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ORIGINAL ARTICLE

# Host plant penetration, development and life cycle of a *Heterodera schachtii* population from the Western Cape province, South Africa

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## Abstract

*Heterodera schachtii* (Tylenchida: Heteroderidae), also called the sugar beet cyst nematode, are of economic importance worldwide. Oilseed rape (*Brassica napus* L.), which is a crop of global importance, suffers yield loss when it is infected with *H. schachtii*. The first report of the occurrence of *H. schachtii* in South Africa was made in 1968 on the Greater Cape Flats of the Western Cape province of South Africa, showing the widespread occurrence and the high nematode numbers on cabbage, cauliflower, beetroot and Brussels sprouts. The penetration, development and life cycle of a local *H. schachtii* population on seven vegetables (beetroot, broccoli, Brussels sprout, cabbage, cauliflower, lettuce and turnip) and three weeds (black nightshade, purslane and shepherd's purse) commonly grown, or found, in the Greater Cape Flats region of the Western Cape, South Africa, and two candidate trap crops (white mustard and oilseed radish) were examined under laboratory conditions. All the vegetables and weeds were found to be good hosts of *H. schachtii*, with the exception of lettuce, which was found to be a non-host. Oilseed radish and white mustard allowed penetration and some nematode development, but no, or little, cyst formation. Female bodies filled with eggs were observed from 31 to 34 days after inoculation onwards on all the vegetables and weeds, except for on beetroot, on which female bodies filled with eggs were observed a few days later. The results from this study are important, especially in terms of the adaptation of vegetable crop rotation cycles that are practised on the Cape Flats, so as to prevent the build-up of *H. schachtii* populations in agricultural settings.

**Keywords** Beet cyst nematode · *Heterodera schachtii* · Reproduction · Trap crops · Vegetables · Weeds

## Introduction

The beet cyst nematode, *Heterodera schachtii* Schmidt 1871, is a major pest of agricultural crops. Unlike most cyst nematodes, it has a wide host range (Steele 1965; Lilley et al. 2005; Turner and Subbotin 2013). *Heterodera schachtii* is an important pathogen of sugar beet (*Beta vulgaris* L.), oilseed rape (*Brassica napus* L.), Chinese cabbage (*Brassica rapa* subsp. *pekinensis* (L.) Hanelt) and spinach (*Spinacia oleracea* L.) (McCann 1981; Cooke 1993; Evans and Russell 1993; Müller 1999; Kakaire et al. 2012; Hauer et al. 2016; Kwon et al. 2016; Hemayati et al. 2017). At present, *H. schachtii* has been described from more than 50 countries and regions on six continents, covering all major agricultural production areas of the world (Subbotin et al. 2010; Turner and Subbotin 2013). Coetzee (1968) first reported the presence of *H. schachtii* in South Africa in the Greater Cape Flats region of the Western Cape province. Since then, its threat to vegetable production in the Western Cape in the south and to Gauteng to the north of South Africa has been established (Daiber 1990, 1992; Van Zyl and Meyer 2000). *Heterodera schachtii* is easily spread undetected in infested soil, in part due to its persistent dormant stages (consisting of microscopic cysts filled with eggs) that enable the

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nematodes to withstand desiccation for long periods (Masler and Perry 2018).

In cyst nematodes, the eggs are retained in a cyst, consisting of the body wall of the dead female (Moens et al. 2018). Inside the eggs, the embryos develop into the first-stage juveniles (J1), which moult into the second-stage juveniles (J2). Under favourable conditions, the eggs hatch within the cysts, with the J2 emerging from the cysts and migrating in the soil towards the roots of a suitable host plant. About 7 days after first penetration of the roots, the J2 moult into the third-stage juveniles (J3). At this developmental stage, the genital primordia of either a male or a female can be observed. The J3 become saccate, moulting into the fourth-stage juveniles (J4), which moult into either an adult male or female. The adult males, which revert to a non-feeding vermiform body shape, leave the roots. The adult females remain saccate, with their posterior end protruding through the root cortex in the soil. The eggs remain in the posterior, saccate part of the females. When the females die, the bodies persist in the soil as cysts that are filled with eggs, which can survive in the soil for many years. The number of generations per crop cycle varies among cyst nematode species and depends upon the coevolution of the species with its host plants, under the prevailing environmental conditions (Moens et al. 2018). In temperate regions, the life cycles are completed within about 30 days, although, in warmer climates, the duration of the life cycle may be shorter (Kakaire et al. 2012).

In addition to preventing *H. schachtii* from spreading into non-infested fields and experiencing the need to apply nematicides, a number of cultural control practices (such as crop rotation with non-hosts, resistant, tolerant, trap crops or no crops and weed management) can suppress *H. schachtii* population densities below economic threshold levels (Back et al. 2018; Pickup et al. 2018). In terms of nematode management, trap crops are plant species that stimulate hatching and that allow root penetration, but that prevent the completion of the life cycle (Back et al. 2018). Population densities of *H. schachtii* can be reduced with some brassica intercrops, such as oilseed radish (*Raphanus sativus* L. var. *oleifera*) and white mustard (*Sinapis alba* L.), albeit sometimes with mixed results (Gardner and Cashwell-Chen 1993; Hemayati et al. 2017; Wright et al. 2019).

The choice of an effective nematode management practice will also be influenced by the local production and marketing conditions. In South Africa, input costs for the production of vegetables in the Greater Cape Flats region are already high, while vegetables are most often produced in either homestead and community gardens or smallholder fields. In addition to the specific local conditions, the effective management of *H. schachtii* requires thorough knowledge of the biology of the local populations. *Heterodera schachtii* is globally distributed, with populations living in highly contrasted environments (Fournet et al. 2018). In Africa, *H. schachtii* has, apart from for South Africa, also been reported from a few countries in North (Algeria, Libya, Morocco, Tunisia) and West (Gambia, Senegal) Africa (CAB Direct 2020), but few comprehensive studies on the biology and host plant interactions of the African *H. schachtii* populations have been undertaken.

Apart from Daiber (1990, 1992), few studies of *H. schachtii* have yet been undertaken in South Africa. The host range of *H. schachtii* on vegetables and weeds in the Greater Cape Flats region (Van Zyl and Meyer 2000) indicates that the local populations can multiply on a wide range of vegetables and weeds in the region. The number and rate of J2 penetrating the roots, and the number of generations that can develop on a host plant, can be used to predict the rate of population density build-up, and subsequent crop damage and yield loss (McSorley and Duncan 1995). The main objective of the current study was to examine the penetration, development and life cycle of local *H. schachtii* on vegetables and weeds commonly grown, or found, in the Greater Cape Flats region, and on two candidate trap crops.

## Materials and methods

### Host plants

Seven vegetables, three weeds and two trap crops were included in the present study (Table 1). The seeds of the weeds were collected from the field and stored at 18 °C until use. They were germinated on moist filter paper placed in 9-cm-diameter Petri dishes at 25 °C in a growth chamber and then transplanted (as soon as the radicule was twice the diameter of the seed coat) in plastic vials for the trials.

### Cyst extraction and inoculum preparation

The *H. schachtii* population used in the current study was originally collected from a cabbage field in Lynedoch (18°65′E, 34°00′S), Western Cape province, and cultured on cabbage in a greenhouse. Cysts were extracted from the soil using the method implemented by Van Zyl and Meyer (2000). Each soil sample was passed through a 4-mm-aperture sieve to remove any coarse organic material present. The soil was then thoroughly mixed, with 500 g of the soil being air-dried at room temperature (2 ± 25 °C). Then, 50 g of the air-dried soil was added to a 1-L Erlenmeyer flask that was half-filled with water, vigorously shaken for 30 s, left for 5 min and then filled, almost to the brim, with water. After another 5 min to allow the cysts and light debris to float to the water surface in the neck of the Erlenmeyer flask, the supernatant was decanted through a pair of nested 850- and 150-µm-aperture sieves.

**Table 1** Vegetables, weeds and trap crops included in the study

| Type of host | Common name | Scientific name/variety/cultivar |
|---|---|---|
| Vegetable | Beetroot | *Beta vulgaris* L. var. *conditiva* cv. Red Ace |
| | Broccoli | *Brassica oleracea* L.var. *cymosa* cv. Viking |
| | Brussels sprouts | *B. oleracea* var. *gemmifera* cv. Odette |
| | Cabbage | *B. oleracea* var. *capitata* cv. Green Coronet |
| | Cauliflower | *B. oleracea* var. *botrytis* cv. Frisby |
| | Lettuce | *Lactuca sativa* L. cv. Snowball |
| | Turnip | *Brassica rapa* (L.) Hanelt |
| Weed | Black nightshade | *Solanum nigrum* L |
| | Purslane | *Portulaca oleracea* L |
| | Shepherd's purse | *Capsella bursa-pastoris* (L.) |
| Trap crop | White mustard | *Sinapsis alba* L. cv. Emergio |
| | Oilseed radish | *Raphanus sativus* L. cv. Adagio |

Following repetition of the entire process, the residue on the 150-µm-aperture sieve was washed with a jet of water onto fluted filter paper placed in a glass funnel. After removal of the filter paper with the collected cysts, it was dried at room temperature, with the cysts being counted, using a stereomicroscope (Leica MX75; Wetzlar, Germany) at 40 times magnification. To obtain J2, the cysts were placed in a $ZnCl_2$ solution (408 mg/L) to stimulate egg hatching (Müller 1992). The infected roots were then washed free of sand and stained with acid fuchsin (Byrd et al. 1983). The number of J2 that had penetrated and the other developmental stages (J3, J4, males, females) that had developed in the roots were counted by means of clamping the stained roots between two glass plates for visual examination, using a light microscope (Leica DM2000, Wetzlar, Germany). The developmental stages were classified as J2 (early J2) or swollen (late J2, J3) as described by Gardner and Cashwell-Chen (1993).

## Penetration of J2 and early nematode development in roots

Seedlings were transplanted in plastic vials, each containing 400 $cm^3$ of a steam-sterilised sandy soil. The vials were inoculated with a concentration of 11 J2s/$cm^3$ in Trial 1 and with a concentration of 22 J2s/$cm^3$ in Trial 2. After inoculation, the vials were placed at 25 °C in a growth chamber, which is the optimum temperature for the development of *H. schachtii* (Moens et al. 2018). At 5 days after inoculation (DAI), the root systems were gently removed from the soil, washed free of sand and stained with acid fuchsin, and the number of J2s that had penetrated the roots (early J2s) and of swollen J2s and J3s was assessed, as described above. Trial 1 consisted of 10 replicates, while Trial 2 consisted of 15 replicates.

## Nematode development in roots

Lettuce was not included in this part of the study. Seedlings were transplanted in plastic vials, each containing 600 $cm^3$ of a steam-sterilised sandy soil. The vials were inoculated with a concentration of 10 J2s/$cm^3$ in Trial 1 and with a concentration of 22 J2s/$cm^3$ in Trial 2. After inoculation, the vials were placed at 25 °C in a growth chamber. After 38 DAI, the root systems were gently removed from the soil, washed free of sand and stained with acid fuchsin, and the number of J2s (early J2s), of swollen J2s and J3s and of cysts was assessed, as described above. Trial 1 consisted of 10 replicates, while Trial 2 consisted of 15 replicates.

## Life cycle

Lettuce and the trap crops were not included in this part of the study. The experiment was conducted in a walk-in sterile room (5 × 12 m), located on the campus of Elsenburg Experimental Farm, Stellenbosch, South Africa, at an ambient temperature of 25 °C. Five hundred seeds of each plant species were germinated and grown for 2 weeks on 350 mL of White's agar medium in 1-L plastic beakers. The medium in each beaker was covered with a black plastic disc, with a slit allowing for the seed to germinate; the bottom of the beaker at the level of the agar was painted black to imitate the dark soil conditions. Continued root growth was maintained by adding, weekly, a thin layer of fresh sterile White's agar medium. After 2 weeks, the seedlings were inoculated with 50 J2s per seedling. From 1 until 50 DAI, 10 infected plants of each plant species were removed daily from the agar. The root systems were washed free of agar with running tap water and stained with acid fuchsin, after which the nematode developmental stages were counted, as described above. The cysts were then counted visually, using a stereomicroscope.

### Statistical analysis

The data obtained were checked for non-formality using the Shapiro–Wilk test (Shapiro and Wilk 1965). Fisher's least significant difference (LSD) tests were performed to separate the means at the 5% ($P \leq 0.05$) confidence level.

## Results

### Penetration of J2s and early nematode development in roots

At 5 DAI, no juveniles were extracted from the roots of the lettuce plants in both trials. For the other vegetables, the total number of nematodes penetrating the roots 5 DAI (including early J2s and swollen J2s and J3s) ranged from the lowest in turnip (66.9) in Trail 1 to the highest for cabbage (218.4) in Trial 2. In the case of weeds, purslane had the least number of nematodes penetrated (33.9). For the trap crops, the lowest penetration of nematodes was for oilseed radish (30.8) in Trail 1 and the highest (113.9) for white mustard in Trail 2. A significantly ($P \leq 0.05$) lower total number of nematodes were found in broccoli and turnip in both trials, compared with all other vegetables, except for beetroot at 5 DAI in Trial 1. The number of nematodes was significantly ($P \leq 0.05$) lower in turnip, compared with the total number in broccoli in Trial 1, and vice versa in Trial 2. Among the weeds, the number of nematodes was significantly ($P \leq 0.05$) lower in the case of purslane and shepherd's purse, compared with black nightshade in Trial 2, although no significant difference between the three weeds was observed in Trial 1. No difference in in the penetration of nematodes was observed between white mustard and oilseed radish in either of the trials. The total number of nematodes observed in both trap crops was significantly ($P \leq 0.05$) lower compared with all the vegetables in both trials, except for in the case of turnip in Trial 1, and in the case of broccoli and turnip in Trial 2, with the total number being significantly ($P \leq 0.05$) lower compared to in the case of the weeds in Trial 1 (Table 2).

At 5 DAI, the number of J2s in the oilseed radish was significantly ($P \leq 0.05$) lower compared with all the except for broccoli, turnip in the vegetables and purslane in the weed of Trial 1 and for broccoli and shepherd's purse in Trial 2. The number of J2s in white mustard was also significantly ($P \leq 0.05$) lower compared to the number with all vegetables and weeds, except for with broccoli, turnip and shepherd's purse in Trial 1. However, in Trial 2 the number of J2s in white mustard only differed significantly ($P \leq 0.05$) from broccoli and shepherd's purse and oilseed radish. In Trial 2, the number of J2s in the purslane was also significantly ($P \leq 0.05$) lower compared with the number in the oilseed radish (Table 2).

**Table 2** Penetration and development of the J2 larvae of *Heterodera schachtii*, 5 days after inoculation. The number of early second-stage juveniles (J2), penetrated, swollen nematodes, the total number of nematodes and the percentage development in the root hosts, grown at 25 °C, 5 days after inoculation. In Trial 1, a concentration of 11 J2s/cm$^3$ soil and, in Trial 2, a concentration of 22 J2s/cm$^3$ soil were inoculated

| Host | Early J2s/plant | | Penetration rate (%) | | Swollen J2s, J3s/plant | | Total nematodes/plant | | Development (%) | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Trials | | | | | | | | | |
| | 1 | 2 | 1 | 2 | 1 | 2 | 1 | 2 | 1 | 2 |
| Vegetables | | | | | | | | | | |
| Beetroot | 87.3 b | 157.7 c | 19.8 | 17.9 | 54.2 d | 37.3 cd | 141.5 bc | 195.0 c | 38.3 | 19.1 |
| Broccoli | 94.2 ab | 52.5 b | 21.4 | 6.0 | 33.2 bc | 23.2 b | 127.4 b | 75.7 a | 26.1 | 30.6 |
| Brussels sprouts | 148.1 c | 173.1 c | 33.7 | 19.7 | 42.1 c | 21.8 b | 190.2 c | 194.9 c | 22.1 | 11.2 |
| Cabbage | 155.8 c | 179.1 c | 35.4 | 20.3 | 51.4 cd | 39.3 d | 207.2 c | 218.4 c | 24.8 | 18.0 |
| Cauliflower | 121.2 bc | 167.1 c | 27.5 | 19.0 | 53.7 cd | 25.8 bc | 174.9 c | 192.9 c | 30.7 | 13.4 |
| Lettuce | – | – | – | – | – | – | – | – | – | – |
| Turnip | 43.2 a | 103.2 c | 9.8 | 11.7 | 23.7 b | 17.2 ab | 66.9 a | 120.4 b | 35.3 | 14.3 |
| Weeds | | | | | | | | | | |
| Black nightshade | 97.8 b | 177.6 c | 22.2 | 20.2 | 36.8 c | 28.1 bc | 134.6 b | 205.7 c | 27.3 | 13.7 |
| Purslane | 84.2 ab | 12.7 a | 19.1 | 1.4 | 31.5 bc | 21.2 b | 115.7 b | 33.9 a | 27.2 | 62.5 |
| Shepherd's purse | 102.7 b | 69.2 b | 23.3 | 7.9 | 19.6 b | 42.1d | 122.3 b | 111.3 ab | 16.0 | 37.8 |
| Trap crops | | | | | | | | | | |
| White mustard | 42.1 a | 113.4 c | 9.6 | 12.9 | 20.3 b | 0.5 a | 62.4 a | 113.9 b | 32.5 | 0.4 |
| Oilseed radish | 21.7 a | 75.3 b | 4.9 | 8.6 | 9.1 a | 6.5 ab | 30.8 a | 81.8 ab | 29.5 | 7.9 |

Means in a column followed by different letters are significantly ($P \leq 0.05$) different, according to Fisher's least significant difference (LSD) formula. The percentage development: the number of swollen J2s and J3s, expressed as a percentage of the total number of nematode developmental stages observed 5 days after inoculation

At 5 DAI, the penetration by J2s ranged from the lowest of 4.9% (oilseed radish) to the highest of 35.4% (cabbage) both in Trial 1. Among the vegetables, the lowest percentage penetration was observed in both trials on turnip (9.8%, 11.7%), with the exception of broccoli in Trial 2 (6%), while the highest percentage penetration was observed in both trials on cabbage (35.4%, 20.3%). In Trial 1, the lowest percentage penetration was observed on oilseed radish and white mustard; in Trial 2, a lower percentage penetration was observed in three and four vegetables and weeds, compared with in oilseed radish and white mustard, respectively. In one vegetable and two weeds, a substantial difference was observed in the percentage penetration that was observed in Trial 1 and Trial 2: 21.4 versus 6% (broccoli), 19.1 versus 1.4% (purslane) and 23.3 versus 7.9% (shepherd's purse), respectively (Table 2).

The highest percentage development at 5 DAI among the vegetables was observed in broccoli (30.6%) and among the weeds in purslane and shepherd's purse (62.5% and 37.8%, respectively). The lowest percentage development among all plant species was observed in white mustard and oilseed radish (0.4% and 7.9%, respectively). In Trial 2, the highest percentage development among the vegetables was observed in beetroot (38.3%), followed by in turnip (35.4%) and cauliflower (30.7%), and among the weeds in black nightshade (27.3%) and purslane (27.2%). The percentage development observed in white mustard and oilseed radish was 32.5% and 29.5%, respectively (Table 2).

## Nematode development in roots

At 38 DAI, the highest number of cysts extracted from the vegetables ranged from 53 (broccoli) to 179 (cabbage) in Trial 1 and from 67 (turnip) to 214 (cabbage) in Trial 2 and, from the weeds, from 53 (purslane) to 174 (black nightshade) in Trial 1 and from 89 (purslane) to 135 (shepherd's purse) in Trial 2. On oilseed radish, 14 and 3 cysts were observed in Trial 1 and Trial 2, respectively, while no cysts were observed on white mustard in either of the trials. In both trials, no significant differences were observed in the number of cysts among the vegetables and weeds, except that the number of cysts in turnip (67) was significantly ($P \leq 0.05$) lower compared to in the case of cabbage (214) in Trial 2 (Table 3).

At 38 DAI, with a few exceptions, no significant difference in the number of early J2s and swollen J2s and J3s was observed among all the plant species in either of the trials. The number of J2s ranged from 2 (broccoli) to 55 (cauliflower) in Trial 1 and from 1 (white mustard, broccoli, cabbage) to 37 (shepherd's purse) in Trial 2. In the vegetables, the percentage development ranged from 63.4% (turnip) to 97.8% (broccoli) in Trial 1 and from 95.5% (beetroot) to 99.3% (cabbage) in Trial 2; the range stretched, in the weeds, from 72.2% (black nightshade) to 96.5% (purslane) in Trial 1 and from 67.4% (shepherd's purse) to 94.8% (black nightshade) in Trial 2; in the trap crops, the range stretched from 24.6% (oilseed radish) to 65.8% (white mustard) in Trial 1 and from 91.9% (oilseed radish) to 99.6% (white mustard) in Trial 2 (Table 3).

**Table 3** The number of early second-stage juveniles (J2), swollen J2s and third-stage juveniles (J3), the percentage development and number of cysts of *Heterodera schachtii* per plant in roots of vegetables, weeds and trap crops, grown at 25 °C, 38 days after inoculation with either 10 J2s/cm$^3$ soil (Trial 1) or 22 J2s/cm$^3$ soil (Trial 2)

| Host | Early J2s/plant | | Swollen J2s, J3s/plant | | Development (%) | | Cysts/plant | |
|---|---|---|---|---|---|---|---|---|
| | Trial 1 | Trial 2 | Trial 1 | Trial 2 | Trial 1 | Trial 2 | Trial 1 | Trial 2 |
| Vegetables | | | | | | | | |
| Beetroot | 53.2 c | 7.8 ab | 92.6 abcd | 165.9 bc | 63.5 | 95.5 | 175.6 bc | 144.2 bcd |
| Broccoli | 2.2 a | 1.1 a | 99.4 abcd | 51.9 ab | 97.8 | 97.9 | 53.4 ab | 98.6 abcd |
| Brussels sprouts | 18.5 abc | 7.3 ab | 144.2 cd | 171.2 bc | 88.8 | 95.9 | 141.2 abc | 162.7 bcd |
| Cabbage | 45.8 c | 1.3 a | 157.1 d | 179.6 c | 77.4 | 99.3 | 179.2 bc | 214.1 d |
| Cauliflower | 55.2 c | 4.1 ab | 121.1 cd | 172.3 bc | 68.7 | 97.7 | 170.2 bc | 172.2 cd |
| Turnip | 24.9 abc | 3.1 ab | 43.1 abc | 108.3 abc | 63.4 | 97.2 | 112.1 abc | 67.1 abc |
| Weeds | | | | | | | | |
| Black nightshade | 37.4 abc | 9.8 ab | 97.2 abcd | 178.1 bc | 72.2 | 94.8 | 173.5 bc | 133.4 abcd |
| Purslane | 3.2 ab | 2.1 a | 87.2 abcd | 12.9 a | 96.5 | 86.0 | 53.2 ab | 89.2 abcd |
| Shepherd's purse | 21.9 abc | 37.2 b | 114.2 bcd | 76.9 abc | 83.9 | 67.4 | 118.2 abc | 135.2 bcd |
| Trap crops | | | | | | | | |
| White mustard | 21.8 abc | 0.5 a | 41.9 abc | 118.2 abc | 65.8 | 99.6 | 0 | 0 |
| Oilseed radish | 8.6 abc | 6.7 ab | 2.8 a | 75.8 abc | 24.6 | 91.9 | 13.8 a | 2.8 a |

Means in a column followed by different letters are significantly ($P \leq 0.05$) different, according to Fisher's least significant difference (LSD) formula. The percentage development: number of swollen J2s and J3s, expressed as a percentage of the total number of nematode developmental stages observed 5 days after inoculation

## Life cycle

Among the vegetables, the highest percentage of J2s that had penetrated the roots 24 h after inoculation was observed in cabbage (42%), with the lowest being in turnip (12%). Penetration by the J2s of the roots of the weeds ranged from 27 to 39%. The J2s penetrated the area of the root tips in both the lateral and the main roots, inducing the formation of numerous root hairs in both the vegetables and the weeds (Fig. 1).

Among the vegetables, the earliest onset of the second moult (from J2 to J3) was observed at 4 DAI (cauliflower), with the latest being observed at 8 DAI (Brussels sprouts). Among the weeds, the onset of the second moult started at either 7 or 8 DAI. In the vegetables, the time from J2 to J3 ranged from 7 days (cabbage) to 10 days (cauliflower); the amount of time concerned in the case of the weeds was either 7 or 8 days. Sexual differentiation (genital primordia) could be observed in the J3s of both vegetables and weeds from 11 to 13 DAI onwards, at which time the male J3 appeared relatively robust, but still elongate while the female J3 was becoming saccate. Among the vegetables, the earliest onset of the third moult (from J3 to J4) was observed at 13 DAI (cabbage), with the latest being observed at 16 DAI (Brussels sprout, turnip). Among the weeds, the onset of the third moult started at either 15 or 16 DAI. In the vegetables, the length of time from J3 to J4 was either 2 or 3 days, while, in the weeds, it was 3–5 days. No difference was observed in the time of onset of the third moult between the male and the female developmental stages. Among the vegetables, the earliest onset of the fourth moult (from J4 to adult) was observed at 16 DAI (cabbage), while the latest was observed at 19 DAI (turnip). Among the weeds, the onset of the fourth moult started at 19, 20 or 21 DAI. In the vegetables, the time from J4 to adult was 1, 2 or 3 days, while, in the weeds, it was either 3 or 5 days. No difference in the time of onset of the fourth moult between male and female developmental stages was observed (Fig. 1).

From the adult developmental stage onwards, the development of the males and females was different. The males appeared fully developed 3 to 4 days after moulting, and they started migrating out of the roots (egressing) from 3 to 5 days after the fourth moult onwards (at 24, 25 or 26 DAI) in both the vegetables and the weeds. Among the vegetables, the males that had developed in cabbage were the first to be observed egressing the roots (at 20 DAI), with the males that had developed in turnip being the last to egress (at 26 DAI). During the first 5 days, the rate of egression was high, but, thereafter, the rate of egression slowed down (data not shown). Saccate-shaped females were observed within 1 to 3 days after the fourth moult, at 18 DAI (cabbage) and at 24 DAI (turnip) in the vegetables and at either 23 or 25 DAI in the weeds. The posterior part of the females burst through the root tissues, becoming visible from the exterior, at 22 DAI in cabbage and at 25, 26 or 27 DAI in the other vegetables and weeds. Female bodies filled with eggs were observed from 31 to 34 DAI onwards in all the vegetables and weeds, except for in beetroot, in which the female bodies filled with eggs were observed much later (from 38 DAI onwards) (Fig. 1).

The first brown cysts were observed on cauliflower at 44 DAI, followed by black nightshade (45 DAI), and then on Brussels sprouts (46 DAI). On the turnip and shepherd's

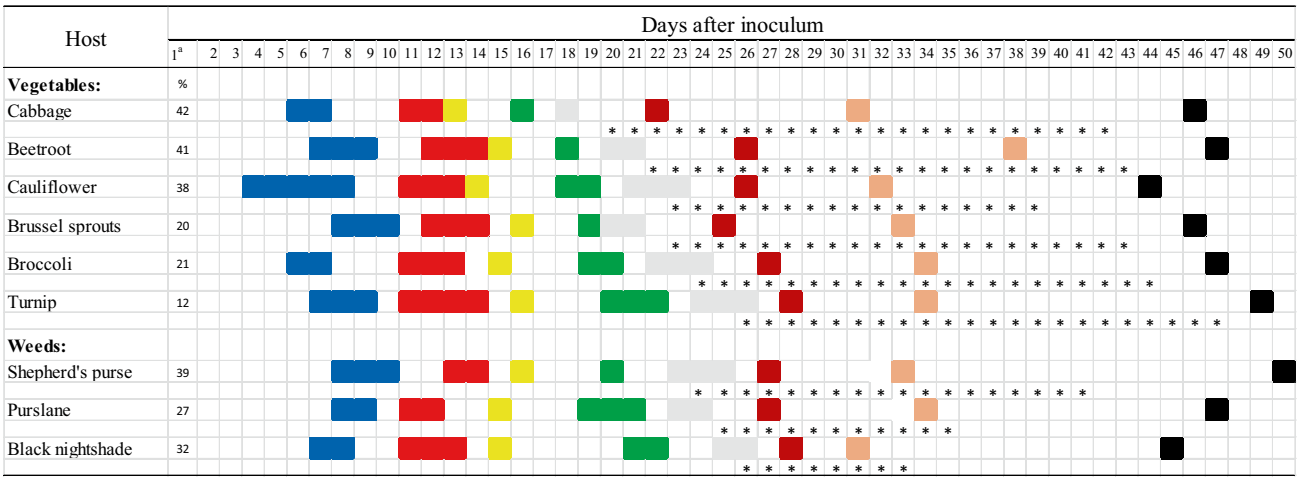


**Fig. 1** Development of a *Heterodera schachtii* population from the Greater Cape Flats, South Africa, on vegetables and weeds, grown at an ambient temperature of 25 °C and inoculated with 50 s-stage juveniles (J2) per seedling. a = % of IJ penetration after 24 h; blue = second moult (J2–J3); red = sexual differentiation; yellow = third moult (J3 to J4); green = fourth moult (J4 to adult); grey = lemon-shaped adults; maroon = female burst through tissue; pink = female body filled with eggs; black = cysts turned brown; * = males

purse, brown cysts were observed the last (at 49 and 51 DAI, respectively) (Fig. 1).

## Discussion

The J2s of the *H. schachtii* population from Lynedoch in the Greater Cape Flats were able to penetrate the roots of all vegetables, weeds and trap crops included in the current study, except for lettuce. The percentage of J2s that had penetrated the roots of the vegetables and weeds at 1 DAI (life cycle experiment) ranged from 12 to 42%. Haydock et al. (2012) found that the highest invasion rate of sugar beet roots by *H. schachtii* occurred at 20 °C to 32 °C, which indicated the temperature range to be the optimum for J2 root invasion. Root penetration (the number, percentage and rate of the J2s invading the roots) can be used to predict the rate of nematode population density build-up, and the subsequent crop damage and yield loss (McSorley and Duncan 1995). However, in the present study, the lowest percentage of root penetration at 1 DAI and the lowest number of J2s at 5 DAI (in terms of the nematode development in the roots experiment) were observed in turnip. However, at the termination of both experiments, the number of cysts and the browning of the cysts that had developed on turnip were not significantly different from what was observed in the case of the other vegetables and weeds. A similar observation was made for cabbage, on which the highest percentage root penetration was observed in both the above-mentioned experiments. However, the observed differences in percentage penetration might have had an effect on the onset of the different nematode developmental stages inside the roots, resulting in the observation that, in many instances, the higher the penetration percentage, the earlier that the different development stages were observed. However, generalising the observation can only be done with care, as there were many exceptions. For instance, on cabbage and beetroot the percentage penetration at 1 DAI was 42% and 41%, respectively, with the browning of the cysts being observed at 46 and 47 DAI. In contrast, on cauliflower, the percentage penetration at 1 DAI was 38%, with the browning of the cysts being observed at 44 DAI.

Significant differences were observed in the percentage of J2s that had penetrated the roots of the plant species included in the current study between Trials 1 and 2 at 5 DAI, such as in broccoli (21.4% vs. 6%, respectively) and in purslane (19.1% vs. 1.4%, respectively). The precise cause of the differences is unclear, since a number of differences in the experimental conditions, such as heterozygosity among the plants used in both trials, as reported by Müller (1986), may have been at the origin of the observed differences.

At 5 DAI, the numbers of J2s that had penetrated the roots of the plant species included in the present study were in both trials among the lowest in the cases of white mustard and oilseed radish, the two trap crops. The result was unexpected, as it was surmised that, as trap crops, the plant species should have the highest penetration percentages and that, after penetration, the development of the J2s should have been inhibited (Viaene et al. 2013).

The presence of swollen J2s and J3s at 5 DAI, and the further development of the J2s until the adult developmental stage, was found to be in agreement with the previous findings made (Raski 1949). Although the two trap crops were not included in the life cycle experiment, some interesting observations concerning their development could be made from the two other experiments. The slowing down of the development process could be observed at 5 DAI in Trial 1, when the percentage of the total number of the developmental stages that had developed from J2s into either swollen J2s or J3s was only 0.4% (white mustard) and 7.9% (oilseed radish) versus < 10% in the vegetables and weeds (with it even being < 30% in the broccoli, shepherd's purse and purslane). However, a similar observation could not be made in the case of Trial 2, when the percentage of the total number of developmental stages that had developed from J2s into either swollen J2s or J3s was similar in the white mustard and the oilseed radish (32.5% and 29.5%, respectively), compared with in the vegetables and the weeds (16–38.3%). In Trial 2, the inoculum level was twice the inoculum level of Trial 1, so it cannot be excluded that a higher inoculum pressure might overcome, at least during the initial stages of the development of the J2, inhibition of its development. However, at 38 DAI (in terms of the nematode in root development experiment), 99.4% and 91.9% of the white mustard and oilseed radish developmental stages, respectively, consisted of either swollen J2s and J3s in Trial 1 versus 99.3–95.9% in the vegetables and versus 72.2–96.5% in the weeds. At 38 DAI, the percentage of the total number of developmental stages that had developed from J2s into either swollen J2s or Js3 in trial 2 was 65.8% (white mustard) and 24.6% (oilseed radish) versus 63.4–97.8% in the vegetables and 72.2–96.5% in the weeds. This observation might indicate that, in Trial 1, the lowest inoculum level development of the J2s in white mustard and oilseed radish was slow in the beginning, but it picked up over time to resemble the development of the J2s of the vegetables and weeds. Since, at 38 DAI, no cysts were observed on the white mustard and only a few cysts were observed on the oilseed radish, the inhibition of the development of the juveniles must have taken place between the third moult (at the stage of the formation of the J4s) and the development of the young females into saccate-shaped, sedentary adult females. The inhibition might have included the poor development and the early death, of syncytia, thus preventing the completion of the life cycle, as was previously reported (Golinowski and Magnusson 1991; Grymaszewska and Golinowski 1998).

With a life cycle somewhat longer than 4 weeks, the *H. schachtii* population from Lynedoch in the Greater Cape Flats is able to complete two generations per crop cycle on most of the vegetables that are commonly grown in the Cape Peninsula. This observation, together with the high number of cysts developed on all the vegetables included in our study, except for lettuce, at 38 DAI (i.e. after one generation), ranging from about 50 to 180 cysts per plant in Trial 1 and from about 65 to 215 cysts per plant in Trial 2, demonstrates the high reproductive and damage potential of, and the substantial threat posed by, *H. schachtii* to vegetable production in the area concerned. Differences in the host response to *H. schachtii* infection might exist among the varieties of the vegetables included in the current study, so that they should be examined. Notably, the lettuce variety included in the present study was found to be a non-host for the Lynedoch *H. schachtii* population. The host response to *H. schachtii* infection of the most common lettuce varieties grown in the Cape Peninsula should be evaluated. Since several pathotypes of *H. schachtii* can, possibly, have developed (Griffin 1981; Müller 1992), the evaluation should include *H. schachtii* populations isolated from the major vegetable-producing areas in the Cape Peninsula. If the non-host status of lettuce to a range of *H. schachtii* populations could be confirmed, lettuce could be an efficient rotation crop to suppress the population densities of *H. schachtii* in infested fields below the damage threshold level.

Weed management is an important practice for managing cyst nematodes, including *H. schachtii*, since many weed species are good hosts of the cyst nematode (Back et al. 2018). All three weed species included in the study are good hosts of *H. schachtii*. The life cycle duration of *H. schachtii* on the weeds was similar to that on the vegetables, while at 38 DAI (i.e. after a single generation), approximately the same number of cysts had developed on the weeds compared with on the vegetables, demonstrating the high reproductive potential of *H. schachtii* on the weeds. To avoid major losses in the subsequent vegetable crops, the producers should keep their fields free of weeds.

No cysts were observed at 38 DAI in both of the trials conducted on the white mustard variety included in the present study, while a limited number of cysts (on average 14 cysts per plant in Trial 1 and 3 cysts per plant in Trial 2) had developed on the oilseed radish variety included in the study. Since the cysts of *H. schachtii* can survive in the soil in the absence of host plants for several years, a lengthy rotation with such trap crops as the oilseed radish and the white mustard varieties included in the current study are likely to stimulate hatching, and to allow for root invasion, but to prevent the completion of the life cycle of *H. schachtii*. The differences in host response to *H. schachtii* infection among the oilseed radish and the white mustard varieties have been reported (Wright et al. 2019). Therefore, as was previously mentioned in the case of the lettuce, the host response to *H. schachtii* infection of the most common white mustard and oilseed radish varieties grown in the Cape Peninsula should be evaluated, using *H. schachtii* populations isolated from the major vegetable-producing areas in the area.

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## Compliance with ethical standards

**Conflict of interest** The authors declare that they have no conflict of interest.

**Human or animal rights** This article contains no reference to any study using human participants or animals as performed by any of the authors concerned.

## References

Back MA, Cortada L, Grove IG, Taylor V (2018) Field management and control strategies. In: Perry RN, Moens M, Jones JT (eds) Cyst nematodes. CAB International, Wallingford, pp 305–336

Byrd DW Jr, Kirkpatrick T, Barker KR (1983) An improved technique for clearing and staining plant tissues for detection of nematodes. J Nematol 15:142–143

CAB Direct (2020) http://www.cabdirect.org/cabdirect

Coetzee V (1968) The distribution of the family *Heteroderidae* (Filipjev, 1934) in South Africa and some host records of *Meloidogyne* species. S Afr J Agri Sci 11:775–778

Cooke D (1993) Nematodes parasites of sugar beet. In: Evans K, Trudgill DL, Webster JM (eds) Plant parasitic nematodes in temperate agriculture. CAB International, Wallingford, pp 133–169

Daiber KC (1990) *Heterodera schachtii* in South African vegetable fields. Phytophylactica 22:133–135

Daiber KC (1992) The decline of *Heterodera schachtii* populations under conditions of vegetable production in South Africa. Phytophylactica 24:321–324

Evans K, Russell MD (1993) The population dynamics in microplots of brassica and beet cyst nematodes in rotations which include oilseed rape. Nematologica 39:411–414

Fournet S, Pellan L, Porte C, Piriou C, Grenier E, Montarry J (2018) Populations of the beet cyst nematode *Heterodera schachtii* exhibit strong differences in their life-history traits across changing thermal conditions. Front Microbiol 9:2801. https://doi.org/10.3389/fmicb/2018.02801

Gardner J, Cashwell-Chen EP (1993) Penetration, development and reproduction of *Heterodera schachtii* on *Fagopyrum esculentum*, *Phacelia tanacetifolia, Raphanus sativus, Sinapsis alba* and *Brassica oleracea*. J Nematol 25:695–702

Golinowski W, Magnusson C (1991) Tissue response induced by *Heterodera schachtii* (Nematoda) in susceptible and resistant white mustard cultivars. Can J Bot 69:53–62

Griffin GD (1981) Pathological differences in *Heterodera schachtii* populations. J Nematol 13:191–195

Grymaszewska G, Golinowski W (1998) Structure of syncytia induced by *Heterodera schachtii* Schmidt in roots of susceptible and

resistant radish (*Raphanus sativus* L. var. *oleiformis*). Acta Soc Botan Poloniae 67:207–216

Hauer M, Koch JJ, Krüssel S, Märländer B (2016) Integrated control of *Heterodera schachtii* Schmidt in Central Europe by trap crop cultivation, sugar beet variety choice and nematicide application. App Soil Ecol 99:62–69

Haydock PPJ, Kakaire S, Grove IG (2012) Effect of temperature on the life cycle of *Heterodera schachtii* infecting oilseed rape (*Brassica napus* L.). Nematology 14:855–867. https://doi.org/10.1163/156854112X631935

Hemayati SS, Akbar MRJ, Ghaemi AR, Fasahat P (2017) Efficiency of white mustard and oilseed radish trap plants against sugar beet cyst nematode. Appl Soil Ecol 119:192–196. https://doi.org/10.1016/japsoil/2017.06.017

Kakaire S, Grove IG, Haydock PPJ (2012) Effect of temperature on the life cycle of *Heterodera schachtii* infecting oilseed rape (*Brassica napus* L.). Nematology 14:855–867. https://doi.org/10.1163/156854112X631935

Kwon OG, Shin JH, Kabir FM, Lee DW (2016) Dispersal of sugar beet cyst nematode (*Heterodera schachtii*) by water and soil in highland Chinese cabbage fields. Korean J Hort Sci Technol 34:195–205

Lilley CJ, Atkinson HJ, Urwin PE (2005) Molecular aspects of cysts nematodes. Mol Plant Pathol 6:77–588. https://doi.org/10.1111/J.1364-3703.2005.00306.X

Masler EP, Perry RN (2018) Hatch, survival and sensort perception. In: Perry RN, Moens M, Jones JT (eds) Cyst nematodes. CAB International, Wallingford, pp 44–73

McCann J (1981) Threshold populations of *Heterodera cruciferae* and *H. schachtii* causing damage to cabbage seedlings. Plant Dis 65:264–266. https://doi.org/10.1094/PD-65-264

McSorley R, Duncan LW (1995) Economic thresholds and nematode management. Adv Plant Pathol 11:147–170. https://doi.org/10.1016/S0736-4539(06)80010-3

Moens M, Perry RN, Jones JT (2018) Cyst nematodes: life cycle and economic importance. In: Perry RN, Moens M, Jones JT (eds) Cyst nematodes. CAB International, Wallingford, pp 1–26

Müller J (1986) Integrated control of the sugar beet cyst nematode. In: Lamberti F, Taylor CE (eds) Cyst nematodes. Plenum Press, New York, pp 235–250

Müller J (1992) Detection of pathotypes by assessing the virulence of *Heterodera schachtii* populations. Nematologica 38:50–64. https://doi.org/10.1163/187529292X00045

Müller J (1999) The economic importance of *Heterodera schachtii* in Europe. Helminthologia 36:205–213

Pickup J, Roberts AMI, Den Nijs LJMF (2018) Quarantine, distribution patterns and sampling. In: Perry RN, Moens M, Jones JT (eds) Cyst nematodes. CAB International, Wallingford, pp 128–153

Raski DJ (1949) The life history and morphology of the sugar-beet nematode, *Heterodera schachtii*. Phytopathology 40:135–152

Shapiro SS, Wilk MB (1965) An analysis of variance test for normality (complete samples). Biometrika 52:591–611

Steele AE (1965) The host range of the sugar beet nematode, *Heterodera schachtii* Schmidt. J Am Soc Sugar Beet Technol 13:573–603

Subbotin SA, Mundo-Ocampo M, Baldwin JG (2010) Systematics of cyst nematodes (Nematoda: Heteroderinae). In: Hunt DJ, Perry RN (eds) Nematology monographs and perspectives 8B. Brill, Leiden

Turner SJ, Subbotin SA (2013) Cyst nematodes. In: Perry RN, Moens M (eds) Plant nematology, 2nd edn. CAB International, Wallingford, pp 109–143

Van Zyl J, Meyer AJ (2000) Distribution and host range of the sugar beet nematode, *Heterodera schachtii,* in the Western Cape Province, South Africa. Afr Pl Prot 6:47–51

Viaene N, Coyne DL, Davies KG (2013) Biological and cultural management. In: Perry RN, Moens M (eds) Plant nematology, 2nd edn. CAB International, Wallingford, pp 383–410

Wright DJ, Back MA, Stevens M, Sparkes DL (2019) Evaluating resistant brassica trap crops to manage *Heterodera schachtii* (Schmidt) infestations in eastern England. Pest Manag Sci 78:303–564. https://doi.org/10.1002/ps.5134