

# Effect of the Nematode *Contortylenchus brevicomi* on Gallery Construction and Fecundity of the Southern Pine Beetle<sup>1</sup>

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**Abstract:** Field-collected *Dendroctonus frontalis* were reared in a controlled environment. Male-female beetle pairs retrieved from galleries 1, 2, or 3 wk after introduction into pine bolts were examined for nematode parasites. Data were obtained for each pair on gallery length, egg niche construction, egg viability, and progeny survival. In a separate study, beetle pairs were reared under laboratory conditions for 10 wk. The number of emerged adult progeny of each pair was recorded. *Contortylenchus brevicomi*, a nematode parasite, was found in 25% of all beetles that established galleries. After 2 and 3 wk, female beetles infected with the nematode had produced fewer eggs and shorter galleries than did uninfected females. Uninfected females mated with nematode-infected males showed similar trends, although the differences in the 2- and 3-wk tests were not significant. Progeny survival or egg viability was not affected by nematode parasitism of either parent beetle. *Unikaryon minutum*, a microsporidian parasite found in 65% of all colonizing beetles, had no effect on measured variables. The lower fecundity of beetles parasitized by *C. brevicomi* continued throughout the insect's reproductive cycle. After 10 wk, nematode-infected beetle pairs produced fewer emerged adult progeny than did uninfected pairs.

**Key Words:** *Dendroctonus frontalis*, population dynamics, nematode-insect interaction.

The nematode *Contortylenchus brevicomi* (Massey) Rühm parasitizes *Dendroctonus frontalis* Zimmerman, the southern pine beetle, in 11 southern states (6,9, unpublished data). Despite the widespread occurrence of the *C. brevicomi*-*D. frontalis* interaction, the effect of nematode infection on the distribution and abundance of southern pine beetle populations is not known.

*C. brevicomi* females parasitize all stages of the southern pine beetle but do not commence oviposition until the beetle host reaches maturity. *C. brevicomi* resembles *Contortylenchus elongatus* (Massey) Nickle in life cycle (7). According to Nickle (10), many adult female nematodes and several thousand eggs and larvae may be present in the hemocoel of an infected host. The

nematode progeny exit the host as fourth-stage larvae. Adult males and preparasitic adult females are found within beetle galleries. Males are short-lived and die soon after mating; impregnated females enter immature stages of the beetle.

Nematode parasitism affects the flight ability (1,3), gallery construction (12), emergence (1,11), and longevity (1,12) of some bark beetles. Reduced fecundity as a result of nematode parasitism has been observed also in several species of bark beetles (1,11,12,14).

The purpose of this study was to determine the effect of *C. brevicomi* infection of *D. frontalis* on the gallery construction, egg production, egg viability, and progeny survival of beetles reared for 1, 2, or 3 wk under laboratory conditions. Also investigated was the effect of nematode parasitism on the number of progeny produced in one generation by the southern pine beetle.

## MATERIALS AND METHODS

Beetles used in these tests emerged from one loblolly pine (*Pinus taeda*) tree brought

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to Gainesville, Florida, from the Homochito National Forest, Bude, Mississippi. Five groups of loblolly pine were felled on five different dates (August-September) to provide beetle rearing habitats for the experiment. Bolts cut from each tree group were designated as group I, II, III, IV, and V bolts. Each bolt, about 20 cm long and 19 cm in diameter, was dried for 2 to 4 d in a 37 C room before beetle introduction. One female beetle was placed in each of two gelatin capsules positioned over predrilled entrance holes 180° apart on each bolt. One male was added to every gelatin capsule 24 h after the female entered the bolt and began gallery construction. Bolts were placed in individual emergence cages and held in a room at about 25 C with a 16-h photoperiod. After 1, 2, or 3 wk the bark was stripped from each bolt and the lengths of beetle egg galleries were measured. Parent beetles were removed from the galleries or emergence cages and dissected for internal parasites. Egg niches, empty egg niches, larval galleries, eggs, and larvae were counted in each gallery. The number of egg niches in a gallery was used as an estimate of the number of eggs produced by the female beetle inhabiting that gallery (fecundity). The number of eggs that hatched was estimated by counting the number of egg niches that were connected to a larval gallery. An egg niche was considered empty if it was neither connected to a larval gallery nor occupied by an egg.

Preliminary studies showed that 65% of the Bude southern pine beetle population was infected with a microsporidian parasite *Unikaryon minutum* Knell and Allen. To determine whether microsporidian infection altered the egg production of nematode-infected and uninfected females, observations from all beetle pairs were classified according to microsporidian and nematode infection in female beetles. Because microsporidian parasitism did not significantly affect the fecundity of beetles in the preliminary studies we conducted, observations from all pairs were reclassified considering only nematode parasitism in male and female beetles.

Beetles were reared for 10 wk by the same methods as in the 1-, 2-, and 3-wk study except that only one male-female beetle pair

was introduced into each pine bolt. Progeny of each pair were collected as they emerged. Bark was stripped from all bolts, and the galleries were measured when no beetles had emerged for 1 wk.

## RESULTS

Fifty-three percent (182/348) of the beetle pairs placed in bolts for 1, 2, or 3 wk began gallery construction. There was considerable variation in the mean gallery length and number of egg niches constructed between like-infected (or uninfected) beetles reared on bolts cut from different tree groups during the study. Data are presented herein only from beetles reared on group I bolts. All trends and significant differences observed from beetles reared in these bolts were similar to the results from beetles reared on all other bolt groups. Microsporidian infection did not affect gallery or egg niche construction by females infected or uninfected with the nematode.

After 2 and 3 wk, galleries of pairs in which the female was infected with the nematode were respectively 34 and 27% shorter and contained 46 and 41% fewer egg niches than did galleries of pairs with uninfected males and females (Table 1). Reduced gallery and egg niche construction in pairs with nematode-infected males were indicated in beetles reared for 2 and 3 wk. Although there were no data on nematode-infected females reared for 1 wk in group I bolts, there were no differences in measured variables between pairs with nematode-infected and uninfected females reared for 1 wk in all bolt groups.

Four bolts assigned to the 3-wk rearing schedule each contained one nematode-infected and one uninfected female. Table 2 shows that with beetles in a relatively homogeneous environment, the effect of nematode parasitism on the fecundity of females is more striking than with beetles reared on many different bolts.

*C. brevicomi* exit both the male and female host within the galleries and infect immature stages of the beetle. Therefore, data on egg hatch and larval survival were combined for pairs with the male or female infected (Table 3). Fewer ( $P = 0.01$ ) eggs of nematode-infected beetle pairs hatched after 2 and 3 wk than did eggs of uninfected

Table 1. Mean gallery length and number of egg niches constructed by male-female pairs of *Dendroctonus frontalis* reared in group I pine bolts for 1, 2, or 3 wk and either infected or uninfected with *Contortylenchus brevicomi*.

Nematode infection status	1 wk		2 wk		3 wk	
	GL*	EN†	GL	EN	GL	EN
Male and female uninfected	21.6 ± 1.4 a (n = 8)	49.5 ± 3.4 a	48.9 ± 5.4 a (n = 11)	116.0 ± 13.7 a	56.6 ± 5.34 a (n = 8)	145.8 ± 11.5 a
Male only infected	19.5 ± 3.9 a (n = 4)	46.3 ± 15.2 a	38.8 ± 12.0 ab (n = 3)	82.7 ± 25.2 ab	51.5 ± 15.5 a (n = 2)	117.0 ± 21.0 ab
Female only infected			32.2 ± 7.6 b (n = 6)	62.2 ± 16.7 b	41.1 ± 3.5 a (n = 5)	85.8 ± 9.5 b

Numbers within a column followed by the same letter are not significantly different ( $P = 0.05$ ) by Duncan's multiple range test.

\*Mean gallery length (cm) ± standard error. n = number of beetle pairs.

†Mean number of egg niches ± standard error.

Table 2. Comparison between male-female pairs of *Dendroctonus frontalis* uninfected with *Contortylenchus brevicomi* and pairs with the female only infected with *Contortylenchus brevicomi* reared in the same pine bolt for 3 wk.\*

Uninfected pair		Pair with infected female	
Gallery length (cm)	No. egg niches	Gallery length (cm)	No. egg niches
70.0	204.0	41.0	68.0
83.0	167.0	30.5	70.0
62.0	171.0	44.5	58.0
63.0	155.0	37.5	63.0
Av. 69.5	174.3	38.4†	64.8†

\*Observations in the same row are from two beetle pairs reared within one pine bolt.

†Mean values significantly different ( $P = 0.01$ ).

pairs. However, there was no significant difference in percentage of egg hatch between nematode-infected and uninfected pairs after 1, 2, or 3 wk. Similarly, fewer ( $P = 0.01$ ) larval progeny of nematode-infected pairs were alive after 2 and 3 wk than were progeny of uninfected beetles. When the number of surviving larvae was considered in terms of the number of eggs hatched, however, no difference was found between nematode-infected and uninfected pairs.

Some established parent beetles died within galleries or emergence cages before the study was completed. Chi-square tests show that the probability of dying was no greater for nematode-infected than for uninfected beetles ( $\chi^2 = 0.553, 0.066, \text{ and } 0.104$  for weeks 1, 2, and 3, respectively).

Table 3. Viability of eggs and larval progeny of *Contortylenchus brevicomi* infected (male or female) and uninfected pairs of *Dendroctonus frontalis*.

Rearing schedule and nematode infection	Av. egg hatch		Av. larvae surviving	
	No.	%	No.	%
<b>1 wk</b>				
Uninfected (n = 8)	14.6	30.0	10.1	70.0
Infected (n = 5)	14.4	26.0	11.0	61.0
<b>2 wk</b>				
Uninfected (n = 11)	80.9	72.0	45.6	52.0
Infected (n = 10)	45.3*	58.0	23.5*	46.0
<b>3 wk</b>				
Uninfected (n = 8)	127.8	88.0	56.9	44.0
Infected (n = 10)	73.3*	82.0	28.9*	39.0

\*Mean values of nematode-infected pairs significantly ( $P = 0.01$ ) different from uninfected pairs.

Fourteen of eighteen (78%) beetle pairs reared for 10 wk mated and constructed galleries. Because parent beetles were not recovered from all bolts, nematode infection of a beetle pair was inferred by parasitism of emerged progeny of that pair (there was a positive correlation between nematode infection in parents and progeny when parents were recovered). The mean gallery length was 30.3 cm for nematode-infected pairs and 43.4 cm for uninfected pairs. Nematode-infected pairs ( $n = 6$ ) produced an average of 74% fewer ( $P = 0.05$ ) emerged progeny than did uninfected pairs (20.6 vs. 5.7).

### DISCUSSION

The effect of *C. brevicomi* parasitism on the fecundity of the southern pine beetle is most evident when female beetles are infected. Females infected with the nematode construct shorter galleries and lay fewer eggs than do uninfected females. Disparity in the reproductive performance of nematode-infected and uninfected females is evident at least 2 wk after females initiate attack on host trees and continues throughout the beetle's reproductive cycle. *Contortylenchus reversus* (Massey) has a similar effect on its host *Dendroctonus pseudotsugae* (14). Lower egg production by nematode-infected females may be due to depletion of hemolymph protein by developing nematodes (13), suppression of oocyte development by nematode toxins (2), or may result from nematodes feeding on gonadal tissue (5).

Data from this study suggest that nematode infection also affects the reproductive system of *D. frontalis* males. Fecundity of uninfected females mated with nematode-infected males was reduced after 2 and 3 wk. Nematode parasitism may cause partial sterility in male beetles (8) or may possibly affect the vigor of males, resulting in reduced gallery construction. Lower *C. brevicomi* incidence in trapped *D. frontalis* males than in reared beetles suggests that nematode infection affects male behavior (4).

Nematode parasitism did not affect egg viability (egg hatch) or survival of adult beetles and their progeny in our study. It seems then that the primary impact of the nematode on southern pine beetles reared in the laboratory is to reduce the egg production of infected beetle pairs.

Microsporidian infection did not influence the reproductive performance of *D. frontalis* in our studies. It is possible, however, that microsporidian parasites do have some effect on the physiology and behavior of the southern pine beetle under field conditions.

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