Neth. J. Pl. Path. 99(1993) 13–22

The development of juveniles of Heterodera schachtii in roots of resistant and susceptible genotypes of Sinapis alba, Brassica napus, Raphanus sativus and hybrids

C.L.C. LELIVELT* and J. HOOGENDOORN

DLO Centre for Plant Breeding and Reproduction Research (CPRO-DLO), P.O. Box 16, 6700 AA Wageningen, the Netherlands

Accepted 15 December 1992

Abstract

The development of *Heterodera schachtii* Schm. (beet cyst nematode, BCN) juveniles in roots of resistant and susceptible genotypes belonging to cruciferous crop species and hybrids was studied from 4 to 28 days after inoculation. No difference in root penetration by larvae was observed between resistant and susceptible plants.

The development of nematodes in roots from resistant plants of *Raphanus sativus* L., resistant ×*Brassicoraphanus* Sageret and a resistant hybrid ×*Brassicoraphanus* × *Brassica napus* L. was similar. BCN resistance in these three sources of plant material appeared to be related to an increased male:female nematode ratio as compared to the ratio found in susceptible *R. sativus* plants.

Also in resistant plants of *Sinapis alba* L. and a resistant intergeneric hybrid *S. alba* × *B. napus* the increase in male:female nematode ratio, as compared to the ratio found for susceptible *S. alba* cultivars and a susceptible intergeneric hybrid *S. alba* × *B. napus*, seemed to be related with the observed resistance. In roots of the resistant *S. alba* and of a resistant hybrid *S. alba* × *B. napus*, however, BCN resistance might also be due to a slower development of larvae and increased necrosis of root cells at the site of larval penetration.

Additional keywords: intergeneric crosses, male:female nematode ratio, resistance mechanism

Introduction

The white beet cyst nematode, *Heterodera schachtii* Schmidt (abbrev. BCN) is an important pathogen of sugar beet (*Beta vulgaris* L.). It can cause severe crop losses in the sugar beet growing areas of northwestern Europe (Heijbroek et al., 1988) and the USA (Steele and Savitsky, 1981). At present, resistant sugar beet varieties are not yet available. Oilseed rape, *Brassica napus* L. ssp. *oleifera* (Metzg.) Sinsk., is a tolerant, but good host for the multiplication of this nematode. For this reason, it is advisable only to include resistant oil-seed rape in a narrow rotation scheme with sugar beet as a main crop. High levels of BCN resistance have been observed, but only in related species such as *Raphanus sativus* L. ssp. *oleiferus* (DC.) Metzg. (fodder radish) and *Sinapis alba* L. (white mustard) (Baukloh, 1976; Lubberts and Toxopeus, 1982) and not in the species *B. napus* L. itself (Harrewijn, 1987).

With the aim to transfer BCN resistance to *B. napus*, sexual hybridization between *B. napus* and resistant *S. alba*, and between *B. napus* and the resistant hybrid ×*Bras-*

* Present address: Royal Sluis, Biotech Center, P.O. Box 22, 1600 AA Enkhuizen, the Netherlands.

sicoraphanus Sageret (Raparadish, AARR, $2n = 38$), derived from crosses between *Brassica rapa* (AA, $2n = 20$) and *R. sativus* (RR, $2n = 18$), has been carried out successfully (Dolstra, 1982; Lange et al., 1989; Lelivelt et al., 1993). F_1 hybrids and first backcross plants, with *B. napus* as the male recurrent parent, have been obtained. Some of these hybrids have demonstrated a high level of BCN resistance, similar to the resistant *S. alba* or ×*Brassicoraphanus* parent (Dolstra, 1982; Lange et al., 1989; Lelivelt et al., 1993).

H. schachtii is a bisexual nematode and its multiplication is dependent on the development of male and female nematodes. The life cycle has been described by Raski (1950). From the eggs, J_2 larvae hatch, penetrate root systems and establish a feeding site. After molting of J_2 larvae differentiation to male and female nematodes occurs. The J_3 male and female larvae undergo two more molts before they reach the adult stage. After fertilization of the eggs within the adult female nematodes by the adult free-living male nematodes, the females die and the eggs survive within the remains of the female bodies, which are then called cysts.

The mechanism of resistance to BCN in *S. alba* and *R. sativus* is thought to be related to an increase of the male:female nematode ratio (Müller, 1985) and not to an inability of larvae to penetrate the roots or a hypersensitivity reaction followed by necrosis and dying of the juveniles or adults. However, indications for the death of female larvae of *H. schachtii* in resistant *S. alba* plants as a consequence of necrosis of root tissue have also been reported (Lubberts and Toxopeus, 1982). Inhibition of growth and differential death rate of larvae of the two sexes, resulting in unbalanced sex ratios, have been described for many host–pathogen interactions with *Heterodera* species (Von Sengbusch, 1927; Johnson and Viglierchio, 1969; Kerstan, 1969; Bridgeman and Kerry, 1980; Steele and Savitsky, 1981). However, there is much diversity of opinion as to the explanation of the cause of the shifted sex ratios in cyst nematodes, with as a basic question whether sex determination is genetically ruled (Koliopanos and Triantaphyllou, 1972; Bridgeman and Kerry, 1980), or environmentally controlled (Ellenby, 1954; Trudgill, 1967; Müller, 1985; Grundler et al., 1991), or whether sex determination might be under both environmental and genetical control (Johnson and Viglierchio, 1969).

In this paper studies are described on the development of juveniles of *H. schachtii* in roots of resistant and susceptible intergeneric sexual hybrids with genomes from *B. napus* and *S. alba* or *B. napus* and *R. sativus*, in comparison to the development of juveniles observed in the resistant and susceptible parental species with the aim to gain more knowledge about the cause of the low multiplication rate of BCN observed in resistant species and hybrids.

Materials and methods

Plant material. Seedlings and cuttings, propagated in vitro through axillary bud or meristem culture were used as material to study host–pathogen interactions (see also Lelivelt and Krens, 1992). Table 1 presents an overview of the material used. Both seedlings and cuttings were used from a resistant population of ×*Brassicoraphanus*, from susceptible and resistant cultivars of *S. alba* L. (white mustard), from susceptible and resistant cultivars of *R. sativus* L. (fodder radish) and from a suseptible cultivar of *B. napus* L. (oil-seed rape). From three intergeneric hybrids only cuttings were used as material in experiments.

Table 1. Materials used in the experiments.

Species	Genotype/Cultivar	
	Resistant	Susceptible
Raphanus sativus	Nemex	Siletina
Sinapis alba	Maxi	Gisilba
Brassica napus	–	Jet Neuf
×*Brassicoraphanus*[a]	pop 4(b)	–
S. alba × *B. napus*[b]		
Maxi × Tantal	–	$H_{sex}3$
Emergo × Jet Neuf	$H_{sex}2$	–
×*Brassicoraphanus* × *B. napus*	hybrid	–

[a] Lange et al. 1989; [b] Lelivelt et al., 1993.

Beet cyst nematode resistance tests. Seeds were sown and cuttings were individually transplanted into 36-ml PVC tubes, filled with sterilized silver sand, moistened with Steiner I (Steiner, 1968) nutrient solution and kept in a greenhouse at a 10 h light regime, a constant temperature of 18 °C and a relative humidity of 85–90%. Twenty-one rows of eight PVC tubes with seedlings or cuttings from each genotype were randomly placed within trays in the greenhouse. After 2 weeks each seedling or cutting was inoculated with 2 ml of a suspension containing approximately 300 pre-hatched J_2 larvae of *Heterodera schachtii* Schm. using a veterinary syringe. As inoculum the population that has been the standard for the last 15 years at our institute was used, a mixture from collections carried out in 1974, 1975 and 1976 at various locations in the Netherlands, and subsequently multiplied on various susceptible plant species in the greenhouse at Wageningen. Larvae were reared on susceptible *B. vulgaris*, *B. napus* or *S. alba* plants. After inoculation the temperature in the greenhouse was raised to 22 °C during the day (Toxopeus and Lubberts, 1979).

Several preliminary tests were performed to establish the optimal sampling frequency for collecting roots with larvae of different stages and to determine the optimal conditions for staining of root tissue, which has led to the following protocol: at 4, 7, 11, 14, and 18 days after inoculation (DAI), 10 to 15 seedlings and 10 cuttings from each of the parental species, and between 5 and 10 cuttings from the three intergeneric hybrids were collected. The root systems were washed free from sand, stained for 1–1.5 min in an acid fuchsin–lactophenol solution at 80 °C in a water bath, and subsequently rinsed thoroughly with distilled water. To achieve an optimal contrast of stained larvae within colorless root tissue, redundant pigment in the root tissue was extracted by the incubation of roots in glycerol: water (50:50, v/v) during 2–3 days at room temperature followed by storage in a fresh glycerol:water solution at 6–8 °C until analysis (Goodey, 1937). For observation each root system was gently squashed between two microscopic slides and the total number of nematodes at different developmental stages, as defined by Raski (1950), was scored using a light microscope. Acid fuchsin–lactophenol solutions, water and glycerol solutions were also checked for the presence of nematodes, since larvae might be lost during the staining procedure (Müller, 1985). At 4 DAI the number of larvae that had

penetrated the root system of each seedling or cutting was scored, and at 7–14 DAI also the number of larvae at different developmental stages and the proportion of sex differentiated larvae were determined. In addition, relative numbers of larvae at different developmental stages as a percentage of the total number of larvae observed on each sampling date are presented, to be able to compare the results of seedlings and cuttings, and the results of different sampling dates. At 28 days after inoculation the root systems of ten cuttings and ten seedlings of resistant and susceptible parental genotypes and of cuttings of intergeneric hybrids were evaluated for the appearance of mature females. The J_3 and J_4 female larvae were not included in the score at 28 DAI. Therefore, the average number of mature females observed at 28 DAI is lower than the total number of females observed at 14 DAI.

Results

Penetration and establishment of second stage larvae. The observed average numbers of J_2 larvae per root system were similar for most of the susceptible and most of the resistant host plants. For all genotypes under study, a large variation between plants from the same genotype for the number of larvae penetrated into the root system was observed. Between 20 and 36% of the approximately 300 nematodes used to inoculate a plant had penetrated the roots of seedlings or cuttings at 4 DAI. Root systems of cuttings were usually much larger than roots of seedlings and were found to contain on average more J_2 larvae.

Rate of larval development. No differences between seedlings and cuttings could be observed for the relative numbers of larvae at different developmental stages. Therefore, since cuttings were tested for the hybrid genotypes, only the results of cuttings are shown in Fig. 1.

At observation dates later than 7 DAI the total number of larvae within the roots was found to have declined slightly, being 10–15% lower than the number of larvae observed in the roots at 4 DAI. To some extent this was found to be due to losses of larvae during the staining procedure. At 11 DAI mainly J_4 and at 14 and 18 DAI also adult male and female nematodes that had been washed off the roots were present in the solutions and represented approximately 5–10% of the total number of larvae observed.

At 4 DAI, swollen J_2 larvae, which had started their second molt, were found in root systems of all genotypes under study. In most susceptible genotypes this frequency was found to be higher than in resistant plants of species or hybrids. The frequencies of J_2, J_3 and of J_4/adult larvae presented in Fig. 1 also include the larvae that had started to molt. At 7 DAI root systems of plants from all species studied contained J_3 larvae and occasionally also J_4 male larvae.

At 11 DAI, the majority of larvae in root systems of susceptible genotypes and of BCN resistant ×*Brassicoraphanus*, *R. sativus* and ×*Brassicoraphanus* × *B. napus* were at the J_3 molting or the J_4 stage, whereas in roots of resistant plants of *S. alba* cv. Maxi and the resistant $H_{sex}2$ more than 50% of the larvae were still at the J_2 stage. At 14 DAI approximately 90% of the larvae present in roots of susceptible and resistant plants of species and hybrids were at the J_4/adult stage, except for resistant *S. alba* cv. Maxi and hybrid $H_{sex}2$, where 50% of the larvae had reached these developmental stages. The adult male score

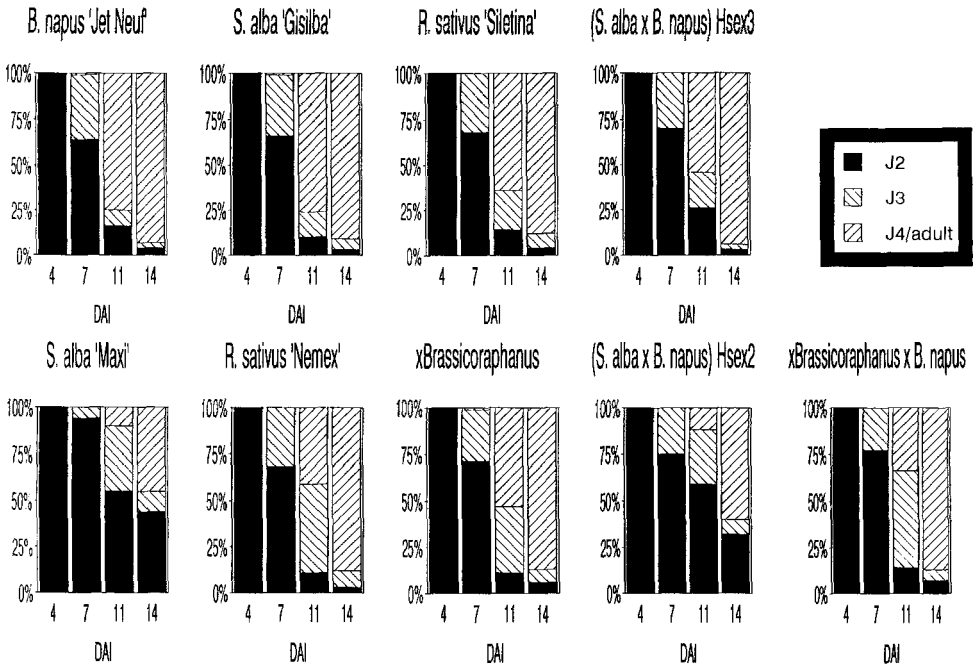

Fig. 1. Frequencies of various developmental stages of *Heterodera schachtii* Schm. larvae in root systems of resistant and susceptible cultivars of *Sinapis alba*, *Raphanus sativus*, *Brassica napus*, of ×*Brassicoraphanus* and of intergeneric hybrids *S. alba* × *B. napus* and ×*Brassicoraphanus* × *B. napus*. Data are means of 5–10 cuttings at 4, 7, 11 and 14 days after inoculation (DAI) with 300 larvae per plant.

includes the number of empty skins found in solutions or present on root systems. Since mainly empty skins of adult male larvae were observed in the solutions or attached to the root systems, and very few adult males were observed, it is assumed that the chance of overestimating the total male score will be only very low.

In roots of resistant plants of *S. alba* cv. Maxi and $H_{sex}2$ many J_2 larvae and also J_2 molting larvae were still found at 14 DAI. The development of larvae, reflected by the frequency of J_3 and J_4/adult staged larvae at different DAI, in roots of resistant plants of *S. alba* cv. Maxi seemed to be slower than in roots of susceptible plants of *S. alba* cv. Gisilba. In the resistant hybrid $H_{sex}2$ this slower development was also observed, but was less conspicuous (Fig. 1). Also, already at 7 DAI, necrosis of root cells near the head of larvae at the site of penetration could be observed in resistant plants of *S. alba* cv. Maxi. Early necrosis was less frequently seen in roots of the hybrid $H_{sex}2$ and in roots of the other resistant and susceptible genotypes. The developmental rate of larvae in roots of resistant and susceptible plants of *R. sativus* did not differ much, as could also be observed by comparison of the intergeneric hybrids ×*Brassicoraphanus* and ×*Brassicoraphanus* × *B. napus* (Fig. 1).

At 18 DAI it was difficult to determine the total number of larvae per root system, since many early-stage larvae could no longer be recognized. Also necrosis of root tissue (syncytia) around the adult male and female nematodes was observed.

Table 2. Sex differentiation of larvae from *Heterodera schachtii* Schm., in root systems of resistant and susceptible cultivars of *Sinapis alba*, *Raphanus sativus*, *Brassica napus*, of ×*Brassicoraphanus* and of intergeneric hybrids. Data are means of 5–10 cuttings at 11, 14 and 28 days after inoculation (DAI) with 300 larvae per plant.

Genotype	Sex differentiated nematodes as a percentage of the total number of larvae observed						Average number of female nematodes	
	11 DAI			14 DAI			14 DAI J_3+J_4+adult	28 DAI mature females
	Male	Female	Ratio	Male	Female	Ratio		
BCN-susceptible								
B. napus cv. Jet Neuf	30.5	49.4	0.62	36.8	57.4	0.64	49.5 (±28.6)	39.9 (±17.1)
S. alba cv. Gisilba	29.5	51.9	0.59	35.2	55.2	0.64	30.6 (±13.1)	23.6 (±11.3)
R. sativus cv. Siletina	34.6	42.0	0.82	44.3	46.4	0.95	27.5 (±12.0)	16.8 (±13.7)
S. alba × *B. napus* $H_{sex}3$	18.9	40.8	0.46	36.7	57.8	0.63	43.3 (±18.0)	37.4 (±24.0)
BCN-resistant								
S. alba cv. Maxi	10.0	0.6	16.7	39.5	5.9	6.7	3.7 (±7.5)	0.1 (±0.6)
R. sativus cv. Nemex	66.4	5.3	12.5	82.6	8.0	10.3	5.1 (±3.9)	0.2 (±1.0)
×*Brassicoraphanus*	68.3	7.7	8.9	82.9	7.1	11.7	3.7 (±4.0)	3.4 (±6.8)
S. alba × *B. napus* $H_{sex}2$	13.2	2.7	4.9	49.3	12.9	3.8	6.9 (±6.6)	0.1 (±0.6)
×*Brassicoraphanus* × *B. napus*	63.2	5.7	11.1	76.6	13.2	5.8	7.0 (±4.8)	2.6 (±6.4)

[a] 95%-confidence interval of the mean is given in parentheses.

Sex differentiation. Table 2 shows the proportion of sex differentiated larvae, i.e. the sum of sex differentiated J_3 to adult larvae relative to the total number of larvae observed in root systems. The root systems of susceptible plants of *B. napus* cv. Jet Neuf, *S. alba* cv. Gisilba and *R. sativus* cv. Siletina, and of the susceptible hybrid $H_{sex}3$ were found to contain less male than female larvae, resulting in a male:female sex ratio at 11 DAI ranging from 0.5 to 0.8. In root systems of resistant plants of *R. sativus* cv. Nemex, ×*Brassicoraphanus* and the hybrid ×*Brassicoraphanus* × *B. napus*, the proportion of sex differentiated nematodes at 11 DAI was similar to that observed in the susceptible genotypes, but since more male than female nematodes were present in root systems, the male:female ratio varied between approximately 9 and 13. For resistant plants of *S. alba* cv. Maxi and hybrid $H_{sex}2$ the frequencies of male and female nematodes at 11 DAI were much lower than those found in the other resistant and susceptible genotypes. In addition, there was an excess of male nematodes, as similarly was observed for resistant *R. sativus* and hybrids (Table 2).

The sex ratios in susceptible genotypes at 14 DAI were not much different from those found at 11 DAI. The proportion of male and female nematodes in susceptible plants of *B. napus*, *S. alba* and $H_{sex}3$ were equal, while for *R. sativus* cv. Siletina a slightly higher frequency of male nematodes and a lower frequency of females was observed. The male:female ratios at 14 DAI in resistant plants of *S. alba* cv. Maxi and in the hybrid ×*Brassicoraphanus* × *B. napus* were found to be lower than those calculated at 11 DAI, whereas for the other resistant genotypes the ratios of male:female were similar for both dates (Table 2).

The increase in the percentage of sex differentiated nematodes from 11 to 14 DAI in resistant genotypes was mainly due to an increase in the proportion of male nematodes. The largest increase in the proportion of males was observed for the resistant *S. alba* cv.

Maxi and $H_{sex}2$. For the susceptible genotypes in general an increase of both male and females was observed (Table 2). The ratio of male:female nematodes was not influenced by whether seedlings or cuttings from the parental species were used for the experiments.

At 28 DAI a clear difference in the average numbers of females on root systems of resistant and susceptible species was found (Table 2). On root systems of resistant plants occasionally a few females were visible, and in general clustered on the same lateral root.

Discussion

In the experiment described above it was investigated whether the resistance to BCN in *R. sativus* and *S. alba* can be explained by a decreased root penetration by the nematodes, an impaired larval development or a changed sex ratio.

No difference between resistant and susceptible genotypes was observed for the number of J_2 larvae of *H. schachtii* that had penetrated the root system, which is in accordance with results by Müller (1985). The differences in root size of seedlings and cuttings may explain the difference in frequency of root penetration, but was not found to have an effect on the rate of larval development or on the ultimate ratio of male to female nematodes. This indicates that, for the method applied in this study, both seedlings and cuttings are suitable to assess the level of BCN resistance.

A decrease in total number of larvae per root system, observed for all genotypes at later observation dates than 7 DAI might be explained by losses during the staining procedure, by migration of nematodes out of the roots or by death of larvae, which consequently could not be stained any more. At 4 and 7 DAI the larvae were at the J_2 and J_3 stage, harboring within the roots and not on the outside, and presumably did not come off easily by the staining procedure.

The development of juveniles of *H. schachtii* in genotypes of *R. sativus* and the intergeneric hybrids ×*Brassicoraphanus* and ×*Brassicoraphanus* × *B. napus* indicates that the mechanism of resistance to BCN in *R. sativus* is related to a shift towards a higher male:female nematode ratio, but not by a decrease of the total proportion of sex differentiated nematodes, which again is in agreement with results by Müller (1985). For *R. sativus* cv. Siletina the male:female ratio was slightly higher than that observed for susceptible *S. alba* and *B. napus*. This might be explained by the fact that some partial resistance is thought to be present within this cross-pollinating *R. sativus* cultivar (Toxopeus, personal communication, 1992). This is also indicated by the lower multiplication rate of BCN observed at 28 DAI as opposed to the other susceptible genotypes (Table 2). For *S. alba* also more males than females were observed in resistant genotypes, but the frequency of sex differentiated larvae at 14 DAI in roots of resistant plants of cv. Maxi and of *S. alba* × *B. napus* was lower than in the other resistant and susceptible genotypes (Fig. 1, Table 2). These results are in contrast to those by Müller (1985), who indicated that the mechanism of resistance to BCN in *S. alba* and *R. sativus* was similar, and only related to a shift in the sex ratio.

The observed lower frequencies of sex differentiated nematodes in resistant *S. alba* genotypes and $H_{sex}2$, as compared to the situation in susceptible *S. alba* genotypes, might be explained by a delayed penetration by larvae or by a stronger migration of J_2 larvae in search for a suitable feeding site, which ultimately results in a lower developmental rate.

An indication for these assumptions is that in resistant plants of *S. alba* or $H_{sex}2$ many larvae were still at the J_2 stage at 14 DAI. The difference in sex ratio of resistant *S. alba* and hybrid $H_{sex}2$ at 11 and 14 DAI (Table 2) are likely to be due to large differences in the total proportion of sex differentiated larvae at both observation dates, i.e. 10.6% at 11 DAI and 45.4% at 14 DAI for *S. alba* cv. Maxi, and in the large difference between the proportion of female nematodes and male nematodes in the resistant species and hybrids.

For resistant *S. alba* plants, but not for resistant *R. sativus*, also necrosis of root cells at the site of penetration and subsequent deterioration of nematodes was observed. A necrotic reaction has been observed in in-vivo experiments by Lubberts and Toxopeus (1982). The necrosis of root tissue at the site of larval penetration may be ascribed to a hypersensitive reaction of root cells during or after penetration by larvae, similar to that described for BCN invasion in monosomic additions of *Beta vulgaris* × *B. procumbens* (Heijbroek et al., 1988) and for other host–parasite interactions involving *Heterodera* and *Globodera* species (Gommers, 1981). From the presented results it remains unclear if predominantly males or females in resistant *S. alba* had died as a consequence of necrosis, since it is difficult to assess the sex of larvae at an early J_3 stage. The results of the proportion of male nematodes in resistant *S. alba*, being similar to that found in susceptible *S. alba* (Table 2), and the indication that female nematodes require approximately 40 times more food than males (Müller et al., 1981) support the assumption of death of predominantly female nematodes as a consequence of necrosis (Lubberts and Toxopeus, 1982). However, since the rate of development in resistant *S. alba* was slower than in susceptible plants, higher frequencies of males might develop after 14 DAI. Further detailed studies on the determination of the sex of stagnated J_3 larvae will be necessary to support these assumptions.

The difference in developmental rate of larvae in the experiments by Müller (1985), as opposed to the experiments reported here, might be explained by the difference in the methods used to grow the inoculated plant material. This may result in a different host plant metabolism, which is suggested to have a major impact on the development and the sex determination of *H. schachtii* (Betka et al., 1991; Grundler et al., 1991). If it is assumed that the sex determination of J_2 larvae of *H. schachtii* takes place within 2–3 DAI and that sex determination is influenced by environmental conditions, i.e. favorable conditions resulting in relatively more female determined larvae and unfavorable conditions in more males (Müller, 1985; Betka et al., 1991; Grundler et al., 1991), the present divergent results for resistant *S. alba* and *R. sativus* may be explained by a different rate of penetration and by a divergent host plant metabolism. The intermediate response for rate of larval development and necrosis in the intergeneric hybrid between a resistant plant of *S. alba* and a susceptible plant of *B. napus* would indicate the involvement of resistance genes with additive effects.

For introgression of BCN resistance in *B. napus* and subsequent elimination of undesired *S. alba* and *R. sativus* traits, further backcrossing of hybrids with *B. napus* as the recurrent parent is required. Furthermore, it will be necessary to evaluate if backcross genotypes can be selected carrying very little of the *S. alba* or *R. sativus* genome, except for those parts of the genome carrying the gene(s) for BCN resistance present in the *S. alba* or *R. sativus* parents. The results of the *S. alba* × *B. napus* hybrid and the ×*Brassicoraphanus* × *B. napus* hybrid evaluated in this study indicate nevertheless that the two different mechanisms of resistance present in *S. alba* and *R. sativus* can be transferred to hybrid and

possibly to backcross progeny, strongly suggesting that it may be possible to obtain BCN resistant oil-seed rape cultivars from these intergeneric hybrids.

Acknowledgements

The authors are grateful to Prof. J.G.Th. Hermsen, J.P.F.G. Helsper and H. Toxopeus for the comments and critical reading of the manuscript.

References

Baukloh, H., 1976. Untersuchung zur Wirtspflanzeneignung der Kruziferen gegenüber dem Rübennematoden, *Heterodera schachtii* (Schmidt), unter besonderer Berücksichtigung der Resistenzzüchtung. Dissertation Georg-August-Universität Göttingen. 72 pp.

Betka, M., Grundler, F. & Wyss, U., 1991. Influence of changes in the nurse cell system (syncytium) on the development of the cyst nematode *Heterodera schachtii*: single amino acids. Phytopathology 81: 75–79.

Bridgeman, M.R. & Kerry, B.R., 1980. The sex ratios of cyst-nematodes produced by adding single second-stage juveniles to host roots. Nematologica 26: 209–213.

Dolstra, O., 1982. Synthesis and fertility of ×*Brassicoraphanus* and ways of transferring *Raphanus* characters to *Brassica*. Agricultural Research Reports 917, 90 pp.

Ellenby, C., 1954. Environmental determination of the sex ratio of a plant parasitic nematode. Nature 174: 1016–1017.

Gommers, F.J., 1981. Biochemical interactions between nematodes and plants and their relevance to control. Helminthological Abstracts. Series B, Plant Nematology, 50 (1): 9–24.

Goodey, T., 1937. Two methods for staining nematodes in plant tissues. Journal of Helminthology 15: 137–144.

Grundler, F., Betka, M. & Wyss, U., 1991. Influence of changes in the nurse cell system (syncytium) on sex determination and development of the cyst nematode *Heterodera schachtii*: total amounts of proteins and amino acids. Phytopathology 81: 70–74.

Harrewijn, J.L., 1987. Screening for resistance against the beet cyst nematode, *Heterodera schachtii* Schm., in *Brassica napus* L. Mededelingen van de Faculteit voor Landbouwwetenschappen van de Rijksuniversiteit Gent 52 (2a): 587–592.

Heijbroek, W., Roelands, A.J., De Jong, J.H., Van Hulst, C., Schoone, A.H.L. & Munning, R.G., 1988. Sugar beets homozygous for resistance to beet cyst nematode (*Heterodera schachtii* Schm.), developed from monosomic additions of *Beta procumbens* to *B. vulgaris*. Euphytica 38: 121–131.

Johnson, R.N. & Viglierchio, D.R., 1969. Sugar beet nematode (*Heterodera schachtii*) reared on axenic *Beta vulgaris* root explants. II. Selected environmental and nutritional factors affecting development and sex-ratio. Nematologica 15: 144–152.

Kerstan, U., 1969. Die Beeinflussung des Geschlechterverhältnisses in der Gattung *Heterodera*. II. Minimallebensraum – selektive Absterberate der Geschlechter – Geschlechterverhältnis (*Heterodera schachtii*). Nematologica 15: 210–228.

Koliopanos, C.N. & Triantaphyllou, A.C., 1972. Effect of infection density on sex ratio of *Heterodera glycines*. Nematologica 18: 131–137.

Lange, W., Toxopeus, H., Lubberts, J.H., Dolstra, O. & Harrewijn, J.L., 1989. The development of Raparadish (_*Brassicoraphanus*, 2*n*=38), a new crop in agriculture. Euphytica 40: 1–14.

Lelivelt, C.L.C. & Krens, F.A., 1992. Transfer of resistance to the beet cyst nematode (*Heterodera schachtii* Schm.) into the *Brassica napus* L. gene pool through intergeneric somatic hybridization with *Raphanus sativus* L. Theoretical and Applied Genetics 83: 877–894.

Lelivelt, C.L.C., Leunissen, E.H.M., Frederiks, H.J., Helsper, J.P.F.G. & Krens, F.A., 1993. Transfer of resistance to the beet cyst nematode (*Heterodera schachtii* Schm.) from *Sinapis alba* L. (white mustard) to the *Brassica napus* L. gene pool by means of sexual and somatic hybridization. Theoretical and Applied Genetics (in press).

Lubberts, J.H. & Toxopeus, H., 1982. [The development of beet cyst nematode-resistant fodder rape and white mustard]. Zaadbelangen 36(3): 66–69. In Dutch.
Müller, J., 1985. Der Einfluss der Wirtspflanze auf die Geschlechtsdeterminierung bei *Heterodera schachtii*. Mitteilungen der Biologischen Bundesanstalt für Land- und Forstwirtschaft 226: 46–63.
Müller, J., Rehbock, K. & Wyss, U., 1981. Growth of *Heterodera schachtii* with remarks of amounts of food consumed. Revue Nematologie 4: 227–234.
Raski, D.J., 1950. The life history and morphology of the sugar-beet nematode, *Heterodera schachtii* Schmidt. Phytopathology 40: 135–152.
Steele, A.E. & Savitsky, H., 1981. Resistance of trisomic and diploid hybrids of *Beta vulgaris* and *B. procumbens* to the sugarbeet nematode, *Heterodera schachtii*. Journal of Nematology 13 (3): 352–357.
Steiner, A.A., 1968. Soilless culture. Proceedings of the 6th Colloquium of the International Potash Institute, Florence. p. 324–341.
Toxopeus, H. & Lubberts, J.H., 1979. Breeding for resistance to the sugar beet nematode (*Heterodera schachtii* Schm.) in cruciferous crops. Proceedings Eucarpia *Cruciferae* Conference, Wageningen (Post Conference Edition), p. 151.
Trudgill, D.L., 1967. The effect of environment on sex determination in *Heterodera rostochiensis*. Nematologica 13: 263–272.
Von Sengbusch, R., 1927. Beitrag zur Biologie des Rübennematoden *Heterodera schachtii*. Zeitschrift für Pflanzenkrankheiten und Pflanzenschutz 37: 86–102.