

Growth of *Heterodera schachtii* with remarks on amounts of food consumed

Joachim MÜLLER *, Klaus REHBOCK ** and Urs WYSS **

* *Biologische Bundesanstalt, Institut für Nematologie, Toppheideweg 88, 4400 Münster*
and ** *Institut für Pflanzenkrankheiten und Pflanzenschutz der Universität Hannover, Herrenhauser Str. 2, 3000 Hannover, Bundesrepublik Deutschland.*

SUMMARY

The development of the sugar-beet cyst nematode *Heterodera schachtii* on excised roots of *Brassica napus*, grown under gnotobiotic conditions in agar culture, was observed from root invasion of infective second-stage juveniles until death of females. Most of the observations were by time-lapse cinemicrography which revealed a characteristic growth pattern for all developmental stages. This pattern consisted of more or less regular alternations between gradual increases in body volume, followed by sudden collapses. The gradual increase in body volume was always associated with a continuous pumping action of the median oesophageal bulb. As soon as pumping stopped, growth ceased. The sudden collapse in body volume, about 20 min after cessation of bulb pumping, was most probably due to defaecation, although the expulsion of faeces could not be seen. Growth of female juveniles was arrested only during moulting which, at 25°, occurred at rather regular intervals of 3-4 days.

From the average data of continuous food ingestion and changes in body volume, the approximative amounts of daily and overall food consumption for the different developmental stages could be calculated. Total food consumption of males was about 29 times less than that of females.

RÉSUMÉ

*Sur la croissance d'*Heterodera schachtii* et la quantité de nourriture consommée*

Le développement du nématode à kyste de la betterave, *Heterodera schachtii*, élevé sur colza (*Brassica napus*) dans des conditions aseptiques sur un milieu nutritif gélosé, a été observé à partir de la pénétration des larves jusqu'à la mort des femelles. La plupart des observations ont été faites par cinématographie accélérée, ce qu'a permis de déceler une croissance caractéristique à tous les stades de développement. Cette croissance se manifeste par des alternances régulières entre un gonflement continu du corps suivi d'un dégonflement brutal. Le gonflement est toujours associé à l'activité du bulbe oesophagien médian. La croissance s'arrête dès que le bulbe médian ne pompe plus. Le dégonflement du corps, qui a lieu environ 20 minutes après cessation de l'activité du bulbe médian, est sans doute la conséquence de la défécation ; toutefois, les fèces n'ont pas été observées. La croissance des larves femelles ne s'arrête que pendant la mue, qui a lieu, à 25°, à intervalles réguliers de 3-4 jours.

A partir des données provenant de l'ingestion permanente et des changements de volume du corps, les quantités approximatives de nourriture consommée, par jour et en totalité, ont été calculées pour les différents stades de développement. La consommation totale d'un mâle est environ 29 fois moindre que celle d'une femelle.

The early thorough studies by Strubell (1888), Chatin (1891) and von Sengbusch (1927) on the biology of *Heterodera schachtii*, the most important nematode pest of sugar-beet production, still deserve our admiration, in spite of several inaccuracies, concerning, for instance, the number of developmental stages. Raski's fundamental contribution (1950) clearly demonstrated five stages and four moults in the life cycle of

females and males. The sugar-beet cyst nematode may thus represent the idealized growth curve of nematodes (Rogers, 1962 ; Rogers & Sommerville, 1963) which consists of five stages of growth, each being terminated by a moult during which growth is temporarily arrested. Within the Heteroderoidea this curve is not followed by *Meloidogyne* spp. (Bird, 1959 ; Triantaphyllou & Hirschmann, 1960) which,

after having established themselves at a feeding site, undergo three moults in quick succession with no growth between these moults. Other nematodes may continue to grow during moults, as shown by the rat lung-worm *Angiostrongylus cantonensis* (Mackerras & Sandars, 1955).

As *H. schachtii* can be well maintained in agar culture under gnotobiotic conditions (Johnson & Viglierchio, 1969; Müller, 1978), it serves as a good model for direct growth observations of a cyst nematode throughout its life cycle. A complete picture of the nematode's development from root invasion until death of the adult stage can be obtained from time-lapse cinemicrography (Müller, Wyss & Inst. wiss. Film, 1981). This paper reports results from cinéfilm analysis and direct observations on growth and food consumption of *H. schachtii*.

Materials and methods

Cultures of *H. schachtii* were maintained on excised *Brassica napus* cv. 'Akela' roots in plastic Petri dishes, as described by Müller (1978). The nematode's development was filmed at the Institut für den wissenschaftlichen Film, Göttingen, BRD, using time-lapse cinemicrography ranging from 1 frame/s to 4 frames/h. (For further details see Müller, Wyss & Inst. wiss. Film 1981). During filming the cultures were kept at approximately 25°. The film was analysed with a nac Film Motion Analyser. Direct microscopic observations and measurements were made at 160 to 640 × magnification. At 640 × magnification the error in measurement was ± 0.5 µm.

The volume of *H. schachtii* at different developmental stages was estimated by applying the formulae mentioned below, where ℓ = maximum length and w = maximum width.

For the volume of the second and third juvenile stage ($V_{2,3}$), the formula for the volume of a cylinder was used :

$$V_{2,3} = \frac{\pi}{4} \cdot w^2 \cdot \ell$$

The fourth-stage female juvenile resembles an ellipsoid whose volume is $\frac{\pi}{6} \cdot w^2 \cdot \ell$. As this

stage is initially still cylindrical, its volume (V_4) was calculated as the mean value from a cylinder and an ellipsoid :

$$V_4 = \frac{5 \cdot \pi}{24} \cdot w^2 \cdot \ell$$

The volume of the female ($V_{\text{♀}}$) was estimated as follows. The body outline of three females, recorded on cinéfilm, was projected onto paper, cut out and weighed (Bird, 1959). From one body half the volume of the female was then calculated according to the Guldin rule for solids of revolution. The centre of gravity of the generating area was determined empirically. In this way the following formula for volume of the female was derived :

$$V_{\text{♀}} = 0.54 \cdot \frac{\pi}{4} \cdot w^2 \cdot \ell$$

The amount of food withdrawn (F) for an individual ingestion phase (during which the median oesophageal bulb pulsed continuously) was determined by : $F = V_2 - V_0$ where V_2 = maximum volume at the end of an ingestion phase and V_0 = minimum volume at the beginning of an ingestion phase. For each developmental stage average values for F ($n > 25$) were deduced from the analysis of cinefilm material. Average continuous ingestion phases for each stage were measured under the microscope. From these data average values of daily food consumption could be obtained. To estimate the approximate amount of food withdrawn per developmental stage, the average duration of the active phase (days during which food was ingested, but excluding moulting time in case of juveniles) of the developmental stages was determined at 25°.

Results

Once the body of the nematodes broke through the roots, which was usually in the third juvenile stage within the rather thin *B. napus* roots, the only recognizable movement under direct microscopic observation was the rapid pulsation of the median oesophageal bulb. This pulsation was, however, only visible in individuals which had their head region not

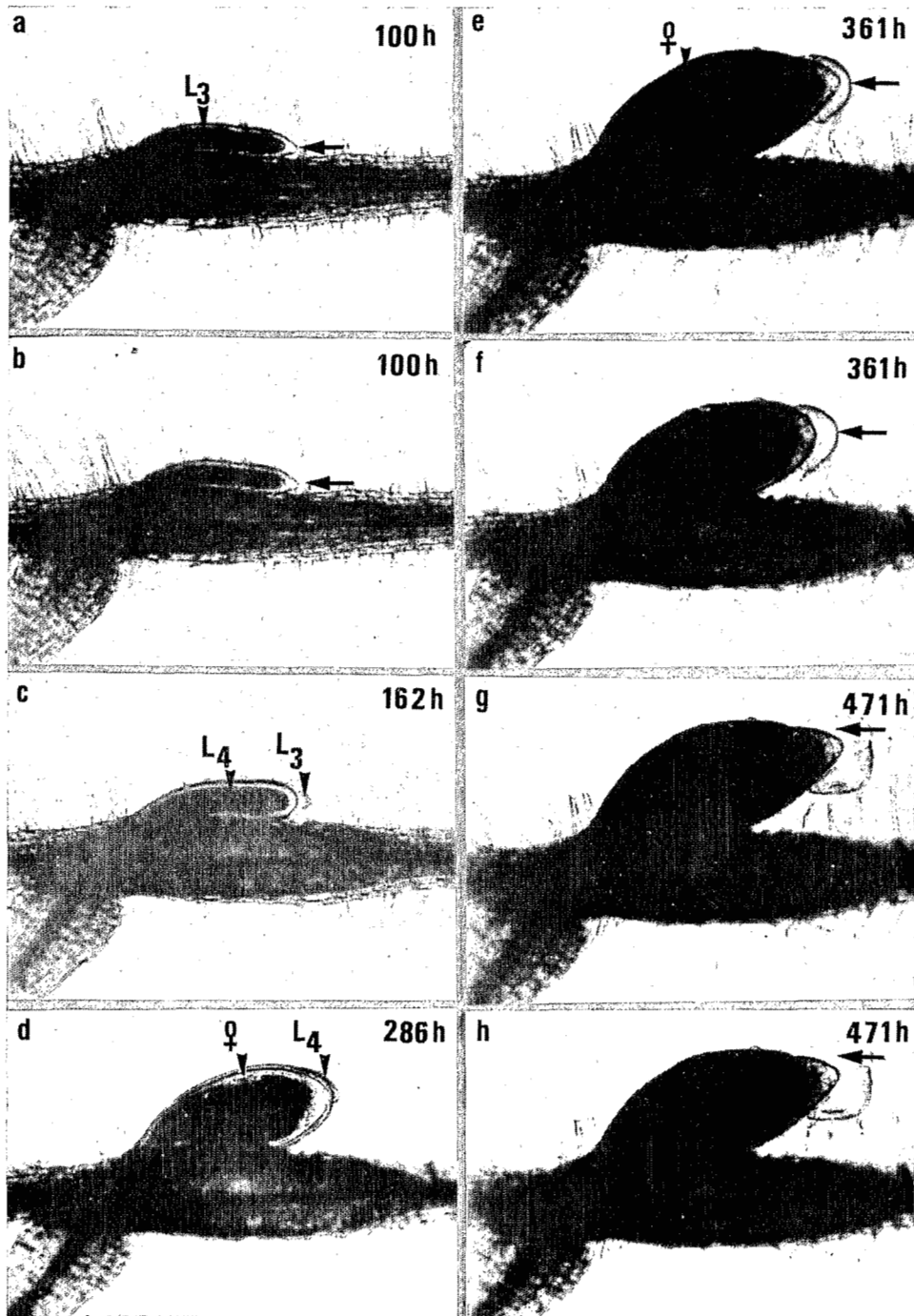


Fig. 1. Growth of *Heterodera schachtii* on rape roots in sterile agar culture, here 100 h after emergence of a third-stage female juvenile from the root. Typical examples of sudden shrinkage in body volume are shown in a/b; e/f and g/h (compare position of the nematode's hind end in relation to arrows); c and d show moulting to fourth-stage juvenile and female respectively. L_3 = third-stage juvenile; L_4 = fourth-stage juvenile. Times in hours on right.

deeply embedded in the roots. Time-lapse filming at 4-30 frames/h revealed a characteristic, more or less rhythmical, growth pattern : a gradual increase in body volume was suddenly followed by a marked decrease. Typical dimensions of such collapses in body volume are shown in Figure 1, 100, 361 and 471 h after the emergence of a third-stage female juvenile from the root. The figure also indicates the pattern of the nematode's growth which is only arrested during moulting (Fig. 1 c, d). In this particular case moulting from the third to the fourth juvenile stage lasted 22 h, and 35 h from the fourth juvenile stage to the female. This corresponds in general to other observations, the last moult had the longest duration, though it was also recorded as being as short as 21 h ($n = 5$). It should be pointed out that during filming the temperature was kept at about $25^{\circ} (\pm 2^{\circ})$, and so no accurate figures can be given. Figure 1 is not typical for the increase in volume of the young female after the last moult, as this was usually more pronounced.

A characteristic pattern of the alternations between increase and decrease of body volume during growth, as revealed by time-lapse filming at 20 frames/h, is shown in Figure 2 for a third-stage female juvenile, 24 h before the onset of moulting. This rather regular pattern could also be observed for all female juvenile stages, females, and the two male juvenile stages before

the third moult. Second-stage juveniles, still within the root tissue, also showed a similar pattern when this became discernible in the few cases of rather shallow root penetration. Changes of body size were always simultaneous, for both length and width.

Growth phases (interval between two onsets of body size increase) are shown in Figure 3, here for a fourth-stage female juvenile from the beginning of growth resumption after the third moult until the onset of the last moult. A total of 41 phases was recorded within this period with an average phase duration of 114 minutes. It may be noteworthy that the two longest growth phases occurred immediately before the final moult.

The data presented so far were all obtained from the analysis of time-lapse film. Direct continuous observation was, however, necessary to determine the character of the growth phases. Figure 4 represents two typical successive growth phases of a third-stage female juvenile whose increase in body length was recorded every five minutes. The curve, fitted by eye, shows the dynamics of a growth phase. A gradual continuous increase in body length finally stopped and was then followed by a slight decrease over about 20 min. Then a pronounced shrinkage occurred within 1-3 seconds after which the nematode started to grow again.

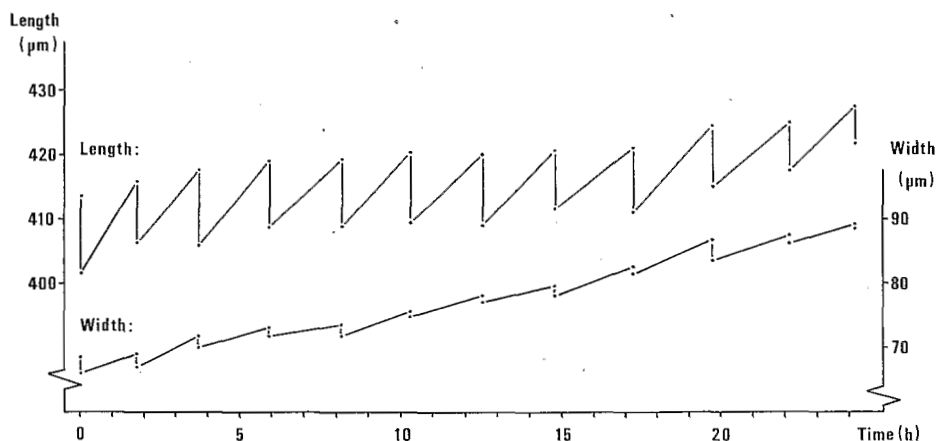


Fig. 2. Growth of *Heterodera schachtii*. Pattern of alternations between increase and decrease of the body size of a third-stage female juvenile, 24 h before moulting. Analysis of time-lapse film (20 frames/h).

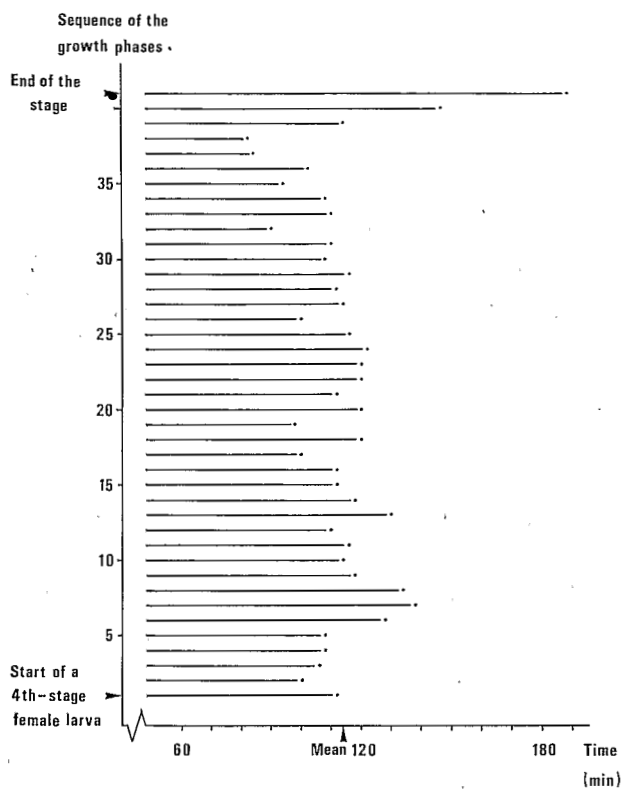


Fig. 3. Growth of *Heterodera schachtii*. Sequence of growth phases (interval between two onsets of body size increase) of a fourth-stage female juvenile from the beginning of growth resumption after the third moult until the onset of the last moult. Analysis of time-lapse film (20 frames/h). Mean duration of a growth phase : 114 min. (arrow).

In some individuals whose head region was not too deeply embedded in the root tissue, the pumping action of the median oesophageal bulb could be well observed. It became evident that under these conditions increase in body size was always associated with continuous bulb pulsation (at a rate of 4-7 pulsations/s), as shown by the dark thick line beneath the growth phase curve. Growth ceased as soon as pumping stopped. The average duration of continuous food ingestion (continuous pulsation of the median oesophageal bulb) and rest period (no bulb pulsation) was measured for individuals of different developmental stages by direct microscopic observation and is represented in Table 1. Shortest phases for both continuous food ingestion and rest period, were recorded for the

second-stage juvenile within the roots. However, as pumping was visible in only a few individuals, the number of observations for this stage was rather low. No measurements of the alternations in body size could be made.

From the average data of continuous food ingestion and changes in body volume, the approximate amounts of food consumed daily by the different developmental stages could be calculated. The average duration of activity (food ingestion and rest periods, but excluding the time spent for moulting) for the stages was determined at a constant temperature of 25°. The 21 females observed fed on average for 19 days. Then they stopped feeding, but remained white for many more days before the cuticle finally turned brown.

Table 2 shows the approximate amounts of food withdrawn by different developmental stages. Although the data are only rough estimates, they indicate that food consumption of the fourth-stage female is about three times that of the third-stage female or male within active periods of equal duration. Males stop feeding after the third larval stage and thus consume about 29 times less food than females. With this figure it is assumed that the second-stage juveniles (for which no data could be obtained) consume about half the amount of third-stage juveniles.

Discussion

The most striking feature throughout the growth of *Heterodera schachtii*, as observed by time-lapse cinemicrography on rape roots in sterile agar culture, was a continuous alternation between increase and decrease of the nematode's body volume at rather regular intervals. Such an alternation was already discernible in second-stage juveniles once they had established themselves at a feeding site within the root. It persisted in male juveniles until termination of the third juvenile stage and in females until ingestion was finally abandoned several weeks after the last moult. Increase in body volume was always associated with a continuous pumping action of the median oesophageal bulb. During moulting growth was always arrested.

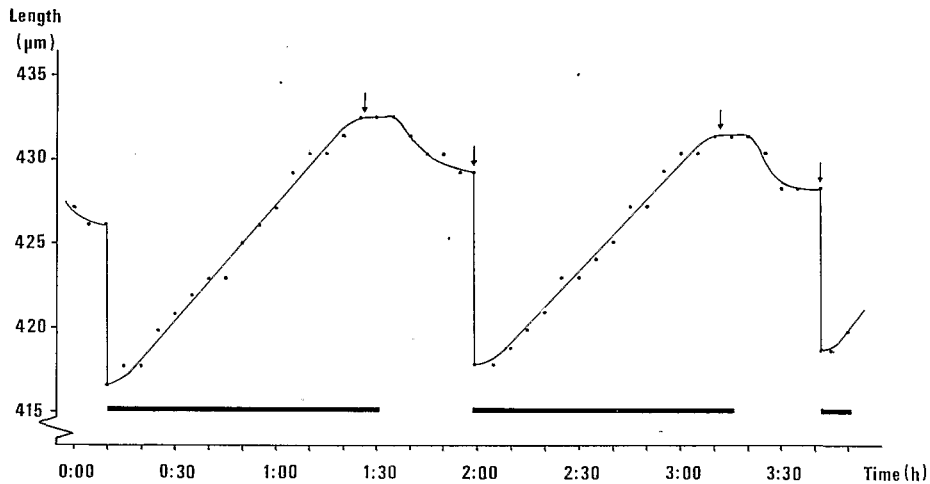


Fig. 4. Growth of *Heterodera schachtii*. Two typical successive growth phases of a third-stage female juvenile whose increase in body length was measured at 5 min intervals. Observations were continuous as soon as the median oesophageal bulb stopped pumping. The sudden shrinkage of the body volume occurred within 1-3 seconds. The thick line beneath the growth curve shows the duration of continuous pumping action of the median oesophageal bulb. The arrows indicate the cessation of body size increase and respectively the onset of the sudden collapse.

Table 1

Feeding of *Heterodera schachtii* on rape roots in sterile agar culture
Phase duration of continuous food ingestion (continuous pulsation of the median oesophageal bulb) and rest period (no bulb pulsation), measured on different developmental stages

Developmental stage	Continuous food ingestion (min)			Rest period (min)		
	$\bar{x} \pm s$	Min.-Max.	n	$\bar{x} \pm s$	Min.-Max.	n
L 2	54 ± 6	43-61	6	17 ± 3	14-24	8
L 3	82 ± 30	41-138	19	24 ± 7	13-49	23
L 4 ♀	89 ± 22	59-146	15	33 ± 10	20-54	20
♀	93 ± 30	44-150	25	34 ± 8	18-52	38

\bar{x} = Mean ; s = Standard deviation ; n = Number of observations.

Table 2

Approximate amounts of food withdrawn by *Heterodera schachtii*, feeding on rape roots in sterile agar culture

Developmental stage	Average duration of the active stage at 25° (days) *		Amount of food withdrawn per day (10 ⁻⁶ ml)	Amount of food withdrawn per developmental stage (10 ⁻⁶ ml)	Body volume ** at the end of each developmental stage (10 ⁻⁶ ml)
	\bar{x}	n			
L 2	3-5	26	?	?	0.25 ***
L 3	3	25	1.8	5.4	1.4
L 4 ♀	3	9	5.8	17.4	6.8
♀	19	21	11.3	214.7	52.0

* Stage that feeds, i.e. excluding moulting time, in the case of juveniles.

** Approximate calculation from our data and from data of Raski (1950) and Günther (1967).

*** The body volume of the infective juvenile is about 0.15 × 10⁻⁶ ml.

A similar growth pattern was observed for the free-living nematode *Panagrellus silusiae* (Mapes, 1965). As long as the nematode ingested food by fairly continuous pumping a gradual increase in body volume of about 10% occurred and the contents of the rectum were then expelled. Mapes suggested that defaecation is associated with the attainment of a critical limit in the increase of body volume and this has since been supported by Bird (1971), Crofton (1971) and Lee and Atkinson (1976). When feeding is continued over long periods, defaecation will thus take place at regular intervals.

The sudden decrease in body volume of *H. schachtii* at rather regular intervals strongly suggests expulsion of faeces, although we were not able to recognise this, even with the aid of Nomarski interference-contrast microscopy. As already noted for *Ditylenchus dipsaci* (Doncaster & Seymour, 1976), *H. schachtii* may mainly defaecate water as a waste product. According to Crofton (1971), Seymour and Doncaster (1972) and Lee and Atkinson (1976) defaecation is preceded by a shortening of the body. The gradual decline in body volume of *H. schachtii* after cessation of food ingestion may represent the onset of a defaecation cycle (Seymour & Doncaster, 1972) which is terminated by a drastic reduction in body volume of about 4-22% (depending on the developmental stage) when the body pressure drops sharply within 1-3 seconds. Such a marked decrease in body volume points to the participation of a pre-rectum in defaecation (Seymour & Doncaster, 1972).

During the rest periods that follow phases of continuous food ingestion, salivary secretions will most probably be injected into the feeding site for maintenance and extension of the syncytium. *Helicotylenchus* spp. that feed for several days from a single cell within roots of wheat showed a similar alternation between phases of continuous ingestion and rest periods and were observed to salivate during the latter (Jones, 1978). Data obtained from film analysis and direct observation suggest that *H. schachtii* continually removes food from its feeding site (syncytium) with regular, but relatively short interruptions (defaecation/salivation) and longer interruptions only during moulting. This forms a strong contrast to the life-cycle of *Meloidogyne*

spp. where food consumption (and salivation) is restricted to the second juvenile stage and to freshly moulted females.

In *H. schachtii* a female juvenile requires about three times more food than a male juvenile until moulting to the adult stage (Table 2). This high food demand leads to the formation of larger syncytia. This could be well observed when infected roots were kept under light for 18 h per day. The syncytia then turned green due to the development of chloroplasts which was confined to the syncytia and thus they became clearly visible within the roots (Müller & Wyss, unpubl.). The minimal living space for males of *H. schachtii* for development in host roots was estimated to be at least 60 times less than that of females (Kerstan, 1969).

From our calculations the daily food consumption of a female was about 11×10^{-6} ml (Tab. 2). Assuming a field soil with sugar-beet, heavily infested by *H. schachtii* to a depth of 0.5 m with 500 feeding females per 100 ml, a total of 25×10^9 females would then remove from the crop about 280 l per hectare. In the summer a sugar-beet crop transpires about 50 000 l or more per hectare (Ehlers, 1976). The wilt symptoms evoked by *H. schachtii* in sunny days should thus have other causes than simply loss of liquid through nematode attack. Seymour and Doncaster (1978) also thought that withdrawal of cell sap *per se* by *Ditylenchus dipsaci* contributes little to plant damage when they estimated the quantity ingested.

REFERENCES

- BIRD, A.F. (1959). Development of the root-knot nematodes *Meloidogyne javanica* (Treub) and *Meloidogyne hapla* Chitwood in the tomato. *Nematologica*, 4 : 31-42.
- BIRD, A.F. (1971). *The Structure of Nematodes*. New York & London, Academic Press, 318 p.
- CHATIN, J. (1891). L'anguillule de la betterave (*Heterodera schachtii*). *Bull. ann. Min. Agric.*, 10 : 457-506.
- CROFTON, H.D. (1971). Form, Function, and Behavior. In : Zuckerman, B.M., Mai, W.F. & Rohde, R.A. (Eds). *Plant Parasitic Nematodes* Vol. I. New York & London, Academic Press : 83-113.

- DONCASTER, C.C. & SEYMOUR, M.K. (1976). Defaecation in the stem nematode, *Ditylenchus dipsaci*. *J. Zool., Lond.*, 180 : 9-14.
- EHLERS, W. (1976). Evapotranspiration and drainage in tilled and untilled Loess soil with winter wheat and sugar-beet. *Z. Acker-u. PflBau*, 142 : 285-303.
- GÜNTHER, B. (1967). *Beiträge zur Morphologie und Histologie von Heterodera schachtii* (Schmidt). Dissert., Universität Greifswald, 291 p.
- JOHNSON, R.N. & VIGLIERCHIO, D.R. (1969). Sugar beet nematode (*Heterodera schachtii*) reared on axenic *Beta vulgaris* root explants. I. Selected environmental factors affecting penetration. *Nematologica*, 15 : 129-143.
- JONES, R.K. (1978). The feeding behaviour of *Helicotylenchus* spp. on wheat roots. *Nematologica*, 24 : 88-94.
- KERSTAN, U. (1969). Die Beeinflussung des Geschlechterverhältnisses in der Gattung *Heterodera*. II. Minimallebensraum - selektive Absterberate der Geschlechter - Geschlechterverhältnis (*Heterodera schachtii*). *Nematologica*, 15 : 210-228.
- LEE, D.L. & ATKINSON, H.J. (1976). *Physiology of Nematodes*. 2nd ed. London & Basingstoke, MacMillan Press, 215 p.
- MACKERRAS, M.J. & SANDARS, D.F. (1955). The life history of the rat lung-worm *Angiostrongylus cantonensis* (Chen) (Nematoda : Metastrongylidae). *Aust. J. Zool.*, 3 : 1-21.
- MAPES, C.J. (1965). Structure and function in the nematode pharynx. II. Pumping in *Panagrellus*, *Aplectana* and *Rhabditis*. *Parasitology*, 55 : 583-594.
- MÜLLER, J. (1978). L'élevage monoxénique d'*Heterodera schachtii* sur crucifères et son application pour la sélection des plantes résistantes. *Revue Nématol.*, 1 : 47-52.
- MÜLLER, J., WYSS, U. & INST. WISS. FILM (1981). Film C 1387 'Entwicklung des Zystennematoden *Heterodera schachtii*' des IWF, Göttingen, *Publ. Wiss. Film, Sekt. Biol.* (in press).
- RASKI, D.J. (1950). The life history and morphology of the sugar-beet nematode, *Heterodera schachtii* Schmidt. *Phytopathology*, 40 : 135-152.
- ROGERS, W.P. (1962). *The nature of Parasitism, The Relationship of some Metazoan Parasites to their Hosts*. New York & London, Academic Press.
- ROGERS, W.P. & SOMMERVILLE, R.I. (1963). The infective stage of nematode parasites and its significance in parasitism. *Adv. Parasit.*, 1 : 109-177.
- SENGBUSCH, R., von (1927). Beitrag zur Biologie des Rübennematoden *Peterodera schachtii*. *Z. PflKrankh PflPath. PflSchutz.*, 37 : 86-102.
- SEYMOUR, M.K. & DONCASTER, C.C. (1972). Defaecation behaviour of *Aphelenchoides blastophthorus*. *Nematologica*, 18 : 463-468.
- SEYMOUR, M.K. & DONCASTER, C.C. (1978). Quantity of sap withdrawn by *Ditylenchus dipsaci*. *Rothamsted exp. Stn. Rep.* for 1977 : 172-173.
- STRUBELL, A. (1888). Untersuchungen über den Bau und die Entwicklung des Rübennematoden, *Heterodera schachtii* Schmidt. *Bibliotheca zool.*, 1 : 1-52.
- TRIANAPHYLLOU, A.C. & HIRSCHMANN, H. (1960). Post-infection development of *Meloidogyne incognita* Chitwood, 1949 (Nematoda : Heteroderidae). *Ann. Inst. phytopath. Benaki, N.S.* 3 : 3-11.

Accepté pour publication le 31 mars 1981.