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# Endoparasitic Nematodes of *Ips* Bark Beetles<sup>1</sup> in Eastern Texas<sup>2</sup>

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## ABSTRACT

East Texas *Ips* species contained 4 specific internal nematodes; *I. avulsus* (Eichhoff) were infected with *Parasitylenchus avulsi* Massey, *I. grandicollis* (Eichhoff) with *Contortylenchus grandicollis* (Massey) Rühm, and *I. calligraphus* (Germar) with *Contortylenchus elongatus* (Massey) Nickle and *Parasitaphelenchus* sp. In all 3 bark beetles, infection peaked in July and August when 50–58% of adults from naturally attacked pine trees contained nematodes. Infection levels declined to 20–30% during January and February. Infected *I. grandicollis* and *I. avulsus* adults appeared lighter in color than noninfected adults.

Nematode infection apparently delayed emergence of both sexes of *I. grandicollis* and females of *I. avulsus*. In *I. grandicollis*, nematode infection did not affect ability to construct egg galleries or number of offspring produced. Supercooling temperatures for infected *I. grandicollis* and *I. calligraphus* were no different than those of noninfected beetles.

Nematodes are commonly associated with many species of bark beetles (Massey 1966, 1974) where they may cause reduced fecundity (Oldham 1930, Massey 1956, 1960, Nickle 1963, 1971, Ashraf and Berryman 1970), delayed emergence (Nickle 1963, Ashraf and Berryman 1970), reduced flight ability (Atkins 1961) and decreased adult longevity (Ashraf and Berryman 1970). Reviews of the effects of nematode parasitism on insects have been written by Welch (1963) and Poinar (1972). Massey (1974) has recently reviewed nematode parasitism in bark beetles.

Three species of *Ips* bark beetles are found in southern pine forests of the U.S.: *Ips avulsus* (Eichhoff), *I. calligraphus* (Germar), and *I. grandicollis* (Eichhoff). Work was undertaken to determine the species of nematodes found internally in the adults of the 3 southern *Ips* and to make preliminary assessments of the effects of the nematodes on their hosts. Nematodes found beneath the elytra of the beetles were not studied since these are thought to be only phoretics (Massey 1974).

## Methods

### Collection of Nematodes

Samples of *Ips* beetle and nematode populations were collected from loblolly pine (*Pinus taeda* L.) and shortleaf pine (*P. echinata* Miller) logging debris in Nacogdoches and Angelina counties, TX, at approximately monthly intervals during 1972 and 1973. Internal nematodes in 30 live specimens of each *Ips* species collected in March 1972 were identified by Calvin L. Massey (U.S. Forest Service, Rocky Mountain Forest and Range Experiment Sta-

tion, Albuquerque, NM) and W. R. Nickle (USDA Crops Research Division, Beltsville, MD). During subsequent phases of the study, internal nemas were classified by comparison with those identified by Massey and Nickle.

### Dissection and Slide Preparation Techniques

To determine the presence or absence of internal nematodes, *Ips* beetles were dissected after being pinned, ventral side up, through the center of the thorax and covered with 0.7% physiological saline solution. Under a dissecting microscope, the pygidium and 5th sternite were grasped with forceps and gently pulled dorsally. Most of the midgut, hindgut, Malpighian tubules, and reproductive system were removed with this procedure. If adult or larval nematodes were present, they floated out in solution. The sex of the beetles was determined at the same time from the reproductive organs.

A search for possible damage to epithelial tissues and any other pathologies was made on microscope slides of longitudinal and cross sections of beetles. Slides were prepared by fixing abdominal portions of adults, which contained part of the midgut and all of the hindgut, in Bouin's solution, followed by dehydration in alcohol, infiltration with paraffin, and embedding for microtoming. Fifteen-micron sections were mounted on slides and stained in Delafield's hematoxylin and Eosin B.

### Laboratory Rearing of Bark Beetles

To determine the effects of nematode infections on brood production and development, pine bolts (each ca. 20 cm diam × 46 cm long) were infested with beetles in the following manner. "Starter holes," 0.16 cm diam, were drilled through the outer bark to the pith of each bolt. A recently emerged ♂ beetle was confined in each hole by pinning a size 0 gelatin capsule over the opening. Five h later the capsules were removed, ♀ beetles were introduced into the holes, and the capsule replaced. When boring dust and frass appeared, the capsules were finally

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removed and each bolt was placed in an individual screened rearing cage.

In the brood production experiments, 30 bolts were infested with *I. avulsus* and a like number with *I. grandicollis*. Each bolt contained only 1 ♂ and 1 ♀ of the respective species. Parent adults, whose emergence preceded brood adult emergence by about 20 days, were collected as they emerged from the bolts and examined for nematodes.

In the brood development tests, 1 bolt was infested with 60 ♀ and 30 ♂ of *I. avulsus* and 1 bolt with like numbers of *I. grandicollis*. Adult beetles were collected daily and examined for nematodes.

#### *Effect of Nematodes on Ability of Bark Beetles to Supercool*

Supercooling, the phenomenon wherein the body water of animals remains unfrozen at temperatures less than 0°C (Salt 1961), is a factor determining a species' cold-hardiness. Supercooling may be prevented or reduced by the presence of nuclearites, such as dust and food particles, that initiate ice crystal formation. Internal parasites may function as nuclearites (Salt 1961).

Tests on *I. grandicollis* and *I. calligraphus* were used to determine the possible effects of nematode infection on supercooling of the beetles. After the insects were acclimated for 24 h at 10°C, a microprobe from a thermocouple thermometer (Baily Instruments type MT-2) was inserted directly between the abdominal sternites and sealed with paraffin heated just to the melting point. The beetles were placed between 2 pieces (2.5 cm × 5 cm × 3.1 cm) of styrofoam plastic and held by rubber bands. This assembly was then cooled in a temperature of -20°C, and the insect's body temperature was monitored as it declined to the supercooling point, i.e., the point at which the insect's decreasing body temperature rebounds due to the heat of crystallization liberated by ice formation. After each observation the beetle was dissected to determine if it was infected. Data for *I. grandicollis* were recorded by sex.

### Results and Discussion

#### *Identification of Nematodes*

Four species of nematodes representing 3 genera were recovered from the beetles:

Bark Beetle	Nematode
<i>Ips avulsus</i>	<i>Parasitylenchus avulsi</i> (Massey 1958)
<i>Ips grandicollis</i>	<i>Contortylenchus grandicollis</i> (Massey 1957) Rühm 1960
<i>Ips calligraphus</i>	<i>Contortylenchus elongatus</i> (Massey 1960) Nickle 1963
<i>Ips calligraphus</i>	<i>Parasitaphelenchus</i> sp.

*Contortylenchus elongatus* has not been previously reported as an associate of *I. calligraphus* (Massey 1974).

The 4 nematodes differed markedly in appearance. *P. avulsi* is a robust, donut-shaped nema. The cuti-

cle of the young adult female was initially quite resistant to punctures but became more delicate as the eggs hatched and the dauerlarvae began to develop in the nematode. The dauerlarvae eventually became so numerous that the internal organs of the parent female were obliterated.

*Contortylenchus grandicollis*, *C. elongatus*, and *Parasitaphelenchus* sp. are long, slender worms, with a delicate cuticle in all adult stages. Adults of all 3 species were usually motionless, yellow-brown, and often coiled about the alimentary canal. In these 3 species of nematodes, the eggs hatched in the hemocoel of the host beetle.

The larvae of all 4 species were very active and colorless, and found either free in the beetle's hemocoel, in the intestine, or both (depending on the state of development). Larvae may number hundreds per beetle.

The number of adult nematodes in infected beetles ranged from 1-10. Means and ranges for *I. avulsus* and *I. grandicollis* are:

Species	Sex	Number of nematodes	
		Average	Range
<i>I. avulsus</i>	♂	2.68	1- 7
	♀	3.20	1-10
<i>I. grandicollis</i>	♂	2.24	1- 7
	♀	2.50	1- 9

Means did not differ significantly by sex ( $P < 0.05$ ) in either host species. Numbers of *I. calligraphus* available were insufficient to afford comparable data for that species.

#### *Level of Nematode Infection*

Monthly sampling of the 3 *Ips* spp. was at times limited by the populations available. Data were available, however, for *I. avulsus* throughout the year, *I. grandicollis* for February through November, and for *I. calligraphus* for 9 mo, excluding January, August and December.

The mean levels of infection and the ranges for each beetle species were 38.5% (16.7-50.0) for *I. avulsus*, 40.0% (20.8-58.3) for *I. grandicollis*, and 44.0% (29.2-54.2) for *I. calligraphus*. There appeared to be a consistent trend toward higher infection levels during summer and fall months (Fig. 1).

Massey (1974) summarized levels of nematode infection for a large number of bark beetles and nematodes. From 13.3-16.7% of the *I. grandicollis* were infected with *C. grandicollis* while 20-22.7% of the *I. avulsus* were infected with *P. avulsi*. The magnitude of nematode infection in a bark beetle species varies widely from year to year and in different areas of the bark beetles' range (Massey 1974). Likewise, the level of infection is dependent on density of the host population (Saunders and Norris 1961) and relative maturity of the adults (Furniss 1967).

#### *Effect on Anatomy*

Examinations of longitudinal cross-sections of *I. grandicollis* and *I. calligraphus* showed no damage to the ventricular epithelial layer as reported in *I.*

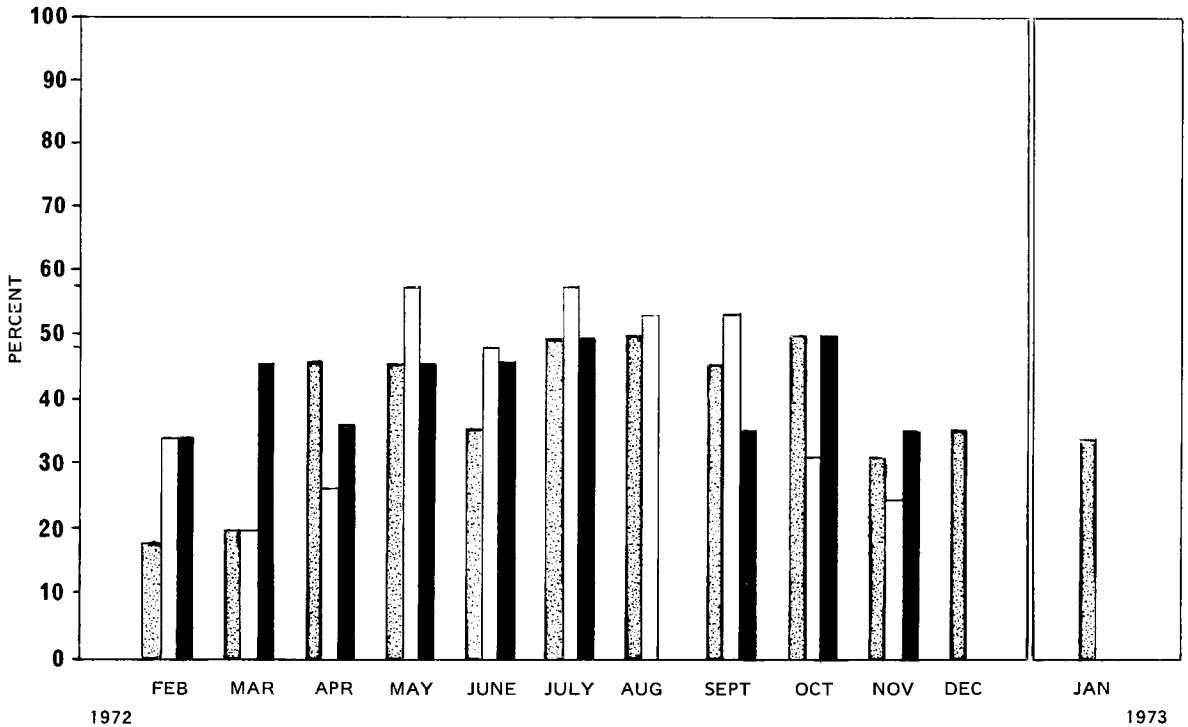


FIG. 1.—Percent of *Ips* beetles infected with nematodes, by months. *I. avulsus* (stippled bars), *I. grandicollis* (white bars), and *I. calligraphus* (black bars).

*paraconfusus* (Leconte) by Nickle (1963). *I. grandicollis* in the present study and *I. paraconfusus* in Nickle's study were both infected with *C. elongatus*.

Hundreds of observations of *I. grandicollis* and *I. avulsus* revealed no differences in size, shape, color, or texture of the ovaries, testes, or antennae between infected and noninfected beetles. Emerged infected brood adults did, however, appear generally lighter in color than noninfected ones. Whether infection causes a thinner exoskeleton or delays its hardening-off in callow beetles as they mature was not determined. A similar effect on exoskeleton coloration in *I. calligraphus* was shown to be due to its nutrition (Richeson, Wilkinson, and Nation 1970).

Reduction in the size of reproductive organs due to nematodes has been reported for *Scolytus scolytus* F. and *S. multistriatus* (Marsham) (Oldham 1930), for *S. ventralis* Leconte (Ashraf and Berryman 1970), and for *I. paraconfusus* (Nickle 1963). Lack of nematode effect on reproductive organs has, however, been reported for *S. multistriatus* (Saunders and Norris 1961), *Dendroctonus brevicomis* Leconte (Nickle unpubl.<sup>4</sup>), as well as for several European bark beetles (Rühm 1956). In other cases (Oldham 1930, Massey 1956, 1966), beetles have been completely sterilized. Apparently, there is variation

in the different parasite-host systems that may not permit generalizations as to the effects of nematodes on bark beetle reproductive organs.

#### Effects on Fecundity

Limited data were gathered in the fecundity study. Nearly all *I. avulsus* aborted egg gallery construction after only a few millimeters. Dissection of the aborting beetles showed about equal numbers infected and noninfected; since this level of infection (ca. 50%) approximates that for summer months (Fig. 1), there is no indication that infection increased the rate of abortion. Six of the 30 *I. grandicollis* aborted, 4 of which were infected. Thus, while 66% of these aborting beetles were infected, the frequency of infection is only slightly higher than that of the summer population of this species (48–58%), and does not suggest that infection affected abortion.

The data for *I. grandicollis* show that infected and noninfected beetles did not differ significantly in gallery length ( $t_{.05} = 1.43$ , 22 df) or number of larval mines ( $t_{.05} = 1.06$ , 22 df):

	Beetle condition	
	Infected	Noninfected
Egg gallery length, cm/bolt	48.16	47.26
Larval galleries, number/bolt	18.00	18.58

Indeed, the number of larvae was highly consistent, averaging about 1 larval mine/cm of egg gallery length.

<sup>4</sup> W. R. Nickle. 1962. The endoparasitic nematodes of California bark beetles, with descriptions of *Bovienema* n.g. and *Neoparasitylenchus* n. subg., and with the presentation of new information on the life history of *Contortylenchus elongatus* n. comb. Ph.D. thesis, Univ. of California, Berkeley. 210 p.

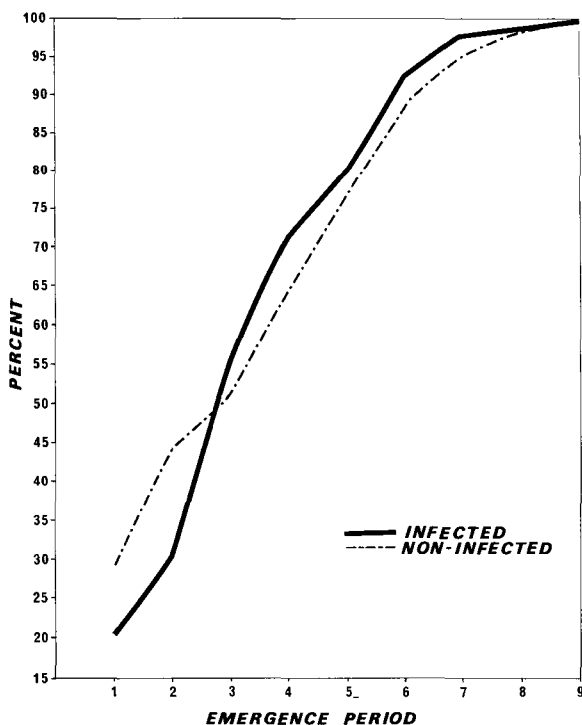


FIG. 2.—Cumulative percentages of 410 infected and 378 noninfected, emergent *Ips grandicollis* brood adults. (1 emergence period = 3 days).

The absence of observed nematode-produced differences in number of brood produced was also reported for *S. multistriatus* infected by *P. oldhami* (Rühm 1956, Saunders and Norris 1961) and *I. paraconfusus* infected by *Parasitaphelenchus* sp. (Massey 1960). Brood was, however, reduced in *I. paraconfusus* infected by *C. elongatus* (Massey 1960) and *S. ventralis* infected by *Parasitylenchus elongatus* (Massey 1958, Ashraf and Berryman 1970).

#### Effect on Brood Development

A total of 788 *I. grandicollis* and 388 *I. avulsus*  $F_1$  adults emerged from the pine bolts. Emergence of *I. grandicollis* began 40 days after introduction of the parent adults, lasted for 27 days, and 52% of the brood were infected with nematodes. Emergence of *I. avulsus* began 35 days after introduction of the parents and lasted for 21 days. Thirty-eight percent of the brood were infected. Males and females of both species were about equally infected.

The cumulative emergence data for infected and noninfected adults of each species were grouped by 3-day periods (Fig. 2 & 3). For *I. grandicollis* (Fig. 2), a greater proportion of noninfected than of infected beetles emerged during the 1st 2 emergence periods (days 0–6). Subsequently, slightly greater proportions of infected beetles had emerged by the end of each period. Among *I. avulsus* (Fig. 3), greater proportions of noninfected than of infected adults had emerged at the end of each successive

period. Emergence of noninfected adults was complete after 15 days; infected adults continued to emerge in the 7th period. Similar delays of emergence have been reported by Ashraf and Berryman (1970).

Examination of the cumulative emergence data by sex showed that for *I. grandicollis*, infected males and females both emerged later than noninfected individuals ( $\chi^2$  males = 19.6,  $\chi^2$  females = 37.8, 8 df). With *I. avulsus*, however, only infected females emerged significantly later ( $\chi^2 = 12.8$ , 6 df).

#### Effects on Ability to Supercool

As only 25 *I. calligraphus* were available for supercooling experiments, sex differences in this species were not tested. Fifty *I. grandicollis* were tested. Results are shown in Table 1. Supercooling temperatures of infected beetles did not differ significantly from those of uninfected ones in either *I. grandicollis* or *I. calligraphus*.

#### Conclusions

Infection of the 3 species of southern *Ips* by nematodes did not produce definite pathologies in the insects. No consistent differences in gonads or antennae were observed, and there was no apparent damage to the ventricular layer as reported by Nickle (1963) for *C. elongatus*. For *I. grandicollis*, neither

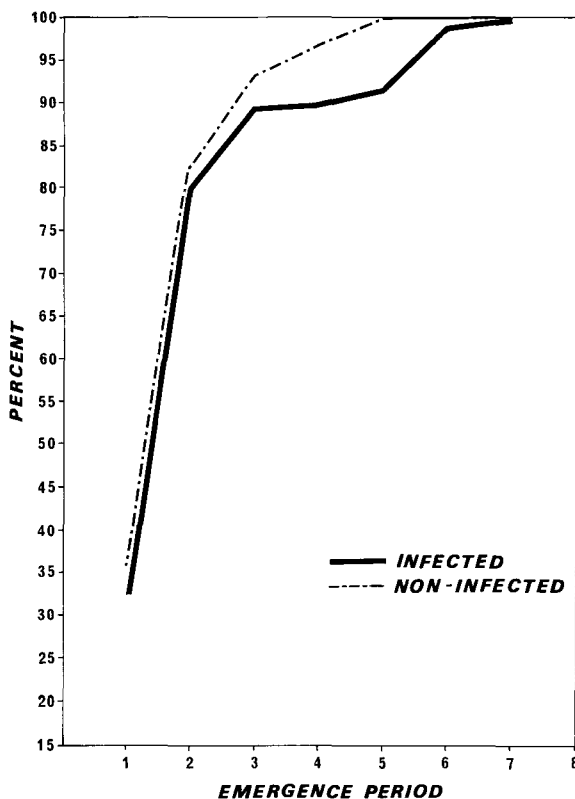


FIG. 3.—Cumulative percentages of 147 infected and 241 noninfected emergent *I. avulsus* brood adults. (1 emergence period = 3 days).

**Table 1.**—Mean supercooling temperature for nematode infected and non-infected *Ips grandicollis* and *I. calligraphus* exposed to  $-20^{\circ}\text{C}$ .

	Sex	Beetle condition	
		Infected	Noninfected
		°C	
<i>I. grandicollis</i>	♂	-12.58	-12.20
	♀	-11.41	-12.00
	Both	-12.00	-12.07
<i>I. calligraphus</i>	Both	-10.33	-12.10

gallery length nor number of larval mines were affected by nematode infection of parent adults; data for *I. avulsus* were insufficient for analysis. The length of the developmental period to adult emergence was, however, delayed. This effect could be important in prolonging the period to which beetles are exposed to other adverse biotic agents.

The effects of nematodes on bark beetles, as reported in the literature, is variable. Long-term studies relating nematode infection to population dynamics are needed for each bark beetle-nematode association to establish the role of nematodes in regulation of bark beetle populations.

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