EFFECT OF INFECTION DENSITY ON SEX RATIO
OF HETERODERA GLYCINES

BY

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Approximately equal numbers of males and females of Heterodera glycines Ichinohe developed
in roots of soybean seedlings at low infection densities. High infection densities significantly
increased the male to female ratio, first in the secondary and tertiary roots and then in the primary
root. Infection density of secondary roots influenced nematode sex ratio in tertiary roots. Increased
male to female ratios were attributed to differential death-rate of male and female larvae under
conditions of food stress created by crowding.

Unbalanced sex ratios, often observed in populations of Heterodera spp., have
been explained in two different ways. Ellenby (1954) became convinced that,
under crowded conditions, female larvae of H. rostochiensis change course of
sexual differentiation and become adult males. He regarded this, and not differen-
tial death-rate of larvae of the two sexes, as the cause of increased male to female
ratios in plants with heavy infection. Den Ouden (1960) indirectly supported
this view by demonstrating that single-larva inoculations of H. rostochiensis result
in the development of many more females than males. Trudgill (1967) and
Ross & Trudgill (1969) confirmed that the environment determines the direction
of sexual differentiation of larvae. They suggested that larvae which find a good
infection site, with enough space to successfully induce the development of a
large group of giant cells, become females. Other larvae, with less space available,
can only produce a small group of giant cells and become males.

Contrary to this view, unbalanced sex ratios in H. schachtii have been attributed
to differential death-rate of male and female larvae under adverse environmental
conditions (Kerstan, 1969; Johnson & Viglierchio, 1969). Female larvae have
higher requirements for space and nutrients than male larvae to develop to adult-
hood. Therefore, under adverse conditions, such as crowding, nutrient deficiencies,
removal of leaves and stems, and resistant hosts, many female larvae die before
they reach maturity, whereas most male larvae manage to become adults.

High male to female ratios have been observed also in H. glycines exposed
to high soil temperatures, particularly during the early stages of larval develop-

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ment (Ross, 1964). Increases in the percentage of males, however, were always accompanied by increases in the percentage of degenerated larvae in the roots. This suggested that high death-rate of female larvae may have been the cause of the increased male to female ratio. The possibility, however, that high temperatures may alter the direction of sexual differentiation of developing larvae was also suggested as partial explanation. The present study was initiated as an attempt to clarify the means by which infection density influences the sex ratio of *H. glycines*.

### MATERIALS AND METHODS

A population of *H. glycines* originating from Wilmington, N. C. and propagated on soybean [*Glycine max* (L.) Merr.] cultivar "Lee" was used in this study. All experimental work was conducted in a greenhouse at 25 to 30°C. Additional fluorescent and incandescent light of 1500 ft-c was provided to extend the light period to 14 hr, and Hoagland's nutrient solution was added twice a week.

For the single-larva inoculations, 200 soybean seedlings derived from pregerminated seed were grown for seven days in 9 × 2.5-cm plastic centrifuge tubes filled with fine quartz sand and then were inoculated with one freshly hatched larva each. Seven days after inoculation, the plants were washed free of sand and transferred to larger containers with the roots immersed in continuously aerated Hoagland's nutrient solution. Male and female nematodes were collected from the solution on the 15th, 20th, and 25th day after inoculation by passing the solution through a 20- over a 325-mesh sieve. Following the last collection, the roots were examined microscopically for female nematodes.

Inoculation of soybean seedlings grown for 7 days in 7.5 × 10-cm plastic pots filled with sand were made also with larval suspensions containing approximately 20, 200, 1000 and 5000 larvae. Five days after inoculation, the plants were washed free of sand and transplanted into pots with new sand. Eighteen days after inoculation, plants inoculated with 20 and 200 larvae were washed and the washings were screened through a 20- over a 325-mesh sieve to recover male and female nematodes. Plants inoculated with 1000 and 5000 larvae were similarly treated 21 and 24 days after inoculation, respectively. The delay was necessary to compensate for the reduced rate of development of larvae under conditions of heavy infection.

After washing, the roots of plants inoculated with 20 larvae were stained for 1 min in boiling acid fuchsin-lactophenol, destained for at least 2 days in clear lactophenol, and examined microscopically for nematodes of all stages of development and sex. The roots of plants inoculated at the other inoculum levels were placed in flasks containing 50 ml of pectolytic enzyme preparation 1) diluted 1 : 4 with distilled water, and macerated under constant agitation for 8 hr. The suspension of each flask was then passed through a 20-mesh sieve and collected.

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1) Pectinol 59-L obtained from Rhom and Haas Company, Philadelphia, Pennsylvania 19105, U.S.A.