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Influence of the host age on development and virulence of *Heterodera zeae* in Egypt

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Abstract

Four ages of corn *Zea mays* L., cultivar Giza 2 were used to evaluate its influence on infectivity, development and virulence of *Heterodera zeae*. The results revealed that the nematode final population and rate of build-up correlated negatively with the host age. The nematode multiplied highly on the youngest seedlings; and was more virulent on the youngest than on the oldest plants.

Successful establishment of a plant parasitic nematode species is depending on the environmental conditions. Such environmental conditions usually involve both biotic and abiotic factors. The biotic factors in a nematode environment are defined as including all other organisms existing in the habitat. The host plant is one of the most important biotic factor influencing the nematode behaviour. The corn cyst nematode (CCN), *Heterodera zeae* has been regarded as one of the most noxious nematodes to corn in several parts of the world. In Egypt, the CCN has been recorded in different localities by Aboul-Eid & Ghorab (1981), Ismail (1985), Abadir (1986), Moussa *et al*., (1988). Some investigators had covered such point using other nematode species on different hosts. Fedorko (1962), Jorgenson & Mussenlman (1966), Griffin (1981) and Olthof (1983) found that sugarbeet seedlings became less susceptible to damage by *Heterodera schachtii* as they grow older and larger. Sensitivity of susceptible tomato or onion to *Meloidogyne incognita* or *Ditylenchus dipsaci* decreased rapidly with increase in age of plant (Bergeson, 1968; Riedel, 1969). When Bhatti & Sasser (1972), Rawsthorne & Hague (1986) used five different host ages, they found that the older seedlings were less prone to infestation by *H. lespedezae* or *H. avenae* as compared to the younger and tender seedlings. As no information on the effect of plant age at the time of inoculation on the behavior and infectivity of *H. zeae* to corn is available, so this study was carried out to evaluate the behaviour of *H. zeae* under stress to different corn ages in greenhouse conditions.

Materials and Methods

Cultivar Giza 2 corn, *Zea mays* L. seeds were sown in 10 cm dia. clay pots filled with sterilized sandy loam soil (1:1) at 7 days intervals to form four different sets of the plant ages. The plants of each age were thinned to one plant/pot and inoculated 5 day after the last set of seeds had germinated with approximately 1000 eggs and juveniles of *Heterodera zeae* (Belbais population) which was originally obtained from Belbais district (Sharkia governorate) and inoculated on corn cv. Giza 2 in the greenhouse in Egypt. Inocula were prepared by crushing cysts by a homogenizer according to the technique of Ismail (1985). Treatments including non-inoculated plants (check) were replicated four times. All pots were arranged in a randomized block design on a greenhouse bench at 30 ± 5 ºC.

Bioassay of nematode and plant growth: After three months of inoculation, plants were uprooted and data on the plant growth and the nematode population in soil and roots were recorded. The soil of each pot was mixed thoroughly after taking

off the plant and divided equally into two subsamples. One part was processed for extraction of *H. zeae* juveniles by the sieving and decanting technique (Barker, 1985), while, the other part of soil was processed for cyst extraction using Fenwick can apparatus (Fenwick, 1940). Cysts were separated from debris and other organic materials by using a very fine drawing brush (Ismail, 1985), then the collected cysts were counted from each pot. Roots of the plants were cut off, weighed, measured and stained in hot acid fuchsin-lactophenol and then cleared with plain lactophenol (Taylor & Sasser, 1978). Number of the developmental stages, white females and brown cysts per root system were counted. Shoot system of each plant was also weighed and measured.

Statistical analysis: Data were analyzed statistically (Gomez & Gomez, 1984) using a paired t-test and LSD test to determine differences between the different host ages.

Results

Four different ages of cultivar Giza 2 corn seedlings were obtained to determine the influence of the host age on population behaviour of the corn cyst nematode, *H. zeae*. Data manifested in Table 1 indicated that the nematode succeeded in invading the roots of corn at all the host ages. However, the behaviour of the nematode greatly differed according to the age of the plant. The population component was correlated with the host age at inoculation time, an inverse relationship occurred between the plant age and the nematode population. The developmental stages within roots at 7, 14, 21 and 28 days old seedlings were 74, 70, 37 and 39 nematodes/plant, respectively. Likewise, the average number of white females and brown cysts of the 7 days old plants were higher than those of the plants of the other ages. By increasing the plant age, the average number of both stages were remarkably decreased (Table 1). Similar trend was also found in the nematode total population and rate of build-up. The nematode multiplied highly when inoculated to the youngest seedlings. It folded 4.5, 3.7, 1.6 and 1.2 times on plants of 7, 14, 21 and 28 days old, respectively. No effect of the host age was observed on average number of eggs/cyst. It was interesting to note that the nematode was more influential on the youngest plants than on the oldest ones (Table 1).

Table 1. Effect of plant age on development and reproduction of the corn cyst nematode, *Heterodera zeae*.

Plant age (days)	Average number of nematodes/plant						Rate of build-up
	Soil	Root			Eggs/ cyst	Total	
	J_2	D. S.	W. F.	B.C.			
7	345	74	40	20	200	4459	4.5
14	0	70	34	18	200	3704	3.7
21	160	37	26	7	200	1623	1.6
28	270	39	10	5	200	1219	1.2
LSD 0.05		-	10	11	-	2445	
LSD 0.01		-	22	-	-	-	

D. S. = Developmental stages; W. F. = White females; B. C. = Brown cyst; Total = J2 + D. S. + W. F. + (B. C. × No. eggs/cyst).

The plant growth parameters of the inoculated plants at 7 and 14 days old were significantly decreased ($p \leq 0.05$ and/or 0.01) than those of non-inoculated plants (Table 2). No significant differences in such parameters were obtained between inoculated and non-inoculated plants of 21 and 28 days old. Therefore, the percentage of reduction in plant growth parameters was negatively correlated with the plant age at time of the nematode inoculation. The amount of reduction was more pronounced in the youngest plants than in the oldest ones. Hence, the nematode seemed to be most virulent on the younger plants (Table 2).

Table 2. Growth response of different ages of Giza 2 corn to *Heterodera zeae* infection.

Plant age at inoculation (days)	Shoot				Root			
	Length (cm)		Weight (g)		Length (cm)		Weight (g)	
	Infected	Non-infected	Infected	Non-infected	Infected	Non-infected	Infected	Non-infected
7	72**	85.7	21.3*	25.0	21.0	25.5	11.7	20.5
14	74.3*	83.0	21.0*	24.3	16.8	19.8	13.0*	17.8
21	78.7	85.8	22.1	25.3	19.0	20.8	16.8	18.0
28	78.6	82.6	21.5	22.0	19.7	21.0	15.5	16.5

*Significant at 5% level according to t-test; **Highly significant at 1% level according to t-test.

Discussion

Infectivity, development and virulence of the corn cyst nematode, *H. zeae* have been influenced by several biotic and abiotic factors involving in the nematode habitat. Some of which are dealing with the pathogen capability and the others are concerning with the environment in which the pathogen survive. One of the most effective factor is the host age which influenced on the nematode behavior. When corn seedling age was used as an influential factor on development, reproduction and virulence of the corn cyst nematode, *H. zeae* behaved differently according to the host age. The obtained results showed an inverse relationship between the plant age and the nematode population. Number of nematode developmental stages, white females, brown cysts and the nematode final population on the 7 days old plants were higher than other plant ages. Accordingly, the amount of damage in the plant growth was more pronounced in the younger plants than in the older ones. Similar findings had been reported by several workers on other nematode species on different plants in regard to the host age (Griffin & Hunt, 1972; Fawole & Mai, 1979; Griffin, 1981; Olthof, 1983; Shamim & Israr, 1989). It is acceptable that root exudates are responsible for nematode attraction (Ismail, 1993; Ismail & Hasabo, 1995). Root exudates from the younger roots are chemically quite different from those released from the older one. Root exudates from 7 or 14 days old plants may be more suitable for the juvenile attraction leading to greater nematode final population and greater damage to the plants. Besides, the anatomical structure of the younger roots may be more suitable for nematode invasion (Ismail, 1993; Haroon *et al*., 2009). On the other hand, roots of the older plants may act as a barrier against nematode penetration and most of them fail to overcome such barriers.

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