanged in size and development until the beginning of pupation of the host (which may occur up to 2 years after nematode entry). Changes that do occur after entry but before host pupation are a thickening of the oviduct walls and a slight increase in the number of oocytes present. Spermatozoa remain evenly distributed within the oviduct.

At the commencement of host pupation the nematode reproductive system is only about 1 mm long but (in most strains of *D. siricidicola* when the host is *S. noctilio*) very rapid growth of the ovary then proceeds so that 5 days after host pupation at 24°C it extends for about half the length and after 8 days at 24°C it is about three-quarters of the length of the nematode. Coincidentally with the beginning of ovariole development the body of the nematode frequently becomes distinctly beaded externally.

Within 2 or 3 days of host pupation the nematode oviduct becomes club shaped and spermatozoa are noticeably more numerous anteriorly; after 5 days all the spermatozoa are located in the spermatheca which lies between oviduct and ovary and by about the eighth day a few eggs have passed into the oviduct. The migration of spermatozoa into the spermatheca and development of the ovary follows the same pattern as described for *Contortylus elongatus* (Massey) by Nickle (1966) except that in *C. elongatus* the whole process occurs simultaneously with parent growth and within 2 to 3 weeks of nematode entry. In all of the many pupae of *S. noctilio* examined, nematodes from the same host have been at a very similar stage of development independent of their size or location within the host.
Eggs hatch after 3 to 4 days and during the early stages juveniles are probably released into the host haemocoel via the vulva. However towards the end of pupation when the nematode becomes packed with juveniles, the oviduct ruptures, juveniles penetrate and emerge through the general body surface and rapidly migrate from the host haemocoel to its reproductive organs.

**Effect on larval host**

Apart from scarring the larva (Fig. 3B) and, in heavily parasitized larvae, reducing fat body, developing parasitic nematodes appear to have little effect on the larval host. Unlike Zondag (1969), the author has observed no instances of heavy infection causing death even when more than 100 infectives have invaded very small larvae. However, *Sirex* larvae which have died or become moribund for other reasons are very attractive to all stages of *D. siricidicola* and many thousands of nematodes may be found on the outside of such larvae particularly along the intersegmental membranes. As they are often not apparent until dissection, the nematodes may appear to come from inside the larva and lead to the assumption that they caused larval mortality.

**Effect on adult host**

a. *Female hosts.* The time of release of juvenile nematodes in relation to the stage of development of the host has an important influence upon the effect of parasitism on the host. This is dependent on both strain of nematode and species or strain of host concerned.

In Australian *S. noctilio*, juvenile nematodes of most strains begin to enter the ovaries well before the end of pupation causing suppression of ovarian development and greatly reducing the number and size of eggs produced (Fig. 4D). All eggs usually contain juvenile nematodes (from 50 to 200) (Fig. 4C). Many thousands of juveniles are also found free in the ovary and oviducts. Very rarely eggs not containing juveniles are present but these are nevertheless small and abortive.

In *S. japonicus* and *S. cinnamomeus* most juvenile nematodes are not released into the host haemocoel until just before the host emerges from pupation at which stage the host’s ovaries are fully developed and the eggs fully grown; juvenile nematodes enter host eggs before the insect emerges from the wood and all eggs are infected before oviposition. Few juveniles are present free in the siricid oviducts and ovaries.

In *S. nitobei* parasitized by *D. siricidicola* from Japan, ovaries and eggs are fully developed and no eggs contain nematodes; all juvenile nematodes are located in the ovaries and oviducts and are transmitted with the eggs during oviposition. *D. siricidicola* from the same source behave similarly in Australian *S. noctilio* since juveniles are not released from the parent nematode until well after the host emerges from pupation. In this instance a difference in the strain of nematode is responsible for its differing effects. However, nematodes from Belgian *S. noctilio*
Fig. 3. A. Infective female (left) and free-living mycophagous female (right) of Deladenus stricticidicola ×125. B. Posterior end of a parasitized Sirex melillo larva showing stearing after natural attack by infective Deladenus stricticidicola ×10. C. Section of wood near Sirex oviposition shaft showing eggs of Deladenus stricticidicola within the trichids ×55.
Fig. 4. A. Ovipositing *Sirex mutillo* ×1. B. Adult and juvenile nematodes from a single host ×2.5. C. *Sirex* egg containing juvenile nematodes ×130. D. Ovaries from unparasitized (left) and parasitized (right) ×3. E. Testes from unparasitized (left) and parasitized (right) *Sirex* ×6.
do not enter the eggs of their natural host but do so when parasitizing Australian
*S. noctilio*, indicating that here it is the strain of host which is responsible for the
difference.

Some juvenile nematodes are frequently found in the ooidal glands of para-
sitized *Sirex* species and in all species fat body is noticeably reduced but this
appears to have little effect on host longevity.

In *X. spectrnum* ovarian development is not noticeably suppressed by parasitism;
eggs are of normal size and only 10-30% of the eggs contain nematodes. Most
juveniles are found within ovaries and oviducts. Juveniles are found free in the
haemocoel of the beetle *S. barbutus* and do not enter the reproductive organs.

b. *Male hosts.* In all parasitized male hosts, juveniles migrate from the haemocoel
to the testes which become filled with many thousands of juveniles during
pupation. (Fig. 4E). Contrary to Bedding (1967) and Zondag (1969), males are
not sterilized. In all of 50 parasitized males from each of Australian *S. noctilio*,
Belgian *S. cupido*, and *S. jawnus* the vesiculae seminales were packed with living
spermatocytes, although no spermatocytes were present in the testes. Production
of sperm in the testes of the host and its passage to the vesiculae seminales occurs
within the first few days of pupation before juvenile nematodes invade the testes.
The testes at this stage are very much larger than in the adult insect and are
usually fused. When filled with juvenile nematodes they remain large and fre-
quently fused throughout the life of the host (Fig. 4E). Infected males mate
readily and transmit viable spermatozoa, but no juvenile nematodes are found
within the vesiculae seminales and so cannot be transmitted during copulation.
Examination of 30 non-parasitized female *S. jawnus* mated with parasitized males
showed that no nematodes had been transmitted and Zondag (1969) found no
nematode transmission during copulation of *S. noctilio*. Entry into male insects
is thus a "dead end" for the nematodes since even if the insect dies within the
wood, juvenile nematodes die soon afterwards.

Frequently very small adult nematodes are found coiled within the host’s
testes. These have almost certainly entered the host just before or during pupation
and migrated into the developing testes soon after entry since larvae removed
from timber and left to pupate in tubes away from further nematode infection,
ever contain adult nematodes within their testes.

*Reproductive capacity of parasitic nematodes*

The number of juveniles produced within a single host (Fig. 4B) varies con-
siderably but is not directly proportional to the number of parent nematodes or
to host size. The numbers of adult and juvenile nematodes were counted in 50
male *S. jawnus*. The number of adults varied from 1 to 91 but the greatest number
of juvenile nematodes, 51,510, was found in a medium-sized host with only nine
parent nematodes. The average number of juveniles per single parent nematode
was 750, but where ten or less parents occurred in one host the average was about
2,000 compared with about 500 where there were more than ten parents per host.
DISCUSSION

The interrelationships between nematodes and insects are many and varied, ranging from facultative commensalism through phoresy and gut parasitism to obligate haemocoelic parasitism. Species of *Deladenus* exhibit one of the most extraordinary and interesting types of entohelminthological associations known but may well be typical of several other Neotylenchid genera whose biologies are presently undescribed.

*D. siricidicolus* can be considered as essentially parasitic but able to breed up in large numbers in the environment of the host thus facilitating infection of further hosts, or it can be regarded as primarily a fungal feeding nematode which is transported by an insect to a fresh environment having the added advantage that it also multiplies within the insect. The two cycles are bound together by the complete specificity of the mycetophagous nematode to the symbiotic fungus (*A. arvalatum*) of its host (Bedding, unpublished).

Since either cycle can continue indefinitely without intervention of the other it is possible that one of them could become completely suppressed leaving either a wholly Neotylenchid-like or a wholly Sphaerulariïd-like nematode. (In fact, continual subculturing of the mycetophagous phase of *D. siricidicolus* over several years has led in one strain to the cessation of production of infective forms in normal cultures). Thus *Deladenus* may represent a link between the Neotylenchidae and the Sphaerulariidae although it is possible that most Neotylenchid nematodes have their parasitic counterparts. Rühm (personal communication) has biological evidence that *Stictylus* (Neotylenchidae) and *Sphaerulariopsis* (Sphaerulariidae) are different forms of the same animal and Bedding (unpublished) has found a new genus of Neotylenchid with a type of life history similar to that of *Deladenus*. Many other Neotylenchid species are found in association with insects in their frass or galls, and it seems likely that at least some of these have sphaerulariid forms parasitic in insects.

The short life cycles or unfavourable or transient environments of most insects tend to preclude parasitism by nematodes having a bicyclic life history, but such a life history is ideally adapted to the biology of *Sirex* which takes a year or more to complete its development, and lives in an atmospheric humidity of 100%. In a year, 10 to 20 mycetophagous generations can occur to greatly increase the number of nematodes; the tree can dry out adequately, and the lamellae of the bordered pits can be broken down by the fungus thus permitting the nematodes to migrate to all parts of the tree, and making possible the very high levels of parasitism experienced.

Only because many *Sirex* (some of which may be unparasitized) usually oviposit on the same tree has the evolution of nematode parasites that enter all the host's eggs been possible. Perhaps those strains of *D. siricidicolus* (and other species of *Deladenus*) that do not enter the eggs have evolved in hosts that are normally more isolated.

The existence of female dimorphism (Bedding, 1968) correlated with the two
life cycles constitutes major adaptations of morphology to function. The most obvious of these adaptations is the dimorphism of stylets; the small hyperdermic-like stylets of the mycetophagous female, juveniles and males being suited to withdrawing fluid from delicate fungal hyphae and the much larger and more robust spear-like stylet of the infective female to penetrating the relatively tough cuticle of *Sirex* larvae.

*Deladenus* species appear to be unique in the animal kingdom in having dimorphic spermatozoa. Presumably the large amoeboid spermatozoa are more primitive, but perfectly adequate for the freeliving mycetophagous form, which, although only able to retain 200 to 500 amoeboid spermatozoa, can have these repeatedly replenished. On the other hand, the infective female must contain, prior to entering a host, enough spermatozoa to fertilize the much larger number of eggs she will produce and this has been achieved by reducing the spermatozoa to little more than the nucleus. Another unusual feature of the spermatozoa of *D. siricidicola* is its longevity; since ovarian development may be postponed for up to 2 years, spermatozoa must be maintained alive within the parasitic female for this length of time.

It is noteworthy that the reproductive system of parasitic females remains completely undeveloped and relatively unchanged until pupation of the host, even when the nematode has attained its full size many months previously, although this is obviously necessary to ensure synchrony with the host life cycle. Presumably hormonal changes or changes in haemolymph composition are responsible for initiating ovarian development. Variation in the effect of parasitism on the reproductive system of the female host has been shown to be correlated with the time at which parent nematodes commence reproduction. The suppression of ovarian and egg development which occurs when juveniles are released early in pupation may be due to greatly increased nutritional demands of reproducing adult nematodes or to the activities of the juveniles themselves or to a combination of the two.

Apart from being of great academic interest, the biology of *D. siricidicola* readily lends itself to manipulation for the biological control of *Sirex* (Bedding & Akhurst, unpublished). The mycetophagous cycle has been utilized for mass rearing this nematode and maintaining strains from many countries and already hundreds of millions of nematodes have been reared and distributed throughout many of the *Sirex* infested forests of Australia with encouraging early results.

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ZUSAMMENFASSUNG

Die Biologie von Deladenus siricidicola (Neotylenchidae), einem entomophag-mycetophagen, in Holzwespen (Siricidae) parasitierenden Nematoden


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