Commun. Fac. Sci. Univ. Ank. Series C
V.23 (1-2). pp. 13-20 (2011)

TEMPERATURE AND THE LIFE CYCLE OF *HETERODERA SCHACHTII* (NEMATODA: HETERODERIDAE)

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(Received July 01, 2011; Accepted Aug. 01, 2011)

ABSTRACT

Development of the sugar beet cyst nematode, *Heterodera schachtii*, was studied in growth chambers at 20, 25, 29, 32 and 35 ± 1 ºC on *Beta vulgaris* cv. Saccharifera. The optimum temperature for reproduction appeared to be 32 ºC, at which the life cycle, from second-stage juvenile (J2) to J2, was completed in 16-19 days; at 35 ºC, 20-21 days were required. Juveniles emerged from eggs within 29 days at 29 ºC and after 43 days at 25 ºC. Although J2 were present within eggs after 62 days at 20 ºC, emergence was not observed up to 101 days after inoculation. Female nematodes produced fewer eggs at 20 ºC than at higher temperatures.

KEYWORDS: Sugar beet cyst nematode, *Heterodera schachtii*, life cycle, temperature.

INTRODUCTION

The sugar beet cyst nematode, *Heterodera schachtii* Schmidt, 1871 is a major parasite and production problem for sugar beet growing areas across the world. First discovered near Halle, Germany in 1859 by H. Schacht, it was named and described by A. Schmidt in 1871. The nematode was later determined to be the major cause of “beet weariness” responsible for the

closure of at least 24 sugar beet processing factories in Germany after 1876 (Franklin 1977; Shadmehr et al. 2007) .

Heterodera schachtii is a parasitic roundworm that overwinters in soil as dormant eggs or juveniles in cysts, which is the dead body of the female. When the root of a host plant contacts or grows near the cyst, combined with adequate soil moisture and temperatures above 10 ºC, the J2 are stimulated by root exudates to hatch from their eggs and emerge from cysts. They migrate to roots, infecting near the tips. After entering roots, they migrate deeper into the cortex and begin developing into swollen third stage juveniles (J3). After the fourth molt, females become lemon-shaped and can be seen as small white dots attached to young fibrous roots. Adult males emerge from roots, enter soil and are attracted to females where fertilization occurs (Asbach et al. 2004; De Greef and Jacobs 1979; Ferris and Williamson 2004).

Females produce an average of 200 eggs each, the majority of which remain inside their bodies. When they die, their body wall thickens and is transformed into a leathery, brown-reddish lemon-shaped cyst, completing the cycle. The life cycle normally takes four to six weeks, depending upon soil temperatures. Sugar beet cyst nematode reproduces optimally between 26.6-32.2 ºC, and is capable of producing three cycles in a season (Gamborg et al. 1976; Southey 1970).

Limited information regarding the biology and temperature requirements of *H. schachtii* is available, and that pertains primarily to the Turkey population. The objective of this study was to investigate certain aspects of the biology of *H. schachtii*, including the influence of temperature on its life cycle.

MATERIALS AND METHODS

Cultures of *H. schachtii* were initially established on *Beta vulgaris* cv. Saccharifera (Taşova-Turkey, 40° 46.2´ N ; 36° 19.2´ E) in the greenhouse. Cysts from these cultures were the source of J2 which were used as inoculum in these studies. Cysts collected from cultures were blended in tap

water in a Waring blender for 90 seconds. The blended suspension was poured through a 45 µm pore sieve, and the residue was incubated on modified Baermann funnels for 6-8 days at 22 ºC. Juveniles were collected daily from the funnels and stored in tap water at 7 ºC until used as inoculum. Prior to inoculation, aliquots of the suspension were examined microscopically to ensure that the J2 were active. The nematode suspension was adjusted to a density of 300 J2 / ml water.

Sugar beet seeds were germinated in flats of acid-washed sand at 25 ºC. Seedlings 13-15 days old were inoculated by pipeting an aqueous suspension containing 1500 active J2 onto roots supported on sterile sand in 100 cm^3 plastic cups. Roots were covered with moist sand and seedlings were placed in growth chambers at 20, 25, 29, 32, or 35 ± 1 ºC, with a 12-hour photoperiod, for 48 hours. Following the infection period the seedlings were removed from the sand, the roots were rinsed thoroughly to remove any J2 that had not penetrated, and the seedlings were planted in sterile sand in 4 cm diameter pots and returned to the growth chambers. Three plants were sampled every 24 hours at 35, 32, 29, and 25 ºC and every 48 hours at 20 ºC until the nematode life cycle was completed and than at intervals of 3-7 days until the experiments were terminated. Roots of infected plants were prepared for examination using the method of Byrd et al. (1983). Nematodes were dissected from roots and observed microscopically to determine the state of development. Washings from sand surrounding the roots of test plants were passed through a 45 µm pore sieve and incubated on modified Baermann funnels in order to extract J2 and males. Residue from a 250 µm pore sieve was examined for females and cysts. The timing of the developmental stages of the nematode life cycle were delineated on the basis of the first observance of each molt and the presence of nematodes in the succeeding developmental stage. The life cycle was considered to be completed when the first emerged J2 was observed. Experiments at each temperature were conducted twice. The data presented in Table 1 are the range of days observed for all plants in the two experiments.

RESULTS

Nematodes penetrated sugar beet seedling roots and developed to maturity at all temperatures (20-35 ºC) used. The optimum temperature for development appears to be 32 ºC at which the life cycle was completed in 16-19 days (Table 1). Development was also rapid at 35 ºC, at which the life cycle was completed in 20-21 days. Emergence of J2 occurred after 29 days at 29 ºC and after 43-44 days at 25 ºC. Although J2 in eggs were present after 62 days at 20 ºC, hatch did not occur even up to 101 days after inoculation.

Table 1. Development time and egg production of *Heterodera schachtii* at five temperatures.

Developmental parameters	Days from inoculation to first observation				
	35 ºC	32 ºC	29 ºC	25 ºC	20 ºC
Second molt	6-8	5-6	7-8	11	13-14
Third molt	8-10	8-9	10-11	15-16	18-19
Fourth molt	10-12	11-12	12-15	20-22	28-29
Egg production	12-14	13-14	16-20	25-29	38-39
First molt (in ggs)	8-20	15-18	27-28	27-38	62-63
Hatch	20-21	16-19	29	43-44	*
Tan cysts	27	31	31	54-58	96
Adult males	15	13-16	16	*	65
Average no. eggs/cyst	171	184	197	276	114

* Not observed

Development of juveniles was uniform and rapid at 29, 32, and 35 ºC (Table 1). Nematodes remained at J2 for 4-7 days, third and fourth juvenile stages were completed within an additional 2-4 days for each stages, and mature females were observed 10-12 days following inoculation. Variation occurred in the time required for development to egg production, embryonation, and emergence of J2 from eggs. Juveniles emerged from eggs 1-2 days following the first molt within eggs, and reinfection of roots occurred almost immediately.

Nematode penetration of roots within the 48-hour infection period seemed to occur less readily and nematodes developed more slowly at 20 and 25 ºC than at higher temperatures. Despite uniform inoculum densities among experiments, roots consistently contained more nematodes at 29, 32, and 35 ºC than at 20 or 25 ºC. At 20 ºC fewer eggs were contained in cysts than at higher temperatures.

A thin subcrystalline layer was present on the body wall of newly developed female nematodes, and gelatinous egg sacs were produced at all temperatures. The maximum number of eggs observed within any single egg sac was 17, most eggs being retained within the female body.

Males were found infrequently at 29, 32, and 35 ºC. A single male was found after 65 days at 20 ºC and none were found at 25 ºC. On several occasions large numbers of males were recovered from stock cultures maintained on Saccharifera sugar beet at 29 ºC in plant growth chambers. Males were never observed to mate with females.

DISCUSSION AND CONCLUSION

The development and reproduction of *H. schachtii* are favored by relatively high soil temperatures. Under the conditions of these experiments, infection of sugar beet roots was heaviest and nematode development most rapid at 32 and 35 ºC. At 20 ºC few J2 penetrated roots within 48 hour, development was slow, and adult females produced fewer eggs than at higher temperatures. A few J2 developed within eggs in females at 20 ºC, but no hatch was observed during the course of the experiments.

The results of my investigations are similar to those of Franklin (1977) who reported that *H. schachtii* completed its life cycle in 17 days following

inoculation at greenhouse temperatures ranging from 21 to 27 ºC. The length of the life cycle varied on the different sugar beet cultivars tested in their studies.

Paul et al. (1990) reported that development of *H. schachtii* J2 to mature egg-filled females occurred in 20 days in the field at temperatures of 22-26 ºC.

Franklin (1977) reported that adult females of sugar beet cyst nematode developed on sugar beet within 18 days of inoculation at 25 ºC and within 17 days at 27 ºC. At 15 ºC, J2 penetrated roots but failed to develop further. Nematode development in his experiments occurred much more rapidly than in my experiments. This variation in nematodes of the two populations can be explored only by comparing his development at a single location under identical environmental conditions.

Baltensperger et al. (2000) studied the development of *H. schachtii* in root explant cultures of *Beta vulgaris* cv. Saccharifera and reported a 20-day life cycle under those conditions at 25 ºC. An average of 200 eggs were deposited within the egg sacs of female nematodes in explant cultures. In contrast, Srivastava and Sethi (1985) observed only 4-15 eggs within egg sacs of female nematodes recovered from greenhouse cultures.

Temperature has been reported to influence the susceptibility of relatively poor host plants to infection by *H. schachtii*. Bajaj et al. (1986) reported that under identical conditions, J2 of *H. zeae* penetrared maize roots but failed to penetrate roots of wheat at 15 and 23 ºC; however, nematode penetration and development were similar at 32 ºC in both corn and wheat roots. Similar behavior was observed during studies on the host range of *H. schachtii*.

The existence of conditions suboptimal for infection of sugar beet roots and development of nematodes, resulting in low soil population densities of *H. schachtii* present in most infested soils, may be responsible for the restricted distribution of this nematode in Taşova. However, the high reproductive potential of *H. schachtii* at soil temperatures of 32-35 ºC suggests that in warmer sugar beet growing regions of Turkey. Sugar beet cyst nematode could develop high soil population densities and be an economically important pathogen of sugar beet.

ÖZET: Şeker pancarı kist nematodu, *Heterodera schachtii*' nin gelişimi, yetiştirme kabinlerinde 20, 25, 29, 32 ve 35 ± 1 ºC de *Beta vulgaris* cv. Saccharifera üzerinde çalışılmıştır. Üreme için optimum sıcaklık 32 ºC gözükmüş, ikinci devre gelişme evresi juveniller (J2)' den J2'ye hayat devri 16-19 günde tamamlanmış olup, 35 ºC de ise 20-21 gün gerekmiştir. Juveniller yumurtadan 29 ºC de 29 günde çıkmıştır. Bu çıkış 25 ºC de 43 gün sonra meydana gelmiştir. J2 20 ºC de 62 gün sonra yumurta içinde bulunmasına rağmen, aşılamadan sonra 101. güne kadar açılım gözlenmemiştir. Dişi nematodlar 20 ºC de, daha yüksek sıcaklıklardakinden daha az yumurta üretmiştir.

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