Root volumes occupied by different stages of *Heterodera schachtii* in sugarbeet, *Beta vulgaris*

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Summary – Sugarbeet plants (*B. vulgaris* cv. US H-11) were grown in PVC tubing 1.5 m long and 15.2 cm diameter containing sterile, silt-loam soil infested with *Heterodera schachtii*. Complete sugarbeet root systems were recovered from the soil. Roots were selected at random, stained with acid fuschin, and the root length and diameter occupied by nematode life stages was measured and corresponding root volumes calculated. A mean root volume of 0.04 mm^3 was occupied by second-stage juveniles (J2), 0.27 mm^3 by third-stage juveniles (J3), 0.5 mm^3 by fourth-stage juveniles (J4), 0.5 mm^3 by adult females (AF), and 0.04 mm^3 by fourth-stage males (M). The mean root diameter occupied by J4 was significantly greater than that occupied by other stages. The mean root length occupied by AF was significantly greater than that occupied by all other stages except for J3. The mean root volume occupied by AF was significantly greater than that occupied by all other stages except the J4. The differences among these parameters may reflect physical and physiological constraints that limit nematode packing along the root, ant the parameters defined will be used to improve an existing simulation model.

Résumé – ***Volume occupé par les différents stades d'*****Heterodera schachtii** ***dans les racines de la betterave,*** **Beta vulgaris** – Des plants de betterave (*Beta vulgaris* cv. US H-11) sont cultivés dans des tubes de PVC (longueur : 1,5 m; diamètre : 15,2 cm) contenant une terre limoneuse stérilisée puis infestée par *Heterodera schachtii*. Le système radiculaire de la betterave est récupéré en entier. Des racines sont choisies au hasard, colorées à la fuchsine acide, puis la longueur et le diamètre occupés dans les racines par les différents stades du nématode sont mesurés et le volume radiculaire correspondant calculé. Le volume moyen occupé par chaque stade est de : 0,05 mm^3 pour le deuxième stade juvénile (J2), 0,27 mm^3 pour le troisième (J3), 0,5 mm^3 pour le quatrième (J4), 0,5 mm^3 pour les femelles adultes (FA) et 0,04 mm^3 pour le quatrième stade mâle (M). Le diamètre radiculaire moyen occupé par les J4 est significativement plus important que chez tous les autres stades. La longueur radiculaire moyenne occupée par les FA est significativement plus grande que chez tous les autres stades, à l'exception des J3. Le volume radiculaire moyen occupé par les FA est significativement plus grand que chez tous les autres stades, à l'exception des J4. Les différences dans ces paramètres pourraient être le reflet de contraintes physiques et physiologiques limitant l'agglomération des nématodes le long de la racine; les paramètres définis pourraient être utilisés pour améliorer le modèle de simulation précédemment publié.

Key-words : Nematodes, *Heterodera*, relation to root.

The sugarbeet cyst nematode, *Heterodera schachtii* Schm., is a sedentary endoparasite. Second-stage juveniles penetrate fibrous roots of sugarbeet, *Beta vulgaris* L., in proximity to lateral roots (Kämpfe, 1960). Previously uninvaded portions of the root system seem to be preferred penetration sites (Kämpfe, 1960). A multinucleate syncytium is induced in the tissues near or within the vascular cylinder (Jones, 1981; Wyss & Zunke, 1986). The syncytium is a strong sink for host assimilates and nutrients, including K, P, glucose, and amino acids, diverting energy from the host plant to the nematode (Betka & Wyss, 1982; Krauthausen & Wyss, 1982).

The minimum feeding-site root volumes required for maturation of male and female *H. schachtii* have been estimated as 0.0011 and 0.07 mm^3, respectively (Kerstan, 1969). Kerstan's minimum root volumes were used in a simulation model as parameter estimates for male and female root-carrying capacities (Caswell *et al.*, 1986).

The research presented here was conducted using *H. schachtii* infecting sugarbeet plants to define the mean (average) root volumes utilized by different stages of the nematode. The results will allow us to improve the carrying capacity parameters in the model, increasing the realism of the simulation.

Materials and methods

Sugarbeet plants (*B. vulgaris* cv. US H-11) were grown in PVC tubing 1.5 m long and 15.2 cm-diameter containing sterile silt loam soil (41.5 % sand, 52 % silt, 6.5 % clay), infested with *H. schachtii* obtained from greenhouse cultures maintained on sugarbeet. Average initial nematode population densities were established as two and six eggs per gram of soil by inoculating the soil

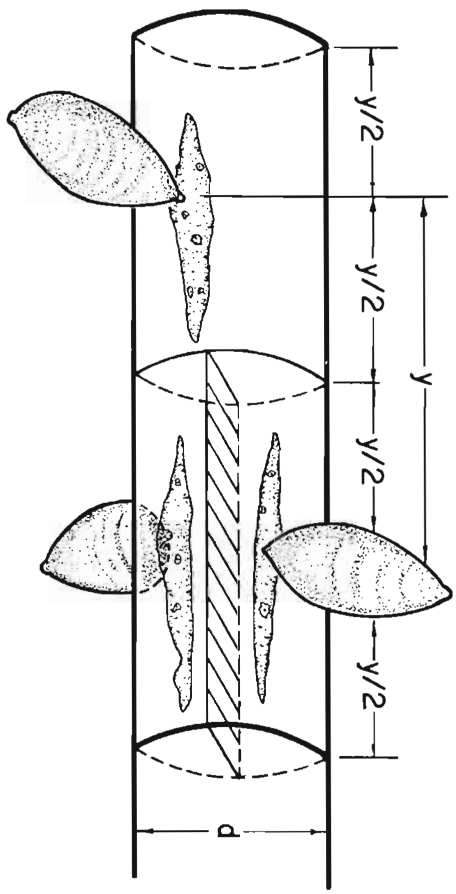

Fig. 1. The spatial model used for calculating root volumes occupied by different stages of *Heterodera schachtii* developing within roots of sugarbeet; y = the distance between the heads of adjacent nematodes measured along the longitudinal axis of a root segment, and d = root diameter.

with cysts, and subsequently sampling to confirm the population density. The entire plant-root systems from both population densities were harvested at 700, 2100, and 2500 degree/days (base 8 °C) following planting, assuring that the full range of the nematode's developmental stages were represented among the samples (Caswell & Thomason, 1991). The soil columns were released by cutting the tubes along the vertical axis, and roots were gently rinsed with water to remove adhering soil (Caswell *et al.*, 1985). Roots were selected at random from the different treatments, were stained with acid fuschin (Byrd *et al.*, 1983), and nematode stages within the roots were counted at high magnification (60 ×) under a dissecting microscope equipped with an ocular micrometer.

The root volume occupied by individual nematodes was calculated by measuring the root diameter at the nematode's head, and the distance (y) between the heads of adjacent nematodes along the longitudinal axis of a root segment (Fig. 1). Each of the respective nematodes of an adjacent pair was then allocated 1/2 this distance (y/2) on either side of its head, and this was considered the length of the root cylinder occupied by the nematode. The maximum root-length distance between any pair of nematodes included in this study was 14 mm. The volume of the cylinder was calculated using the radius (d/2) of the root at the head of the nematode. If two nematodes were opposite each other, a plane was projected across the diameter of the cylinder, and an equal root volume allotted each nematode. This approach was extended for adjacent nematodes found in different spatial arrangements and densities along the root segments.

Measurements were made on 58 second-stage juveniles (J2), 42 third-stage juveniles (J3), 24 fourth-stage females (J4), 109 adult females (AF), and 7 fourth-stage males (M). Root lengths, diameters, and volumes for each stage were subject to pairwise comparisons for unbalanced data using *t* tests (LSD, $p = 0.05$) (Anon., 1988).

Results and discussion

Initial nematode population density did not influence measured root parameters. These parameters are : root length and root volume (Table 1). The mean root diameter occupied by J4s was significantly ($p = 0.05$) greater than that occupied by other stages (Fig. 2A). The mean root length of feeding sites occupied by AFs was significantly greater ($p = 0.05$) than that occupied by all other stages except that occupied by J3s (Fig. 2B). The mean root volume occupied by AFs was significantly ($p = 0.05$) greater than that occupied by all other stages except for J4s (Fig. 2C). The differences among these parameters may reflect important physical and physiological constraints that limit nematode packing along the host root.

Kerstan (1969) reported that males of *H. schachtii* required a minimal root volume of 0.0011 mm^3, and females required 0.07 mm^3, thus the minimum root volume that supported female maturation was 63 times that for males. Acknowledging that we had a limited number of observations on males, our minimum volumes for males and for females was larger than Kerstan's. We observed females occupying minimum root volumes that were only 3.7 times greater than the minimum for males.

The body volume of a male cyst nematodes is 1/40 to 1/100 the volume of a female (Seinhorst, 1986). Although previous estimates of food consumption by *H. schachtii* are probably not entirely accurate (Wyss, 1992), studies on the feeding behavior of *H. schachtii* have suggested that developing females consume 29 times more food than do males (Müller *et al.*, 1981). The absolute energy requirements for development of

Table 1. Diameter, length and volume of root of sugarbeet occupied by juvenile and adult stages of *Heterodera schachtii*.

	J2	J3	J4	Female	Male
Root diam. (mm)	0.35 (0.12-0.67)	0.48 (0.12-0.75)	0.62 (0.27-0.75)	0.51 (0.22-0.8)	0.31 (0.17-0.75)
Root length (mm)	0.70 (0.30-4.00)	1.52 (0.06-12.6)	1.4 (0.17-6.1)	2.34 (0.07-14)	0.16 (0.07-2.3)
Root volume (mm^3)	0.04 (0.002-0.27)	0.27 (0.006-1.56)	0.5 (0.01-2.7)	0.5 (0.01-4.4)	0.04 (0.003-0.16)

male and female *H. schachtii* have not been determined. However, if nematode biomass is assumed proportional to energy requirements, and males and females have similar metabolic efficiencies, then males should require less energy for development than do females. Syncytial volume may be considered approximately proportional to the quantity of nutrients available to the parasite, and consequently males may be expected to occupy less root volume than females. Other observers have noted that the syncytia supporting male development tend to be smaller than those supporting female development (Wyss, 1992). The mean female root volumes we observed were approximately twelve times greater than that required by males. Of course, the root volumes we calculated are not equal to syncytial volumes. If approximately 1/19 of the root volume occupied is considered syncytium (Kerstan, 1969), then we estimate that males used a mean syncytial volume of 0.002 mm^3 and females 0.026 mm^3.

The female sugarbeet-cyst nematode is considered a strong sink for host assimilates (Jones, 1981; Betka & Wyss, 1982; Krauthausen & Wyss, 1982), and interference competition for space and/ or energy among developing nematodes may result in mortality (Wyss, 1992). Because males require less root volume to complete development, they may suffer less mortality due to competition for space or energy than do females. It is possible that juvenile stages were suffering high mortality in our study, as they occupied small root volumes compared to adult females; however the fact that the observed juveniles may have been from a different generation than the females eliminates our capacity to reach an explicit conclusion on this point.

Because of difficulties recovering fourth-stage males our sample size for estimating root volumes for males was small. Despite this fact, our findings support Kerstan's observation that males require less root volume, and probably less energy, than females to complete successful development. Recent research on *H. schachtii* suggests that sex is determined by trophic factors, and that under favorable conditions most juveniles develop as females (Grundler *et al.*, 1991). Our initial population densities in these experiments were close to the damage threshold for *H. schachtii* on sugarbeets in California, consequently we anticipate that the conditions for plant growth in this experiment were " favorable " for nematode development. Conditions favorable for the development of females, combined with the difficulties of recovering fourth-stage males within roots, may explain why we observed so few males in these studies.

Our previous simulation model of *H. schachtii* infecting *B. vulgaris* used a mechanistic, carrying capacity to limit nematode penetration and devolpment within roots (Caswell *et al.*, 1986). Nematodes were allowed to penetrate and develop based on the availability of roots of a given age class, and the root volume available for infection within those roots. We used Kerstan's (1969) minimum volumes as parameter estimates of carrying capacity in the simulation model, and the model yielded unrealistically high nematode population densities (Caswell *et al.*, 1986). The mean root volumes obtained from the research presented here will provide more biologically realistic estimates of the root carrying capacity for the simulation model paremeters, although they do not account for possible variation that may occur under different environmental conditions. Using the mean root volumes as the carrying capacity parameter will decrease the numbers of nematodes allowed to develop in the simulated root system, presumably resulting in more realistic population dynamics. It is possible that the model parameters describing the spatial dimensions of *H. schachtii* penetration and development may be further defined using video-enhanced contrast light microscopy (Wyss, 1992).

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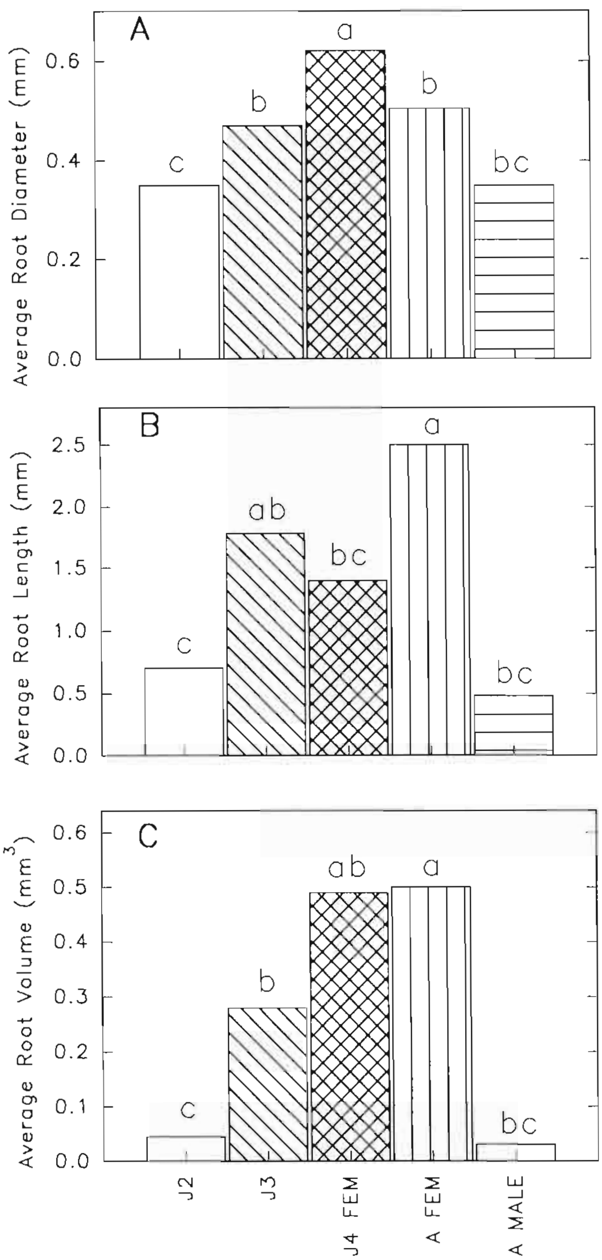

Fig. 2. A : Mean root diameters, as measured at the head of the nematode, occupied by the different stages of *Heterodera schachtii* within sugarbeet roots; J2 = second-stage juvenile; J3 = third-stage juvenile; J4 FEM = fourth-stage female; A FEM = adult female; and A MALE = fourth-stage male. Means represented by columns with different letters above them are significantly different as judged by pairwise *t*-test comparisons (p = 0.05). – B : Mean root lengths occupied by the different stages of *Heterodera schachtii* within sugarbeet roots. J2 = second-stage juvenile; J3 = third-stage juvenile; J4 FEM = fourth-stage female; A FEM = adult female; and A MALE = fourth-stage male. Means represented by columns with different letters above them are significantly different as judged by pairwise *t*-test comparisons (p = 0.05). – C : Mean root volumes required by the different stages of *Heterodera schachtii* within sugarbeet roots. J2 = second-stage juvenile; J3 = third stage juvenile; J4 FEM = fourth-stage female; A FEM = adult female; and A MALE = fourth-stage male. Means represented by columns with different letters above them are significantly different as judged by pairwise *t*-test comparisons (p = 0.05).

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