

Reproduction of *Heterodera schachtii* Schmidt on Resistant Mustard, Radish, and Sugar Beet Cultivars¹

HEIDI J. SMITH,² FRED A. GRAY,³ AND DAVID W. KOCH⁴

Abstract: The reproduction of a Wyoming population of *Heterodera schachtii* was determined for resistant trap crop radish (*Raphanus sativus*) and mustard (*Sinapis alba*) cultivars, and resistant and susceptible sugar beet (*Beta vulgaris*) cultivars in a greenhouse (21 °C/16 °C) and a growth chamber study (25 °C). Oil radish cultivars also were field tested in 2000 and 2001. In the greenhouse study, reproduction was suppressed similarly by the resistant sugar beet cultivar Nematop and all trap crop cultivars ($P \leq 0.05$). In the growth chamber study, the radish cultivars were superior to most of the mustard cultivars in reducing nematode populations. All trap crops showed less reproduction than Nematop ($P \leq 0.05$). In both studies, Nematop and all trap crops had lower Pf than susceptible sugar beet cultivars HH50 and HM9155 ($P \leq 0.05$). In field studies, Rf values of radish cultivars decreased with increasing Pi of *H. schachtii* ($r^2 = 0.59$ in 2000 and $r^2 = 0.26$ in 2001). In 2000, trap crop radish cv. Colonel (Rf = 0.89) reduced nematode populations more than cv. Adagio (Rf = 4.67) and cv. Rimbo (Rf = 13.23) ($P \leq 0.05$) when Pi was lower than 2.5 *H. schachtii* eggs and J₂/cm³ soil. There were no differences in reproductive factors for radish cultivars in 2001 ($P \leq 0.05$); Rf ranged from 0.23 for Adagio to 1.31 for Commodore for all Pi.

Key words: *Beta vulgaris*, cultivar, *Heterodera schachtii*, management, mustard, oil radish, *Raphanus sativus*, reproduction, resistance, *Sinapis alba*, sugar beet cyst nematode, trap crop.

The sugar beet nematode (SBN) (*Heterodera schachtii* Schmidt) is one of the most serious pests of sugar beet (*Beta vulgaris* L.) in the western United States (Griffin, 1981b) and most other sugar beet production areas worldwide, especially where beets have been grown for an extended number of years (Cooke, 1991). This nematode pest causes severe yield reductions when population densities are high (Griffin, 1981a). Crop losses can be reduced by cultural methods such as sanitation and crop rotation, and with pesticide applications.

A recent approach to *H. schachtii* control in the United States is the planting of resistant cultivars of oil radish (*Raphanus sativus* var. *oleifera* L.) and yellow mustard (*Sinapis alba* L.) as trap crops. Trap cropping with resistant radish and mustard cultivars has been practiced in German and other European sugar beet production areas since the 1980s (Schlang, 1985). In Germany, all newly developed trap crop cultivars are rated for resistance to the SBN by the Federal Research Center for Agriculture and Forestry (BBA, Biologische Bundesanstalt für Land- und Forstwirtschaft) (Bundessortenamt, 2002; Müller and Rumpfenhorst, 2000). Nematode soil populations are determined in closed containers before and after growing a trap crop cultivar. Reproductive factors (Rf = Pf/Pi) for each cultivar are calculated and standardized to those of standard resistant trap crop cultivars. Resistance ratings are assigned according to the Rf: 1 = Rf less than 0.1, 2 = Rf from 0.1 to 0.3, and 3 = Rf from 0.31 to 0.5. Cultivars

with Rf above 0.5 are classified as susceptible. Only cultivars with Rf values less than 0.5 are federally registered as resistant in Germany. However, no such restriction has been proposed on varieties being used in the United States.

Schlang (1985, 1989, 1997) observed a high correlation between initial population density and Rf value under field conditions. Reproductive factors increased with low initial populations and decreased with high initial populations. The nematode equilibrium density (Pi at an Rf value of 1.0) indicates the host status of a plant (Ferris, 1985).

Gardner and Caswell-Chen (1993) showed that development of the SBN was suppressed by resistant trap crop cultivars under a controlled environment. Resistant trap crops also have proven effective in decreasing soil populations of *H. schachtii* in Wyoming (Gray et al., 1994, 1997; Koch and Gray, 1997; Koch et al., 1998), in Idaho (Hafez, 1994; Hafez and Sundararaj, 1998, 1999, 2000, 2001), and in Nebraska (Kerr et al., 1995). Currently, the oil radish cultivar Colonel is being used in northwestern Wyoming in sugar beet rotations for management of the SBN.

Another means for controlling the SBN in sugar beets is host resistance, which has only recently become available in Europe (Müller, 1999). In 1996, *H. schachtii*-resistant sugar beet cultivars Evasion and NemaKill were released in France followed in 1998 by the registration of the SBN-resistant cultivar Nematop in Germany (Schlang, 1999). No SBN resistance has been found in *Beta vulgaris* (Roberts, 1992). Savitsky (1975) was the first to produce viable hybrids by the crossing of the wild species *B. procumbens* and *B. vulgaris*. The U.S. Department of Agriculture released Savitsky's cyst nematode resistant sugar beet breeding lines in 1982 (Doney, 1995). Breeding programs in Europe have been using these lines as well as producing further crosses (Heijbroek et al., 1988; Lange and De Bock, 1994; Lange et al., 1990; Löptien, 1984, 1985). Sugar

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² Graduate Assistant, Department of Plant Sciences, University of Wyoming, Laramie, WY 82071.

³ Professor of Plant Pathology, Department of Plant Sciences, University of Wyoming, Laramie, WY 82071.

⁴ Professor of Agronomy, Department of Plant Sciences, University of Wyoming, Laramie, WY 82071.

E-mail: hjsmith@uwyo.edu

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beet cultivars with resistance to the SBN are currently not commercially available in the United States.

Control of *H. schachtii* with resistant trap crops and sugar beet cultivars would reduce environmental risks associated with pesticides (Griffin, 1987; Thomason, 1987) and decrease sugar beet production cost (Held et al., 2000). However, physiological variation is present in *H. schachtii* (Griffin, 1981c), and it is not clear how sugar beet and trap crop cultivars resistant to German SBN populations respond to Wyoming SBN population. The purpose of this study was to evaluate SBN-resistant oil radish and yellow mustard cultivars, and one resistant sugar beet cultivar, for their potential to control Wyoming populations of the sugar beet cyst nematode.

MATERIALS AND METHODS

Two experiments were designed to compare nematode reproduction on resistant oil radish and mustard cultivars and resistant and susceptible sugar beet cultivars in a controlled environment. Information on crop cultivars entered into the studies are shown in Table 1. The resistance ratings presented are according to evaluations done in Germany (Bundessortenamt, 2002; Müller and Rumpfenhorst, 2000).

Experiment 1. Reproduction in the greenhouse: The experiment was a randomized complete block design with eight replications, and each plant was one experimental unit. Four seeds of each cultivar were planted per round pot (15-cm top diam., 10.5-cm bottom diam., 14 cm deep, 1,100 cm³ soil volume) filled with a pasteurized (1:1, v/v) sand and soil mixture (88% sand, 9% silt, 3% clay; pH 7.7). Radish and mustard cultivars were thinned to 1 plant/pot 13 days after planting (DAP), and sugar beet cultivars were thinned 16 DAP. Plants were held at 21 °C during the day and 16 °C during the night with ambient natural light. Pots were irrigated as needed and fertilized weekly with a

0.0002% 21-18-18 N-P-K fertilizer solution. The insecticide malathion, which has shown not to be nematocidal to several plant-parasitic nematodes (Elgin and Evans, 1975), was applied foliarly at a concentration of 400 mg a.i./liter starting 9 weeks after planting in 4 successive weeks for aphid control.

Inoculum was extracted from SBN populations maintained on HH50 sugar beets in a greenhouse by crushing the cysts and sieving to catch eggs and juveniles (Halbrendt et al., 1987). A total of 4,260 eggs and second-stage juveniles (J2) of *H. schachtii* were applied to each plant by pipeting aliquots into 3-cm deep holes in the soil around the basal stem between 29 DAP and 57 DAP: 1,720 eggs and J2 in a 5-ml aliquot 29 DAP, 1,000 eggs and J2 in a 5-ml aliquot 35 DAP, and 1,540 eggs and J2 in a 10-ml aliquot 57 DAP. This resulted in an initial nematode density of 3.87 eggs and J2/cm³ soil.

Plants were maintained under described conditions for 92 days to allow for nematode reproduction. Plant shoots were removed 1 cm above the soil level, successively block by block (block one and two at 92 days, block three and four at 102 days, block five and six at 118 days, and block seven and eight at 134 days after last inoculation). After shoot removal, pots with the remaining roots and soil were stored in a refrigerator at 6 °C, and nematodes were extracted within 2 weeks. Roots were removed and attached soil was collected by shaking into a plastic tray. Roots were then gently rinsed with tap water and observed for female nematodes using a 10× illuminated magnifying glass (Luxo Magnifier, Luxo Lamp Corp., Port Chester, NY). Soil was moved from each pot into the tray and thoroughly mixed, and two subsamples of 250 cm³ each (total of 500 cm³) were taken for nematode extraction. Each subsample was placed on a 2-mm-pore sieve. The soil was washed through the sieve into a 10-liter bucket. Stones and debris in the sieve were discarded. The sieved soil and water were placed into a flotation can

TABLE 1. Trap crop radish and mustard cultivars and sugar beet cultivars evaluated in the greenhouse and the growth chamber studies.

Crop	Cultivar	Origin	Resistance rating
Trap crop oil radish	Adagio	Petersen Saatzzucht, Lundsgaard, Germany	2 ^a
	Arena	Seed Research of Oregon, Corvallis, Oregon	2 ^a
	Colonel	Petersen Saatzzucht	1 ^a
	Commodore	Petersen Saatzzucht	1 ^b
	Rimbo	Seed Research of Oregon	2 ^a
Trap crop mustard	Concerta	Petersen Saatzzucht	2 ^a
	Metex	Petersen Saatzzucht	2 ^{bc}
	Rivona	Seed Research of Oregon	— ^c
	Salvo	Seed Research of Oregon	2 ^a
	Serval	Seed Research of Oregon	2 ^a
	Vertus	Seed Research of Oregon	— ^c
Sugar beet	Nematop	Hilleshog (Syngenta)	resistant ^a
	HH50	Holly Sugar (Imperial Sugar)	susceptible ^d
	HM9155	Hilleshog (Syngenta)	susceptible ^d

^a According to Bundessortenamt (2002).

^b According to Petersen Saatzzucht, Germany: Rating system for resistance in trap crops: 1 = Rf < 0.1, 2 = Rf from 0.1 to 0.3.

^c No rating available.

^d Unknown but presumed to be susceptible since no SBN resistance has been reported in *B. vulgaris* (Roberts, 1992).

apparatus (Roberts and Thomason, 1981) and processed for 4 minutes. Cysts were collected on a 250- μm -pore sieve. Males, juveniles, and eggs were collected on a 25- μm -pore sieve attached below the 250- μm -pore sieve. The cysts located on the sieve surface of the 250- μm -pore sieve were crushed by gently rubbing with a rubber stopper to liberate eggs and juveniles (Halbrendt et al., 1987). The residue on the 25- μm -pore sieve was collected into a beaker. The residue of the second 250- cm^3 subsample was rinsed into the same beaker. After nematodes were separated from remaining soil by centrifugal flotation (Jenkins, 1964), eggs, juveniles, and males were collected in a 25- μm pore sieve and counted using a compound microscope at 100 \times magnification. Final *H. schachtii* populations recovered from each pot were extrapolated.

Variances were unequal and blocks were not efficient; therefore, data were analyzed as weighted one-way analysis of variance (weight = $1/\sqrt{s^2}$) set in a completely randomized design using the general linear models (GLM) procedure in SAS (SAS Institute, Cary, NC). Treatment means were separated on basis of Student's *t*-test ($P \leq 0.05$).

Experiment 2. Reproduction in the growth chamber: To increase nematode reproduction, a number of changes from experiment 1 were made: (i) Soil volume was decreased and initial nematode density was increased, (ii) plants were inoculated at a younger age, and (iii) temperature was increased. The test was a randomized complete block design with 16 replications, and each plant was one experimental unit. Cultivars were planted as 1 seed/pot (Conetainer: 3.8-cm top diam., 2.5-cm bottom diam., 21 cm deep, 164- cm^3 volume, Stuewe & Sons, Corvallis, OR) filled with a pasteurized sand and soil mixture. Pots were placed in a 30-cm by 60-cm holding rack (56 plants/rack) and maintained in a growth chamber (Puffer-Hubbard Environmental, Weaverville, NC) at 25 °C with the following 24-hour light cycle: 1 hour low-light intensity (6 incandescent Slimlite 60-W bulbs), 1 hour medium-light intensity (6 bulbs plus 8 fluorescent Slimlite 160-W bulbs), 10 hours full-light intensity of 500 $\mu\text{E m}^2 \text{sec}^{-1}$ at the pot surface (6 incandescent bulbs plus 16 fluorescent bulbs), 1 hour medium-light intensity, 2 hour low-light intensity, and 10 hours dark. The distance from the pot rim to the lights was 100 cm. Pots were irrigated daily and fertilized weekly with a 0.0002% 15-30-15 N-P-K fertilizer solution (Miracle Gro, Port Washington, NY). Inoculum from the same source was extracted as described under experiment 1. Plants were inoculated by pipeting 2-ml aliquots containing *H. schachtii* eggs and J2 into 3-cm-deep holes in the soil around the basal stem: 550 eggs and J2 11 DAP and 2,000 eggs and J2 17 DAP. This resulted in a total of 2,550 eggs and J2/pot and an initial nematode density of 17 eggs and J2/ cm^3 soil.

Plant shoots from blocks 1 to 16 were cut 1 cm above soil level 33, 36, 41, 49, 64, 72, 80, 93, 99, 107, 113, 117,

130, 135, 144, and 150 days after the second inoculation, respectively. Containers with soil were stored at 6 °C until they were processed to extract *H. schachtii*, generally within 14 days after shoot removal. Soil and roots were placed on three stacked sieves (2-mm-pore, 250- μm -pore, and 25- μm -pore), and roots were gently rinsed free from soil. Females were removed from the roots using a dissecting microscope at 10 \times and counted. Females were crushed in a glass tissue grinder (7-ml Pyrex Tenbroek, Corning, NY), and released eggs and J2 were counted using a 1-ml counting slide (Advanced Equine Products, Issaquah, WA). Sugar beet cyst nematodes of all growth stages were extracted from soil collected on the three stacked sieves (2-mm, 250- μm , and 25- μm) and counted as described for experiment 1. The final nematode density (Pf) was considered to be the sum of eggs and J2 from females plus nematodes of all growth stages extracted from the soil. The reproductive factor

$$Rf = \left(\frac{Pf}{Pi} = \frac{Pf}{\text{number of eggs} + \text{J2 inoculated}} = \frac{Pf}{2,550} \right) \text{ was calculated.}$$

Data on the number of females per root system and reproductive factors were subjected to analysis of variance using the SAS general linear model procedure. Because the variances were not equal and the blocks were not efficient, both variables were analyzed as weighted one-way analysis of variance (weight = $1/\sqrt{s^2}$) set in a completely randomized design. Cultivar means were separated on the basis of Student's *t*-test ($P \leq 0.05$).

Field trials: An experiment was conducted on two adjacent sugar beet production fields in cooperation with John Snyder of the Sage Creek Land & Cattle Company, near Worland, Wyoming, in 2000 (trial 1, field 1) and was repeated in 2001 (trial 2, field 2). The soil of field 1 was a fine-loamy over sandy, mixed, superactive, mesic Typic Haplargid with 59% sand, 18% silt, 23% clay, 1.2% organic matter, and pH 7.9. Field 2 soil was a fine-loamy, mixed, superactive, calcareous, mesic Typic Torrifluent with 39% sand, 36% silt, 25% clay, 1.5% organic matter, and pH 7.8. The *H. schachtii*-resistant radish cultivars Adagio, Arena, Colonel, Commodore, and Rimbo were evaluated for their efficiency in reducing SBN soil populations. Only radish cultivars were tested because this was the trap crop of choice of the local sugar beet growers. Both trials were arranged in a randomized complete block design with five replications. Both test sites had been in a barley-sugar beet rotation for the previous 3 years. The plots measured 9.14 m by 2.13 m. The 9.1-m wide borders and 6.1-m wide alleys between blocks were seeded to the SBN-resistant mustard cultivar Metex. After barley harvest in early August 2000 and 2001, fields were irrigated and

cultipacked. Radish was seeded in both years on 15 August at 25 kg/ha with a double-disk drill. The entire field surrounding the test site was planted to Adagio radish in 2000 and to Colonel radish in 2001 at 25 kg/ha. Ammonium nitrate was applied through the drill at seeding at 67 kg N/ha to the field and to the test site. Following seeding, the field was irrigated with 20 mm of water through a center pivot sprinkler irrigation system, and soil moisture for good plant growth was maintained for 10 weeks with subsequent irrigation until the end of October. Prior to planting on 15 August and after growing trap crops on 21 October 2000 for trial 1 and on 6 October 2001 for trial 2, 12 soil cores to a depth of 30 cm were taken per plot with a 2.5-cm-diam. soil probe and combined, following an x-shaped pattern within plots. Each soil sample was thoroughly mixed, passed through a 4-mm-pore sieve, and allowed to dry at room temperature. A subsample of 200 cm³ was randomly taken after the soil was mixed for 3 minutes in a rotary mixer (Twin shell dru blender, The Patterson Kelley Company Inc., East Stroudsburg, PA). Cysts were extracted from the subsample using a semi-automatic elutriator (Byrd et al., 1976) with a 2-mm-pore sieve for collecting debris nested over a 250- μ m-pore sieve for collecting the cysts. Cysts from the 200-cm³ subsample were crushed in a glass tissue grinder, and released eggs and J2 were counted. This number was multiplied by a correction factor of 1.47 (previously determined by the authors) to adjust for the extraction efficiency. Preplant and final nematode densities were expressed as eggs and juveniles per cm³ of field soil, and reproductive factors were determined.

Preplant nematode densities varied greatly among plots in both years. *Heterodera schachtii* populations in 2000 increased ($R_f > 1$) in most plots where P_i was below a threshold of 2.5 eggs + J2/cm³ soil and decreased ($R_f < 1$) in all plots where P_i was above 2.5 eggs + J2/cm³ soil. Therefore, a binary threshold term was included in the 2000 data analysis: 1 for $P_i < 2.5$ eggs + J2/cm³ soil and 2 for $P_i > 2.5$ eggs + J2/cm³ soil. Because variances were not equal and blocks were not efficient, R_f data were analyzed as weighted analysis of variance (weight = $1/\sqrt{s^2}$) set in a completely randomized design. Due to two P_i values of zero, only 23 observations could be included in the analysis: cultivars Adagio, Colonel, and Commodore with $n = 5$ and cultivars Arena and Rimbo with $n = 4$. The SAS general linear model procedure was used for all statistical analyses with the least square means statement to determine significant differences between cultivar means ($P \leq 0.05$).

In 2001, *H. schachtii* populations increased in only four plots with $P_i < 2.7$ eggs and J2/cm³ soil and decreased in all plots with $P_i > 2.7$ eggs and J2/cm³ soil. However, the threshold factor of 2.7 eggs + J2/cm³ soil was not significant ($P \geq 0.05$) when added to an analysis of variance. Therefore, the covariate P_i was included,

and R_f data were analyzed as analysis of covariance set in a randomized complete block design. Because two plots had P_i values of zero, only 23 observations were used in the analysis: cultivars Colonel, Commodore, and Rimbo with $n = 5$ and cultivars Adagio and Arena with $n = 4$. The SAS general linear model procedure was used for statistical analyses.

RESULTS

Experiment 1: Reproduction in the greenhouse: No *H. schachtii* females were detected on the rinsed roots of any entry. Nematode reproduction could be verified only for one plant of the susceptible sugar beet cultivar HH50. Average P_f values per container were similar for all trap crops ($P > 0.05$) and ranged from 32.7 for Commodore radish to 144.3 for Rivona mustard (Table 2, Greenhouse). Average P_f values of the sugar beets were 59.2 for Nematop, 830.4 for HM9155, and 2078.4 for HH50. The SBN-resistant cultivar Nematop and all trap crop cultivars were similar with regard to reducing soil populations of the SBN ($P > 0.05$) and had lower P_f values than the susceptible beet cultivars ($P \leq 0.05$). Final densities of the SBN-susceptible sugar beets varied, with HH50 having a higher P_f than HM9155 ($P \leq 0.05$).

Experiment 2: Reproduction in the growth chamber: The number of females per trap crop plant ranged from 0.1 for Commodore radish to 8.4 for Serval mustard (Table 2, Growth chamber). Numbers of females per sugar beet root system ranged from 64.6 for SBN-resistant Nematop to 249.4 for susceptible HH50. Numbers of females per root were similar for all radish cultivars, as well as the mustard cultivars Concerta and Salvo ($P \leq 0.05$). All radishes and Concerta mustard supported fewer females per root than mustard cultivars Rivona, Vertus, Metex, and Serval that were equal in number of females ($P \leq 0.05$). For mustard cultivars, Salvo had as many females as Rivona and Vertus but fewer than Metex and Serval ($P \leq 0.05$). Roots of the SBN-resistant Nematop carried more females than any of the trap crop cultivars but fewer than SBN-susceptible HH50 and HM9155 ($P \leq 0.05$). Numbers of *H. schachtii* females per root of HH50 and HM9155 were not different ($P > 0.05$).

Reproductive factors ranged from 0.08 for Colonel radish to 0.94 for Metex mustard and from 3.55 for the resistant sugar beet Nematop to 16.2 for the susceptible sugar beet HH50. Reproductive factors of all radish cultivars were similar ($P \leq 0.05$) (Table 2). However, Colonel radish had a lower reproductive factor than any of the mustard cultivars. Radishes Commodore, Arena, and Adagio performed better than any of the mustards except Concerta. The reproductive factor of Metex mustard was equal to mustard cultivars Serval and Vertus but higher than the R_f values of any of the

TABLE 2. Final *Heterodera schachtii* soil density of cultivars tested in the greenhouse study, and number of females per root system and reproductive factors of cultivars tested in the growth chamber study.

Crop	Cultivar	Reaction to SBN ^a	Greenhouse		Growth chamber			
			nematodes/pot (Pf)		females/root system		Rf value (Pf/Pi)	
Oil radish	Colonel	R	71.8	a	0.31	a	0.08	a
	Arena	R	83.13	a	0.44	a	0.15	ab
	Commodore	R	32.7	a	0.13	a	0.15	ab
	Adagio	R	78.05	a	0.88	a	0.16	ab
	Rimbo	R	86.59	a	0.75	a	0.30	abc
Mustard	Concerta	R	45.54	a	1.31	a	0.28	bc
	Salvo	R	98.09	a	2.19	ab	0.44	cd
	Rivona	R	144.29	a	4.38	bc	0.49	cd
	Vertus	R	108.52	a	5.63	bc	0.74	de
	Serval	R	73.45	a	8.44	c	0.84	de
Sugar beet	Metex	R	96.28	a	7.5	c	0.94	e
	Nematop	R	59.24	a	64.63	d	3.55	f
	HM9155	S	830.42	b	210.38	e	11.20	g
	HH50	S	2,078.42	c	249.38	e	16.20	h

Data are means of 8 replicates for the greenhouse study and 16 replicates for the growth chamber study. Means within a column followed by a common letter are not different according to Student's *t*-test ($P \leq 0.05$).

^a R = resistant, S = susceptible.

other trap crop cultivars ($P \leq 0.05$). Reproductive factors differed among the three sugar beet cultivars, which all had higher Rf values than any of the trap crops.

Average Rf values were less than one for all resistant trap crop cultivars, indicating no population increase. However, Rf values greater than one occurred on single plants of the Arena and Rimbo radish and on all mustard cultivars except Concerta (data not shown). The high average Rf value of 3.55 for the resistant sugar beet cultivar Nematop was caused by a single plant on which *H. schachtii* reproduced at a rate of 39.54. Average Rf value for Nematop without this outlier was 1.15. Rates greater than one occurred on only 5 of 16 Nematop plants.

Field Trial 1 (2000): The relationship between Pi and Rf was expressed by a non-linear statistical model with the equation $Rf = (3.4154 - 1.9765 * Pi^{0.241})^{2.38095238}$ (Fig. 1A). Average reproductive factors differed among radish cultivars ($P = 0.0115$) and between initial nematode densities below and above the threshold of 2.5 eggs + J/cm³ soil ($P < 0.0001$) (Table 3). However, an interaction between cultivars and threshold was detected ($P = 0.02$). At initial nematode densities below the threshold, reproductive factors differed between cultivars with Colonel having a lower reproductive factor than Adagio and Rimbo ($P < 0.05$). Only Colonel decreased *H. schachtii* populations at preplant densities below the threshold with an Rf of 0.89, while the reproductive factors of the other cultivars were 2.81 for Arena and 13.23 for Rimbo. At preplant densities above the threshold, reproductive factors did not differ among cultivars ($P > 0.05$) and ranged from 0.02 for Colonel to 0.44 for Rimbo, indicating that all cultivars reduced SBN populations.

Field Trial 2 (2001): Similarly to field trial 1, a relationship between Rf and Pi existed in field trial 2 with

the non-linear statistical model $Rf = (0.37517 + 0.53031 * Pi^{0.2})^{4.5433893}$ (Fig. 1B). Reproductive factors were similar for all cultivars ($P = 0.37$). At preplant nematode

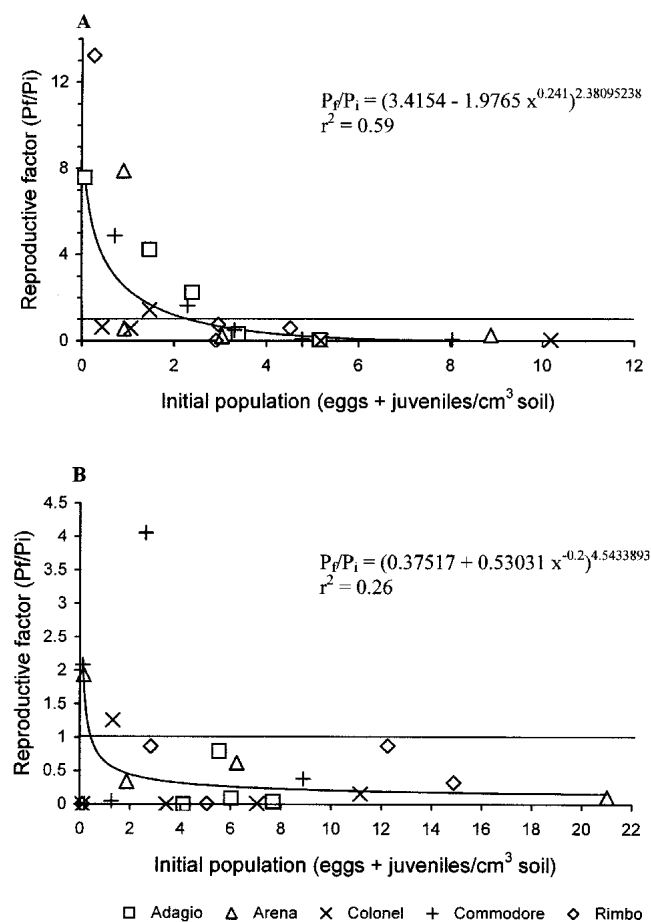


FIG. 1. Relationship between initial population and reproductive factor of *Heterodera schachtii* on five SBN-resistant oil radish cultivars in the field trials in Worland, Wyoming. A) Field Trial 1: August–October 2000 and B) Field Trial 2: August–October 2001.

TABLE 3. Preplant and final *Heterodera schachtii* densities and reproductive factors below and above the Pi threshold of 2.5 eggs + J2/cm³ of soil in the radish cultivar field study in Worland, Wyoming, August to October 2000 (field trial 1).

Radish cultivar	Pi < 2.5 eggs + J2/cm ³ soil					Pi > 2.5 eggs + J2/cm ³ soil				All Pi		
	<i>n</i> ^a	Pi	Pf	Rf		<i>n</i> ^b	Pi	Pf	Rf		<i>n</i> ^c	Rf
Colonel	3	0.99	1.00	0.89	a	2	7.69	0.21	0.02	a	5	0.54
Arena	2	0.92	2.56	2.81	ab	2	5.96	1.42	0.23	a	4	2.22
Commodore	2	1.51	3.63	3.26	ab	3	5.38	0.82	0.21	a	5	1.43
Adagio	3	1.31	4.03	4.67	b	2	4.28	0.62	0.17	a	5	2.87
Rimbo	1	0.26	3.44	13.23	c	3	3.46	1.62	0.44	a	4	3.64

Data are means of *n* replicates. Rf means within a column followed by a common letter are not different according to the LSMEANS option of PROC GLM in SAS ($P \leq 0.05$).

^a Eleven observations had Pi < 2.5 eggs and juveniles per cm³ of soil.

^b Twelve observations had Pi > 2.5 eggs and juveniles per cm³ of soil.

^c Only 23 observations were included in the analysis due to two plots with Pi = 0.

densities above 2.7 eggs + J2/ml soil, reproductive factors ranged from 0.05 for Colonel to 0.52 for Rimbo (Table 4).

DISCUSSION

Results of the greenhouse and the growth chamber studies were different because experimental methods were not the same. Reproduction of *H. schachtii* is dependent on host plant status, plant age at penetration, soil temperature, soil moisture, and presence of predators and parasites of the nematode (Roberts et al., 1981). Lower soil temperature, periods of low soil moisture, and more advanced plant age at inoculation in the greenhouse study resulted in lower nematode reproduction than in the growth chamber study, where environmental conditions were more favorable for nematode reproduction and plants were younger.

The final nematode populations of the greenhouse study indicated that all trap crop radish and mustard cultivars tested, as well as the SBN-resistant sugar beet cultivar Nematop, had similar levels of resistance. The growth chamber study, on the other hand, showed differences between radish and mustard cultivars and Nematop in number of females, as well as in reproductive factors. These findings suggest that the radish cultivars have a higher nematode reduction potential under optimal conditions than most of the mustard cultivars. Radish cv. Colonel decreased nematode

population densities the most, more than any of the mustards but equal to the other radish cultivars. Colonel was the only cultivar with an Rf value less than 0.1, which is in agreement with a nematode population reduction of more than 90% and a BBA resistance rating of 1 (Bundessortenamt, 2002; Müller and Rumpfenhorst, 2000).

Reproductive factors, on which resistance ratings in Germany are based, are determined by BBA under different environmental conditions than in the growth chamber study and are standardized to Rf values of select resistant standard cultivars. However, due to the 25 °C optimal temperature for *H. schachtii* reproduction (Griffin, 1981b) in the growth chamber study, reproductive factors were probably equal to or higher than standardized BBA rates, which were obtained at temperatures of 18 °C to 20 °C day and 14 °C to 16 °C night (Müller and Rumpfenhorst, 2000). Therefore, it can be concluded that the low Rf value of 0.08 for radish cultivar Colonel under favorable conditions for nematode reproduction is in accordance with the resistance evaluation of the BBA (Bundessortenamt, 2002).

Reproductive factors of radish cultivars Commodore, Adagio, Arena, and Rimbo in the growth chamber study were between 0.11 and 0.3. This corresponds to BBA resistance levels of 2 (Rf = 0.1–0.3) and is, with exception of Commodore, also in accordance with BBA evaluations. Under the optimal conditions for nema-

TABLE 4. Preplant and final *Heterodera schachtii* densities and reproductive factors below and above the Pi threshold of 2.7 eggs + J2/cm³ of soil in the radish cultivar field study in Worland, Wyoming, August to October 2001 (field trial 2).

Radish cultivar	Pi < 2.7 eggs + J2/cm ³ soil				Pi > 2.7 eggs + J2/cm ³ soil				All Pi	
	<i>n</i> ^a	Pi	Pf	Rf	<i>n</i> ^b	Pi	Pf	Rf	<i>n</i> ^c	Rf
Colonel	2	0.71	0.83	0.63	3	7.22	0.6	0.05	5	0.29
Arena	2	1.01	0.46	1.14	2	13.64	3.1	0.36	4	0.75
Commodore	4	1.05	2.72	1.55	1	8.89	3.38	0.38	5	1.31
Adagio	0	—	—	—	4	5.84	1.3	0.23	4	0.23
Rimbo	1	0.1	0	0	4	8.76	4.51	0.52	5	0.42

Data are means of *n* replicates. Rf means are not different according to the F-test ($P = 0.37$).

^a Nine observations had Pi < 2.7 eggs and juveniles per cm³ of soil.

^b Fourteen observations had Pi > 2.7 eggs and juveniles per cm³ of soil.

^c Only 23 observations were included in the analysis due to two plots with Pi = 0.

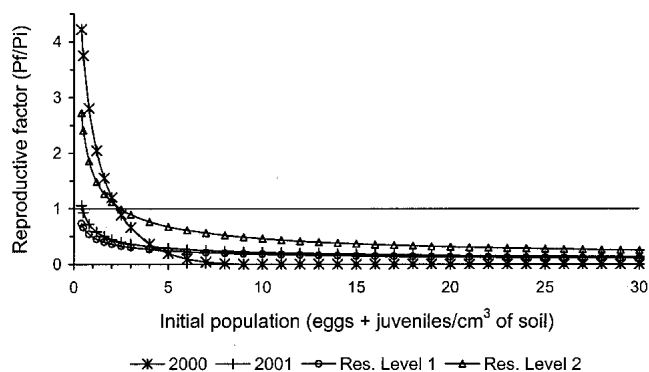


FIG. 2. Relationship between initial population and reproductive factor of *H. schachtii* for oil radish cultivars of the resistance levels 1 and 2 (Schlang, 1997) and for oil radish cultivars Adagio, Arena, Colonel, Commodore, and Rimbo (resistance levels 1 and 2) in the radish cultivar field tests in Worland, Wyoming, in 2000 and 2001.

tode reproduction in our study, Rf values of four out of five radish cultivars tested confirmed the resistance levels found by BBA. The similarity of Rf values obtained from the growth chamber study with the ones reported by Bundessortenamt (2002) leads to the conclusion that resistant oil radish cultivars have comparable resistance to Wyoming and German populations of the SBN. Cultivar information from Bundessortenamt appears to be a valid indicator of nematode resistance in radish for Wyoming growers.

In contrast to the results found on the radish cultivars, the mustards, with the exception of Concerta, showed much higher reproductive factors that were not in agreement with BBA findings. According to Rf values in the growth chamber study, mustards Vertus, Serval, and Metex could not be classified as resistant at all, while Salvo and Rivona both would have a resistance rating of 3 (Rf = 0.3–0.5). This may relate to a higher nematode reproduction on the resistant host plant mustard than on oil radish under optimal temperature.

Resistance of a cultivar is assessed according to its effect on nematode reproduction (Trudgill, 1986). Müller and Rumpfenhorst (2000) found no females on one third of tested Nematop plants, but some beets had 20 to 30 females. In their studies, this relates to an Rf value of 7 to 8 under the assumption that each cyst contains 300 eggs. They questioned if a cultivar that allows this amount of reproduction can be considered as resistant. However, they found many females and cysts to be empty or with few eggs. In the growth chamber study, the average of 64.6 females/plant seems to be high, compared to Müller and Rumpfenhorst's (2000) findings. Temperatures in their studies were lower, the same as for the trap crops. Also, females were counted 6 weeks after inoculation (Müller and Rumpfenhorst, 2000), while counts in the growth chamber were done 1 to 5 months after inoculation. In agreement with Müller and Rumpfenhorst (2000), Nematop had, on average, fewer eggs per female than the susceptible cultivars. There were a few plants with

large reproduction rates, contributing to the high average number of females in the growth chamber study. Fifty-six percent of Nematop plants had 0 to 30 females.

Serendipitously, a difference ($P \leq 0.05$) in final nematode populations and thus Rf values on susceptible sugar beet cultivars was found in greenhouse and growth chamber studies, with HH50 having higher populations than HM9155. This indicates a possible difference in susceptibility to the SBN among U.S. sugar beet cultivars. However, numbers of females in the growth chamber study were the same.

As previously reported (Müller, 1986; Schlang, 1985, 1989, 1997; Steudel et al., 1989), the reproductive factor decreases with increasing initial nematode populations. Gardner and Caswell-Chen (1993) described a similar regression curve between reproductive factor and initial population for resistant and susceptible cabbage cultivars, resistant mustard and oil radish cultivars, as well as for the non-hosts *Phacelia tanacetifolia* and buckwheat. In accordance with these findings, a curvilinear relationship between Rf and Pi was observed in the two radish cultivar field trials 2000 and 2001 (Fig. 1A,B). Schlang (1997) developed regression curves from field data for oil radish cultivars of all resistance ratings. The regression curve for 2001 is in the range of the two curves for oil radish cultivars of resistance rating 1 (Rf < 0.01) and 2 (0.01 < Rf < 0.03), while the regression curve for the 2000 test descends below the curve for resistance level 1 at Pi = 5 eggs and J2/cm³ soil, thus underestimating Rf (Fig. 2). This was caused by population reductions of up to 100% at high Pi.

In the radish cultivar field study in 2000, Colonel had lower reproductive rates than Adagio and Rimbo and also was the only cultivar that reduced nematode soil densities at initial nematode densities below threshold (2.5 eggs and J2/cm³ soil). This is in agreement with Schlang's (1997) regression curve of oil radish cultivars of resistance rating 1 (Fig. 2).

In conclusion, radish cultivars are superior to mustard cultivars relative to their potential in reducing Wyoming SBN populations. Concerning new cultivars that might be marketed in the United States in the future, only varieties with a resistance rating of 1 should be used as trap crops for managing the SBN in Wyoming because they reduce *H. schachtii* soil populations the most and cause a population decrease even at low initial populations (Schlang, 1997).

LITERATURE CITED

- Bundessortenamt. 2002. Beschreibende Sortenliste Getreide, Mais, Ölfrüchte, Leguminosen (grosskörnig), Hackfrüchte (außer Kartoffeln) 2002. Hannover, Germany: Landbuch-Verlag. (In German.)
- Byrd, D. W., Jr., K. R. Barker, H. Ferris, C. J. Nusbaum, W. E. Griffin, R. H. Small, Connie A. Stone. 1976. Two semi-automatic elutriators for extracting nematodes and certain fungi from soil. *Journal of Nematology* 8:206–212.
- Cooke, D. 1991. Europe goes green to control beet cyst nematode. *British Sugar Beet Review* 59:44–47.

- Doney, D. L. 1995. USDA-ARS sugar beet releases. *Journal of Sugar Beet Research* 32:229–257.
- Elgin, J. H., Jr., and D. W. Evans. 1975. Effects of greenhouse sprays on stem and root-knot nematodes. *Plant Disease Reporter* 59:14–16.
- Ferris, H. 1985. Density-dependent nematode seasonal multiplication rates and overwinter survivorship: A critical point model. *Journal of Nematology* 17:93–100.
- Gardner, J., and E. P. Caswell-Chen. 1993. Penetration, development, and reproduction of *Heterodera schachtii* on *Fagopyrum esculentum*, *Phacelia tanacetifolia*, *Raphanus sativus*, *Sinapis alba*, and *Brassica oleracea*. *Journal of Nematology* 24:695–702.
- Gray, F. A., D. W. Koch, L. Yun, and J. M. Krall. 1994. Use of “catch” crops to control the sugar beet nematode, *Heterodera schachtii*. *Phytopathology* 84:1089.
- Gray, F. A., D. W. Koch, L. Yun, and J. M. Krall. 1997. Effect of nematode-resistant fodder radish on soil population dynamics of *Heterodera schachtii* in sugar beet–barley rotations. *Journal of Nematology* 29:580.
- Griffin, G. D. 1981a. The relationship of *Heterodera schachtii* population densities to sugar beet yields. *Journal of Nematology* 13:181–184.
- Griffin, G. D. 1981b. The relationship of plant age, soil temperature, and population density of *Heterodera schachtii* on the growth of sugar beet. *Journal of Nematology* 13:184–190.
- Griffin, G. D. 1981c. Pathological differences in *Heterodera schachtii* populations. *Journal of Nematology* 13:191–195.
- Griffin, G. D. 1987. Efficacy of using split and post plant applications of aldicarb for control of *Heterodera schachtii* on sugar beet. *Annals of Applied Nematology (Journal of Nematology 19, Supplement)* 1:119–122.
- Hafez, S. L. 1994. The use of green manure crops in a sugar beet rotation for sugar beet cyst nematode management. *Journal of Nematology* 26:548.
- Hafez, S. L., and P. Sundararaj. 1998. Differential reaction and antagonistic potential of trap crop cultivars in the management strategy of sugar beet cyst nematode. *International Journal of Nematology* 8:145–148.
- Hafez, S. L., and P. Sundararaj. 1999. Exploitation of nematocidal efficacy of trap crops for the management of *Heterodera schachtii* under sugar beet. *International Journal of Nematology* 9:27–33.
- Hafez, S. L., and P. Sundararaj. 2000. Impact of agronomic and cultural practices of green manure crops for the management of *Heterodera schachtii* in sugar beet. *International Journal of Nematology* 10:177–182.
- Hafez, S. L., and P. Sundararaj. 2001. Impact of green manure crops on sustainable management of sugar beet cyst nematode. *Journal of Nematology* 33:259.
- Halbrendt, J. M., S. A. Lewis, and E. R. Shipe. 1987. A modified screening test for determining *Heterodera glycines* resistance in soybean. *Annals of Applied Nematology (Journal of Nematology 19, Supplement)* 1:74–77.
- Heijbroek, W., A. J. Roelands, J. H. de Jong, C. van Hulst, A. H. L. Schoone, and R. G. Munning. 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.
- Held, L. J., J. W. Jennings, D. W. Koch, and F. A. Gray. 2000. Economics of trap cropping for sugar beet nematode control. *Journal of Sugar Beet Research* 37:45–55.
- Jenkins, W. R. 1964. A rapid centrifugal-flotation technique for separating nematodes from soil. *Plant Disease Reporter* 9:692.
- Kerr, E. D., R. G. Wilson, and D. D. Baltensperger. 1995. Comparison of several trap crop species on soil populations of *Heterodera schachtii*. *Journal of Sugar Beet Research* 32:147.
- Koch, D. W., and F. A. Gray. 1997. Nematode-resistant oil radish for *Heterodera schachtii* control I. sugar beet–barley rotations. *Journal of Sugar Beet Research* 34:31–43.
- Koch, D. W., F. A. Gray, and J. M. Krall. 1998. Nematode-resistant oil radish for *Heterodera schachtii* control II. Sugar beet–dry bean–corn rotations. *Journal of Sugar Beet Research* 35:63–75.
- Lange, W., and T. S. M. De Bock. 1994. Pre-breeding for nematode resistance in beet. *Journal of Sugar Beet Research* 31:13–26.
- Lange, W., C. Jung, and W. Heijbroek. 1990. Transfer of beet cyst nematode resistance from *Beta* species of the section *Patellares* to cultivated beet. Proceedings of the 53rd Winter Congress of the Institute for Sugar Beet Research, Brussels: 89–102.
- Löptien, H. 1984. Breeding nematode-resistant beets. I. Development of resistant alien additions by crosses between *Beta vulgaris* L. and wild species of the section *Patellares*. *Zeitschrift für Pflanzenzüchtung* 92:208–220.
- Löptien, H. 1985. Breeding nematode-resistant beets. II. Investigations into the inheritance of resistance to *Heterodera schachtii* Schm. in wild species of the section *Patellares*. *Zeitschrift für Pflanzenzüchtung* 93:237–245.
- Müller, J. 1986. Integrated control of the sugar beet cyst nematode. Pp. 235–250 in F. Lamberti and C. E. Taylor, eds. *Cyst nematodes*. New York: Plenum Press.
- Müller, J. 1999. The economic importance of *Heterodera schachtii* in Europe. *Helminthologia Bratislava* 36:205–213.
- Müller, J., and H. J. Rumpfenhorst. 2000. Testing of crop cultivars for resistance to noxious organisms at the Federal Biological Research Center. *Mitteilungen aus der Biologischen Bundesanstalt für Land- und Forstwirtschaft* 372. Berlin, Germany: Paul Parey Verlag. (In German.)
- Roberts, P. A. 1992. Current status of the availability, development, and use of host plant resistance to nematodes. *Journal of Nematology* 24:213–227.
- Roberts, P. A., and I. J. Thomason. 1981. Sugarbeet pest management: Nematodes. Special Publication 3272. Division of Agricultural Sciences, University of California.
- Roberts, P. A., I. J. Thomason, and H. E. McKinney. 1981. Influence of nonhosts, crucifers, and fungal parasites on field populations of *Heterodera schachtii*. *Journal of Nematology* 13:164–171.
- Savitsky, H. 1975. Hybridization between *Beta vulgaris* and *Beta procumbens* and transmission of nematode (*Heterodera schachtii*) resistance to sugar beet. *Canadian Journal of Genetics and Cytology* 17:197–209.
- Schlang, J. 1985. Resistenzverhalten verschiedener Ölrettichsorten gegenüber *Heterodera schachtii*. *Gesunde Pflanzen* 37:233–235. (In German; English abstract.)
- Schlang, J. 1989. Zur biologischen Bekämpfung des Weissen Rübenzystemnematoden (*Heterodera schachtii*) durch resistente Zwischenfrüchte. Pp. 249–265 in Proceedings of the 52nd Winter Congress of the International Institute for Sugar Beet Research, Brussels. (In German; English abstract.)
- Schlang, J. 1997. Neue Strategien zur Biologischen Bekämpfung von *Heterodera schachtii*. Pp. 229–242 in Proceedings of the 60th Congress of the International Institute for Sugar Beet Research, Cambridge. (In German; English abstract.)
- Schlang, J. 1999. Keine Chance für Nematoden. *DLZ-Agrarmagazin* 50:62–65. (In German.)
- Studel, W., J. Schlang, and J. Müller. 1989. Studies on the influence of catch crops on population dynamics of the sugar beet nematode (*Heterodera schachtii* Schmidt) in a sugar beet/cereal rotation. *Nachrichtenblatt des Deutschen Pflanzenschutzdienstes* 41:199–203. (In German; English abstract.)
- Thomason, I. J. 1987. Challenges facing nematology: Environmental risks with nematicides and the need for new approaches. Pp. 469–476 in J. A. Veech and D. W. Dickson, eds. *Vistas on nematology*. Hyattsville, MD: Society of Nematologists.
- Trudgill, D. L. 1986. Concepts of resistance, tolerance, and susceptibility in relation to cyst nematodes. Pp. 179–189 in F. Lamberti and C. E. Taylor, eds. *Cyst nematodes*. New York: Plenum Press.