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Observations of Molting and Population Development by *Orrina phyllobia*

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In an earlier report (4), we referred to the juveniles of *Orrina phyllobia* (Thorne) Brzeski that survive desiccation within foliar galls of *Solanum elaeagnifolium* Cav. as fourth stage or J4. The only other documentation of molting in *O. phyllobia* is Bird's description (1) of the first molt within the egg. The following information is presented to confirm the occurrence of three additional molts in *O. phyllobia* and the predominance of the J4 stage among survivors of gall desiccation. In vitro development by *O. phyllobia* has not been observed, and my conclusions are based on morphometric continuities within populations extracted from plant tissue.

Orrina phyllobia were extracted from plant tissue by three methods designed to test separate hypotheses concerning molting. The first method determined whether molts could be distinguished by nematode size differences among age-asynchronous populations. Three leaves of *S. elaeagnifolium,* each ca. 2 g fresh weight and extensively galled by nematode infection, were collected from a naturally occurring plant population in the lower Rio Grande valley. Leaves were macerated individually with water in a blender for 15 seconds, and each macerate was examined microscopically. One thousand nematodes from each leaf were photographed at random (30 × final print magnification). Also from each leaf, 100 nematodes observed to be molting or to have molted but not undergone ecdysis were photographed at 240 ×. Ten adult males and ten adult females were photographed at 240 ×.

The second method provided a basis to conclude which fraction of a nondesiccate population can survive desiccation. Three galled leaves, each ca. 2 g fresh weight and collected at the same time and location as the first three leaves, were allowed to dry to ca. 10% moisture in a greenhouse and then were broken into small pieces and

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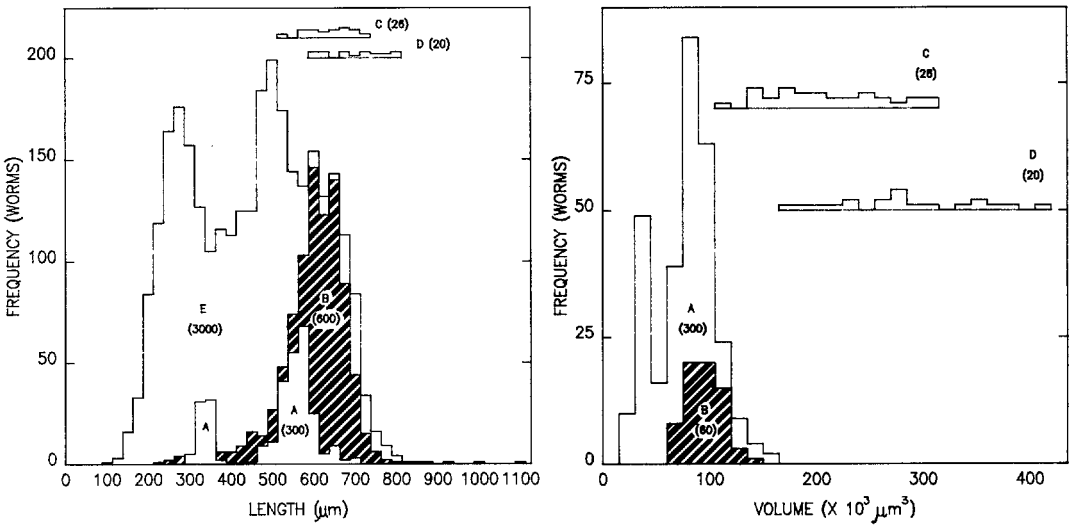

FIG. 1. Frequency distributions for lengths and volumes of *Orrina phyllobia* from *Solanum elaeagnifolium* leaf galls. Distributions are given for molting nematodes from mature leaves (A), juveniles that survived desiccation (B), molting nematodes from newly inoculated rhizome shoots (C), adults (D), and all nematodes from mature leaves (E). The number of individuals in each distribution is given in parentheses. Baselines for distributions C and D are elevated to simplify data presentation; otherwise they are to scale.

placed individually in continuously aerated water at ca. 23 C. After 6 hours, most of the leaf material was removed with a 710-μm-pore sieve. The remaining suspensions were concentrated on a 23-μm-pore sieve and put on aerated Baermann funnels using medium speed filter paper as the separator. After 12 hours, several thousand nematodes (> 95% motile) were recovered from each funnel and examined microscopically. From each leaf, 200 nematodes were photographed at 30 × and 20 nematodes were photographed at 240 ×.

The third method for obtaining nematodes from plant tissue determined whether juveniles that infect a new host molt before reaching reproductive maturity. Four 15-cm-d pots were filled 9 cm deep with soil. Five or six segments of *S. elaeagnifolium* rhizomes (5–10 cm long) were placed horizontally on the soil surface in each pot and inoculated with 3 g of dried nematode-infected foliar material obtained as described above. Rhizome segments were covered with 4 cm of additional soil. Pots were watered from below and held at 20–30 C for 8 days. Two or more shoots that sprouted from buds were dissected at 2, 3, 4, 5, 6, and 8 days after planting and inoculating the rhizomes. Twenty-eight nematodes that were molting or undergoing ecdysis were photographed at 400 ×.

A Zeiss MOP 30® digital image analyzer was used to measure the lengths of all nematodes and the sagittal image areas of nematodes photographed at 240 × and 400 ×. Volumes of nematodes photographed at 240 × and 400 × were calculated by the average radius method described previously (3). For each gall, nematode length and volume measurements were partitioned into frequency classes, based on a length interval of 25 μm and a volume interval of 15 × 10^3 μm^3.

Frequency distributions of lengths and volumes of nematodes from galls processed by the same method were similar to each other and herein are presented as their combined distribution (Fig. 1). Based both on length and volume measurements, nematodes from nondesiccate galls belonged to two large groups separated by a distinct molt at a length of 300–350 μm. A second group of molting nematodes (500–600 μm long) occurred in the middle of the second peak of the overall length distribution, indicating that the second large group of nematodes represented, in addition to J3s, nematodes that necessarily would be classified as J4s and/or adults. Length and volume measurements of

nematodes from desiccated galls placed them on the larger side of the second posthatch molt, indicating they are either J4s or sexually undifferentiated adults.

No molting nematodes from mature leaf galls were observed to be differentiating sexually, and no nematodes from those galls appeared to be differentiated intermediately between J4s and adults. Molting nematodes were not observed until the fourth day after inoculation of shoots. Molting nematodes comprised less than 10% of the populations extracted, the remainder being morphologically indistinguishable from infective juveniles. On days 5, 6, and 8, many molting nematodes were observed and in every case the prodelphic gonad had nearly reached or overlapped the esophagus prior to ecdysis. The volumes of molting nematodes from freshly inoculated shoots, unlike those of molting nematodes from mature galled leaves, were intermediate between the volumes of J4s and adults (Fig. 1). I concluded that infective nematodes are J4 and exactly one molt occurs between the J4 and the adult. This situation is different from that in *Nothanguina cecidoplastes* (Goodey) Whitehead, the only other species of the same genus in which Thorne previously placed *O. phyllobia* (5), wherein the infective juvenile is the J3 and molts twice within plant tissue before reaching adulthood (2). The absence or infrequency of fourth molts among 300 molting nematodes from three large galls, each of which contained at least 10^5 nematodes, suggests developmental arrest, where J4s, which are uniquely capable of desiccation survival, fail to molt into adults when the galled plant tissue in which they live approaches maturity. A more detailed examination of development in *O. phyllobia* will be required to test this hypothesis adequately.

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