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NEMAT.

IMPREGNATION OF HETERODERA TRIFOLII BY MALES OF *H. SCHACHTII* (NEMATODA : HETERODERIDAE)¹

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Abstract

A parthenogenetic nematode, *Heterodera trifolii* (Goffart, 1932) Raski and Hart, 1953, was impregnated by a bisexual nematode, *H. schachtii* Schmidt, 1871, in mixed cultures of these two cyst-forming species. No males occurred among several hundred offspring.

Introduction

Until 1953 the clover-cyst nematode, *Heterodera trifolii* (Goffart, 1932) Raski and Hart, 1953, was considered a variety of the sugar-beet nematode, *H. schachtii* Schmidt, 1871, but is now recognized as a distinct species. These two species of cyst-forming nematodes are markedly different both morphologically and cytologically. *H. schachtii* is a bisexual nematode in which males are common, whereas *H. trifolii* reproduces parthenogenetically (4) and only in a few instances have males been found (1).

This is a report on a study on whether males of *H. schachtii* might impregnate *H. trifolii*.

Materials and Methods

Whether impregnation occurred was tested in mass cultures and in cultures from single cysts. Cysts for cultures of the sugar-beet nematode were obtained from red beet roots and those for the clover-cyst nematode from white Dutch clover roots. All plants were grown in a greenhouse at Ottáwa.

Mass cultures were used as follows. Five-inch pots were nearly filled with the following soil mixtures: (a) sterilized soil plus soil heavily infested with the clover-cyst nematode; (b) sterilized soil plus soil heavily infested with the sugar-beet nematode; (c) a mixture of (a) and (b).

Red beet and white Dutch clover seeds were planted in all pots. Pots in series (a) and (b) were used as checks.

Cultures from single cysts were used as follows: A box was partly filled with a mixture of sterilized sand and soil heavily infested with the clover-cyst nematode. After a few days white Dutch clover seedlings were transplanted into this box. A second box was partly filled with a mixture of sterilized

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sand and soil heavily infested with the sugar-beet nematode. A few days later red beet seedlings were transplanted into this box. After 2 days, during which the second-stage larvae had entered the plant roots, the seedlings were removed and washed free of soil debris and one plant of each kind was replanted in each of twenty-five 5-in. pots containing sterilized soil compost.

Thirty days after replanting, both white Dutch clover and red beet plants were examined for the presence of white female nematodes on the roots. Each plant was carefully washed before examination by immersing the roots in water. The roots were then examined under the microscope and live white females, which were attached to the roots, were removed and immediately placed in Carnoy's fluid. Slide material was prepared for study by the squash technique (5) and in the manner described by Mulvey (2). Each *H. trifolii* female found containing sperm was carefully checked for the number of chromosomes in the oöcytes. *H. schachtii* and *H. trifolii* differ in chromosome number (3, 4).

Results

Mass Cultures

In the mixed culture the females from the red beet plants had well-developed spermathecae, most of which had numerous tailless sperm, and 12 of the 45 females from the white Dutch clover roots showed sperm in their reproductive systems.

In the culture of the clover-cyst nematode, only the white Dutch clover was attacked. Examination of the clover roots and screening of the soil showed that no males were present, and sperm was not found in the reproductive systems of the many females examined. In the culture of the sugar-beet nematode only the red beet was attacked. Both males and females were present, and sperm was abundant in the spermathecae of the females.

Cultures from Single Cysts

Many of the females from red beet roots were fertilized. Six of the 62 females from the white Dutch clover roots had sperm in their reproductive tracts. The chromosome number in the impregnated females was the same as that reported by Mulvey (4) for *H. trifolii*.

These results indicate that *H. trifolii* females may be impregnated by *H. schachtii* males in mixed populations of the two species. However, limited tests have revealed that no males are produced in the several hundred offspring of several impregnated females.

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