Effects of *Crotalaria juncea* and *C. spectabilis* on hatching and population density of the soybean cyst nematode, *Heterodera glycines* (Tylenchida: Heteroderidae)

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Abstract

In order to assess the efficacy of *Crotalaria juncea* and *C. spectabilis* as trap crops against the soybean cyst nematode, *Heterodera glycines*, rates of hatching of *H. glycines* eggs in the root leachate, penetration into *Crotalaria* roots by *H. glycines* juveniles and their development in the roots, and the effects of planting these *Crotalaria* species on population density of *H. glycines* in soil were investigated. The rates of hatching of *H. glycines* eggs in the root leachates from *C. juncea* and *C. spectabilis* were significantly greater than the rates in distilled water and in the leachates from non-host plants. Stimulation of hatching by *C. juncea* was significantly greater than that by *C. spectabilis* and similar to that by the host plant, kidney bean. The number of second-stage juveniles that penetrated *Crotalaria* roots was similar to the number that penetrated soybean roots, but most of the second-stage juveniles in *Crotalaria* roots did not develop to an advanced stage of growth. The population density of *H. glycines* in soil in which *Crotalaria* had been planted decreased significantly compared with that in fallow soil (no plants) in both pot and field plot tests. The effect of planting *C. juncea* on reduction in population of the nematode in soil was generally greater than that of *C. spectabilis*. From the results of these experiments, it was concluded that the use of *Crotalaria*, especially *C. juncea*, as a trap crop is a promising method for reducing the population density of *H. glycines*.

Key words: Cultural control; hatching stimulation; soybean cyst nematode; trap crop

INTRODUCTION

It is well known that the hatching of cyst nematode eggs is stimulated by factors exuded from host plants roots. The hatching stimulants for eggs of the soybean cyst nematode (*Heterodera glycines*), glycinoeclepins A, B and C, have been extracted from kidney bean roots and characterized (Masamune et al., 1982; Fukuzawa et al., 1985). Some hatching stimulants for the potato cyst nematode (*Globodera rostochiensis*) have also been isolated from potato root leachate and characterized (Atkinson et al., 1987; Devine and Jones, 2000a). It has been thought that populations of cyst nematodes can be reduced by applying hatching factors to infested soil in the absence of host plants and inducing suicide hatching of the cyst nematode eggs. Devine and Jones (2000b) showed the possibility of controlling *G. rostochiensis* by using a stimulant, since they found that application of crude ex-

tract from potato or tomato root diffusate to infested fields caused a reduction in the nematode population density. However, there are still no practical control measures using a hatching stimulant that is produced artificially.

There have been many attempts recently to control cyst nematode populations by planting susceptible or resistant host plants with hatching-promoting activity as a trap crop. For the beet cyst nematode (*H. schachtii*), such trap crops have been successfully developed and have been used practically (Koch and Gray, 1997; Hafez and Sundararaj, 1998, 2000; Koch et al., 1998; Krall et al., 2000). For potato cyst nematodes (*G. rostochiensis* and *G. pallida*), cultivation of a susceptible or resistant potato cultivar as a trap crop has been demonstrated to reduce the population density of the nematode in soil (Lamondia and Brodie, 1986; Halford et al., 1999; Scholte, 2000a).

Resistant plant species with hatch-promoting

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activity toward potato cyst nematode or beet cyst nematode have also been used in some studies to control populations of those nematodes (Turner and Stone, 1981; Cooke, 1985; Gardner and Caswell-Chen, 1993; Hafez and Sundararaj, 1998, 2000; Koch et al., 1998; Scholte, 2000b,c; Scholte and Vos, 2000). However, there have been only a few studies on the control of *H. glycines* populations by the use of such resistant plants.

Aiba and Mitsui (1995) reported that some leguminous plants, i.e., *Crotalaria juncea* and red clover (*Trifolium pratense*), had hatch-promoting activity toward *H. glycines* eggs and were resistant to the nematode. Kushida et al. (2002) demonstrated that planting red clover caused a significant reduction in population density of *H. glycines* in soil. Red clover is an important green manure crop in Japan, and species of *Crotalaria* (notably *C. juncea* and *C. spectabilis*) are also widely used as antagonistic green manures to root-knot nematodes. If these *Crotalaria* species are effective in reducing the population density of *H. glycines*, controlling of the nematode using trap crops would be more widely applicable. The objectives of this study were to determine the efficacy of *C. juncea* and *C. spectabilis* for stimulating hatching of *H. glycines* eggs and for reducing its population density in soil.

MATERIALS AND METHODS

Nematode. The soybean cyst nematode (*H. glycines*), cultured on susceptible soybean (*Glycine max* (L.) Merr. cv. Kurosengoku) planted in a field of the National Agricultural Research Center for Hokkaido Region (NARCH), Sapporo, Japan, was used in experiments 1, 2 and 3. Soil infected with the nematodes was sampled from the field in November of the year before each experiment was performed, and maintained at 4°C. Cysts were isolated from the soil using a method described by Yoshihara and Kegasawa (1989) just before each experiment.

Experiment 1: Effect of root leachates on rates of hatching of *H. glycines*. The rates of hatching of *H. glycines* in root leachates from *C. juncea*, *C. spectabilis*, susceptible soybean (cv. Kurosengoku), kidney bean (*Phaseolus vulgaris* L. cv. Honkintoki), wheat (*Triticum aestivum* L. cv. Haruyutaka), tomato (*Lycopersicon esculentum*

Mill cv. Momotaro) and potato (*Solanum tuberosum* L. cv. Irish Cobbler) were investigated. This experiment was performed twice. Each plant was sown in a plastic pot (16 cm in diameter and 20 cm in height) filled with 2 l of autoclaved soil on April 20, 1998 for the first test and on May 28, 1999 for the second test, and maintained in a greenhouse. After emergence, seedlings were reduced to five for *Crotalaria*, two for soybean and kidney bean, six for wheat and one for tomato and potato per pot.

Root leachates were obtained from two pots of each of the plants stated above in the 7th week after sowing. The drain of each pot was closed, and 1,000 ml of tap water was poured into each pot. About 8 h later, root leachates were drained and collected. The leachates were filtered through No. 5 Whatman filter paper and stored at 4°C in the dark.

Hatching tests were carried out using 15-ml vials. Cysts isolated from the field soil were soaked in distilled water at 24°C for a week and then crushed to release the eggs. Egg suspension was passed through a sieve with a pore size of 64 µm to remove debris. Eggs were rinsed three times with 100 ml distilled water. One milliliter of each leachate was added to 1 ml of egg suspension (600 eggs/ml) in the vial. Vials to which distilled water had been added instead of leachate were prepared as controls. All vials were maintained in the dark at 24°C. After 10 days, the numbers of hatched juveniles and unhatched eggs were counted. There were three replicates per treatment.

Experiment 2: Penetration into *Crotalaria* roots by *H. glycines* juveniles and their development in the roots. Seeds of *C. juncea*, *C. spectabilis*, susceptible soybean (cv. Kitamishiro), red clover (cv. Sapporo) and potato (cv. Irish Cobbler) were sown in 9-cm pots filled with autoclaved soil on July 9, 2001 and maintained in a greenhouse. After emergence, seedlings were reduced to three for *Crotalaria*, two for soybean and five for red clover per pot.

Eggs of *H. glycines* collected from crushed cysts were suspended in distilled water and incubated at 27°C, and second-stage juveniles (J2) hatching spontaneously were collected. After all plants except for red clover had grown for 10 days and red clover had grown for 21 days, approximately 2,000 J2 were simultaneously inoculated to each pot.

Inoculated pots were kept in a growth chamber maintained at a temperature of 24°C and a light condition of 16-h L/8-h D. Three replications of each treatment were harvested on the 5th, 14th and 28th days after inoculation. Root systems of the plants were gently washed and soaked in 5% formaldehyde solution until use for investigation. On the 28th day, females and cysts released from the roots were also collected from soil with a 0.21-mm aperture sieve and counted. The roots were stained with lactoglycerol containing 0.05% acid fuchsin (Hooper, 1986) and homogenized in 30 to 50 ml distilled water. Slim second-stage juveniles (J2), swollen J2, third-stage juveniles (J3) or fourth-stage juveniles (J4) and adult females or cysts in 2 ml homogenate were counted six times for each sample. Slim J2 were regarded as J2 that were almost similar to inoculum, and swollen J2 were regarded as J2 that were clearly fatter than inoculum (Fig. 1).

Experiment 3: Effect of cultivation of *Crotalaria* on *H. glycines* population density in pots. *C. juncea*, *C. spectabilis* and wheat (cv. Haruyutaka) were sown in plastic pots (16 cm in diameter and 20 cm in height) containing 2.5 l of soil from a *H. glycines*-infested field belonging to NARCH (P_i =ca. 216 eggs per 1 g of dry soil) on June 8, 1998. Pots without plants were prepared as controls. Three replications of pots were kept in a greenhouse at 20–25°C. After emergence, the numbers of seedlings of these plants were reduced to 5 per pot. Plants were harvested on the 60th day after germination. Cysts were extracted from 50 g of air-dried soil and crushed to count the remaining eggs. P_f/P_i , the ratio of egg density on the 60th day after germination to the initial egg density, was calculated to assess the effect of *Crotalaria*.

Experiment 4: Effect of cultivation of *Crotalaria* on *H. glycines* population density in field plots. This experiment was performed in both fields of NARCH and Ibaraki Agricultural Center, Agricultural Research Institute (IACARI), Mito, Japan. *C. juncea*, *C. spectabilis* and resistant soybean (cv. Suzuhime in NARCH, cv. Tozan 93 in IACARI) were used. Fallow plots were prepared as controls. Three replications were prepared for each treatment.

Test in a field of NARCH. Each plant was cultivated in a 2 m×1.5 m plot in which soybean cyst nematodes had been cultured on susceptible soy-

Fig. 1. Slim and swollen second-stage juveniles. Scale bar=400 μ m.

bean in the previous year. Initial densities of the nematode were 25 to 292 eggs per 1 g of dry soil. *Crotalaria* seeds were sown in rows 30 cm apart at a density of 8 kg of seed per 10 a in the field plots. Soybean was space-planted at 20 cm in rows spaced 50 cm apart. Five kilograms each of N, P and K per 10 a was applied as fertilizer for *Crotalaria*, and 3 kg of N, 22 kg of P and 15 kg of K per 10 a was applied as fertilizer for soybean. *Crotalaria* seeds were sown on June 19, 2000 and soybean seeds were sown on May 26, 2000. Foliage of *Crotalaria* was cut and removed on September 14. Soybeans were harvested on September 25. On October 1, each plot was harrowed, and soil samples were collected and air-dried. Numbers of remaining eggs in cysts in 50 g of soil were determined with three replications.

Tests in a field of IACARI. Tests in a field of IACARI were conducted in 1999 and 2000. Each plant was cultivated in a 3.6 m×5 m plot in which soybean cyst nematodes had been infested. Initial densities of the nematode were 5 to 17 eggs per 1 g of dry soil in 1999 and 2 to 6 eggs per 1 g of dry soil in 2000. *Crotalaria* seeds were sown in rows 60 cm apart at a density of 4 kg of seed per 10 a in the field plots. Soybean was space-planted at 10 cm in rows spaced 60 cm apart. As fertilizers, 4.5 kg of N, 15 kg of P and 15 kg of K per 10 a were applied. Seeds of each plant were sown on June 22, 1999 and on June 21, 2000. Foliage of *Crotalaria* was cut and removed on September 21, 1999 and September 18, 2000. Soybeans were harvested on

November 2, 1999 and October 18, 2000. On November 22, 1999 and November 20, 2000, each plot was harrowed and soil samples were collected and air-dried. Numbers of remaining eggs in cysts in 300 g of soil were determined with three replications.

Statistical analysis was performed by Tukey's test or Dunnett's test using commercial software (Excel Toukei, Esumi Co.).

RESULTS

Effect of root leachates on rates of hatching of *H. glycines*

The rates of hatching in the root leachates from *C. juncea* and *C. spectabilis* were significantly greater ($p < 0.01$) than the rates in distilled water and in the leachates from non-host plants, wheat, tomato and potato (Table 1). *C. juncea* stimulated more hatching of *H. glycines* eggs than did *C. spectabilis* ($p < 0.01$) and less hatching than did soybean, but stimulated hatching to the same degree as that stimulated by another good host, kidney bean, in both tests. The rate of hatching in the root leachate from *C. spectabilis* was not as high as the rates in the leachates from soybean or kidney bean (Table 1). The rates of hatching in the root leachates from non-host plants, wheat, tomato and potato were similar to that in distilled water.

Penetration into *Crotalaria* roots by *H. glycines* juveniles and their development in the roots

The numbers of J2 that penetrated roots of *C. juncea*, *C. spectabilis*, soybean and red clover were almost the same (Fig. 2). Approximately half of the inoculated juveniles were recovered from the roots of those plants on the fifth day after inoculation. About 80% of J2 recovered from roots of *C. juncea* and soybean on the fifth day were swollen J2. The rate of swollen J2 recovered from the roots of *C. spectabilis* on the fifth day was about 35%. On the 14th day, about 80% of the juveniles that had

Table 1. Effects of root leachates on hatching rate of *Heterodera glycines* eggs

Treatment plant (cultivar)	Hatching rate (% mean $\pm$ SE)	
	1st test	2nd test
Distilled water	13.8 $\pm$ 1.6 a	15.4 $\pm$ 1.1 a
<i>Crotalaria juncea</i>	70.6 $\pm$ 2.4 c	70.7 $\pm$ 2.4 c
<i>Crotalaria spectabilis</i>	47.4 $\pm$ 2.0 b	47.0 $\pm$ 3.5 b
Soybean (Kurosengoku)	83.4 $\pm$ 1.8 d	86.1 $\pm$ 1.4 d
Kidney bean (Honkintoki)	68.1 $\pm$ 1.9 c	71.0 $\pm$ 3.0 c
Wheat (Haruyutaka)	11.9 $\pm$ 0.9 a	11.3 $\pm$ 0.9 a
Tomato (Momotaro)	10.9 $\pm$ 0.8 a	11.1 $\pm$ 1.6 a
Potato (Irish Cobbler)	—	11.4 $\pm$ 1.4 a

Hatching rates were examined after exposing *H. glycines* eggs to each leachate for 10 days at 24°C. Means with different letters are significantly different ($p < 0.01$, Tukey's test). —: not examined.

Fig. 2. Penetration of *Heterodera glycines* to the roots of *Crotalaria juncea*, *C. spectabilis*, susceptible soybean (cv. Kitamishiro), red clover (cv. Sapporo) and potato (cv. Irish Cobbler), and their development. Each plant inoculated with 2,000 J2 was grown in a growth chamber at a temperature of 24°C and a light condition of 16-h L/8-h D.

Fig. 3. Effects of planting *Crotalaria* on population density of *Heterodera glycines* in pots. Mean values ($\pm$ SE) with different letters are significantly different ($p < 0.01$, Tukey's test). Pf/Pi is the ratio of egg density at 60 days after germination to the initial egg density. Initial egg density was 216 eggs per 1 g of dry soil. Wheat cultivar was Haruyutaka.

penetrated soybean roots had developed to J3 or J4, but few J2 had developed to J3 or J4 in the roots of *C. juncea* and *C. spectabilis*. On the 28th day, most juveniles recovered from roots of *C. juncea* and *C. spectabilis* were still J2, and a few adult females were isolated only from the roots of *C. spectabilis*. On the other hand, many females and cysts with egg masses were recovered from soybean roots on the 28th day. Most of the juveniles in roots of red clover harvested on the 5th, 14th and 28th days were slim J2. A few slim J2 were recovered from the roots of potato, but none of the J2 developed to an advanced stage of growth.

Effect of cultivation of *Crotalaria* on *H. glycines* population density in pots

Values of Pf/Pi after growing *C. juncea* and *C. spectabilis* for 60 days were about 20% and 42%, respectively (Fig. 3). Those values were significantly lower than the values in pots in which wheat had been planted and in pots with no plants ($p < 0.01$). The *H. glycines* population in pots in which *C. juncea* had been planted was reduced more than was the population in pots in which *C. spectabilis* had been planted ($p < 0.01$). There was no significant difference between the values of Pf/Pi in pots not treated with plants and pots in which wheat had been planted.

Effect of cultivation of *Crotalaria* on *H. glycines* population density in field plots

Cultivation of *C. juncea* and *C. spectabilis* re-

Table 2. Effects of planting *Crotalaria* species on population density of *Heterodera glycines* under field conditions

Treatment	Pf/Pi (% , mean $\pm$ SE)		
	IACARI (Mito)		NARCH (Sapporo)
	1999	2000	
Fallow	59.6 $\pm$ 13.0	55.4 $\pm$ 1.2	61.1 $\pm$ 5.7
<i>C. juncea</i>	13.5 $\pm$ 2.9*	13.5 $\pm$ 2.3*	12.7 $\pm$ 1.0*
<i>C. spectabilis</i>	30.8 $\pm$ 11.2	9.6 $\pm$ 5.0*	24.7 $\pm$ 8.8*
Resistant soybean	18.4 $\pm$ 0.4*	6.3 $\pm$ 0.5*	22.7 $\pm$ 3.3*

IACARI and NARCH are agricultural research centers in which the field tests were conducted. Pf/Pi indicates the ratio of egg density of *H. glycines* after harvest of each plant to initial egg density. The values with asterisks are significantly different from the value of fallow ($p < 0.05$, Dunnett's test). The resistant soybean cultivars used in this experiment were Tozan 93 in IACARI and Suzuhime in NARCH. Each *Crotalaria* species was grown for about 3 months.

duced about 70 to 90% of the density of *H. glycines* eggs in soil (Table 2). The mean value of Pf/Pi in plots of *C. juncea* was significantly ($p < 0.05$) smaller than that in fallow plots, and almost the same as that in plots in which resistant soybean had been planted. Similar results in plots treated with *C. juncea* were obtained from tests performed in both fields and in both tested years. Values of Pf/Pi in plots of *C. spectabilis* were significantly ($p < 0.05$) smaller than those in fallow plots in 2000 of IACARI and NARCH. Though the mean reduction in density of *H. glycines* eggs in *C. spectabilis* plots in 1999 of IACARI was large, no significant difference was found between the Pf/Pi values in the *C. spectabilis* plot and the fallow plot.

DISCUSSION

It was confirmed that *C. juncea* stimulated the hatching of *H. glycines* eggs as Aiba and Mitsui (1995) reported, and it was newly found that *C. spectabilis* also stimulated hatching of the nematode eggs in this study (Table 1). *C. juncea* and *C. spectabilis* were thought to be highly resistant plants since juveniles of *H. glycines* that penetrated the roots of both *Crotalaria* species did not develop into adult females or cysts with eggs (Fig. 2). Schmitt and Riggs (1991) suggested that poor-host plants do not influence the hatching of *H. glycines* eggs. However, results of these experiments indi-

cate that there are some resistant legumes that have high hatch-promoting activity toward *H. glycines*.

The rate of hatching in the leachate from *C. juncea* was significantly higher than that in the leachate from *C. spectabilis* and as high as that in the leachate from the good host plant, kidney bean, in both tests. Since *C. spectabilis* grew slowly compared with the growth rate of *C. juncea*, this plant might not have been able to produce sufficient hatching stimulants by the day that the leachate was collected.

Since similar numbers of J2 penetrated roots of *C. juncea*, *C. spectabilis* and soybean (Fig. 2), it was thought that roots of the two species of *Crotalaria* were recognized as roots of host plants by juveniles of *H. glycines*. However, most juveniles recovered from *Crotalaria* roots were still in the second stage of growth, and no juveniles in the roots of *C. juncea* developed into adult females or cysts. A few adult females were recovered from roots of *C. spectabilis*, but they were considered to be dying because their bodies were empty. These findings indicate that *Crotalaria* catches and kills juveniles of *H. glycines* in its roots.

Since most of J2 recovered from roots of *C. juncea* and *C. spectabilis* on the fifth day after inoculation were swollen as were J2 recovered from soybean roots (Fig. 2), it was thought that juveniles could ingest nutrition from the roots of *Crotalaria* in a short time after their invasion. On the other hand, no juveniles grew in the roots of red clover, which is another resistant legume with hatch-promoting activity toward *H. glycines*. This result indicated that juveniles that had penetrated the roots of red clover could not ingest any nutrition. The very weak hatch-promoting activity of red clover (Aiba and Mitsui, 1995; Kushida et al., 2002) and the results of experiments 1 and 2 suggest that resistant plants in which juveniles grow in roots as they do in good host plant roots have stronger hatch-promoting activity than do resistant plants in which juveniles do not grow in roots. However, Schmitt and Riggs (1991) observed that some juveniles of *H. glycines* developed to the fourth stage of growth in roots of cabbage, which did not stimulate hatching of nematode eggs at all. We have also observed similar growth of juveniles in roots of cabbage (data not shown). Therefore, this hypothesis may be limited to leguminous resistant plants.

Results of the pot tests and field tests demon-

strated that planting of two species of *Crotalaria* reduced population densities of *H. glycines* in soil. The results also indicate that *Crotalaria* stimulates the hatching of *H. glycines* eggs in soil (Table 2, Fig. 3).

The rate of reduction in population density of *H. glycines* in pots in which *C. juncea* had been planted was significantly higher than that in pots in which *C. spectabilis* had been planted (Fig. 3). *C. spectabilis* might not be able to produce sufficient hatching factors to stimulate hatching of eggs, since it grew slowly, especially in its early growth phase. Evans (1983) and Rawsthorne and Brodie (1986) suggested that stimulation of hatching might be affected by the amount of roots. *C. spectabilis* may need to grow for a long period in order to reduce the nematode density to the same extent as that of the reduction caused by *C. juncea*.

The results of field tests also coincided with the results of hatching stimulation in root leachates. Cultivation of *C. juncea* greatly reduced the population of *H. glycines* in soil in all tests performed in fields (Table 2). The Pf/Pi value of the *C. spectabilis* plot was not significantly lower than that of the fallow plot in IACARI in 1999, but mean values of Pf/Pi decreased greatly in all tests. It was confirmed from these results that cultivation of these *Crotalaria* species result in a large reduction in the population of *H. glycines* under field conditions. Cultivation of *C. spectabilis* reduced the population of *H. glycines* more in the field tests than in the pot tests. The reason for this may have been the longer cultivation period in the field tests.

The results of field tests also showed that resistant soybean cultivars could decrease population density of *H. glycines* effectively (Table 2). However, frequent planting of resistant soybean cultivars in the same soil results in an increase of the nematodes that are able to develop and reproduce on them. Therefore, effectiveness of resistant soybean does not last long in Japan. On the other hand, since no juveniles have been able to reproduce on *Crotalaria* it is thought that the effectiveness of *Crotalaria* is much stabler than that of resistant soybean.

The optimal climate for growth of *Crotalaria* species is subtropical or temperate, but the results of this study showed that *Crotalaria* species could greatly reduce the population of *H. glycines* in a low temperature region, Sapporo (NARCH). This

may have been due to the sowing of a double amount of seeds and sowing in half-spaced rows compared with the sowing in the IACARI field in Mito, which is located in a temperate region.

The results of this study indicate that the two species of *Crotalaria*, especially *C. juncea*, are promising trap crops of *H. glycines*. Since *Crotalaria* has been used as a green manure crop in Japan, it might not be difficult to use this crop in existing crop rotation. The use of *Crotalaria* as a trap crop is a simple and effective means for reducing the population of *H. glycines*. Further research is needed to clarify suitable cultivation techniques of *Crotalaria* in crop rotation systems.

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REFERENCES

- Aiba, S. and Y. Mitsui (1995) Effects of some legumes on hatching of soybean cyst nematode (*Heterodera glycines*). *Ann. Rept. Plant Prot. North Japan* 46: 197–199 (in Japanese with English summary).
- Atkinson, H. J., M. Fowler and R. E. Isaac (1987) Partial purification of hatching activity for *Globodera rostochiensis* from potato root diffusate. *Ann. Appl. Biol.* 110: 115–125.
- Cooke, D. A. (1985) The effect of resistant cultivars of catch crops on the hatching of *Heterodera schachtii*. *Ann. Appl. Biol.* 106: 111–120.
- Devine, K. J. and P. W. Jones (2000a) Purification and partial characterization of hatching factors for the potato cyst nematode *Globodera rostochiensis* from potato root leachate. *Nematology* 2: 231–236.
- Devine, K. J. and P. W. Jones (2000b) Response of *Globodera rostochiensis* to exogenously applied hatching factors in soil. *Ann. Appl. Biol.* 137: 21–29.
- Evans, K. (1983) Hatching of potato cyst nematodes in root diffusates collected from twenty-five potato cultivars. *Crop Protection* 2: 97–103.
- Fukuzawa, A., H. Matsue, M. Ikura and T. Masamune (1985) Glycinoeclepin B and C, nortriterpenes related to Glycinoeclepin A. *Tetrahedron Letters* 26: 5539–5542.
- 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*. *J. Nematol.* 25: 695–702.
- 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. *Int. J. Nematol.* 8: 145–148.
- Hafez, S. L. and P. Sundararaj (2000) Impact of agronomic and cultural practices of green manure crops for the management of *Heterodera schachtii* in sugarbeet. *Int. J. Nematol.* 10: 177–182.
- Halford, P. D., M. D. Russell and K. Evans (1999) Use of resistant and susceptible potato cultivars in the trap cropping of potato cyst nematodes, *Globodera pallida* and *G. rostochiensis*. *Ann. Appl. Biol.* 134: 321–327.
- Hooper, D. J. (1986) Preserving and staining nematodes in plant tissues. In *Laboratory Methods for Work with Plant and Soil Nematodes* (J. F. Southey ed.). London, UK, H.M.S.O., pp. 81–85.
- Koch, D. W. and F. A. Gray (1997) Nematode-resistant oil radish for control of *Heterodera schachtii*. I. Sugarbeet-barley rotation. *J. Sugar Beet Res.* 34: 31–43.
- Koch, D. W., F. A. Gray and J. M. Krall (1998) Nematode-resistant oil radish for *Heterodera schachtii* control. II. Sugarbeet-dry bean-corn rotations. *J. Sugar Beet Res.* 35: 63–75.
- Krall, J. M., D. W. Koch, F. A. Gray and J. J. Nachtman (2000) Cultural management of trap crops for control of sugarbeet nematode. *J. Sugar Beet Res.* 37: 27–43.
- Kushida, A., T. Uehara and Y. Momota (2002) Effect of red clover on hatching and population density of *Heterodera glycines* (Tylenchida: Heteroderidae). *Jpn. J. Nematol.* 32: 69–76.
- Lamondia, J. A. and B. B. Brodie (1986) The effect of potato trap crops and fallow on decline of *Globodera rostochiensis*. *Ann. Appl. Biol.* 108: 347–352.
- Masamune, T., M. Anetai, M. Takasugi and N. Katsui (1982) Isolation of a natural hatching stimulus, Glycinoeclepin A, for the soybean cyst nematode. *Nature* 297: 495–496.
- Rawsthorne, D. and B. B. Brodie (1986) Relationship between root growth of potato, root diffusate production, and hatching of *Globodera rostochiensis*. *J. Nematol.* 18: 379–384.
- Schmitt, D. P. and R. D. Riggs (1991) Influence of selected plant species on hatching of eggs and development of juveniles of *Heterodera glycines*. *J. Nematol.* 23: 1–6.
- Scholte, K. (2000a) Effect of potato used as a trap crop on potato cyst nematodes and other soil pathogens and on the growth of a subsequent main potato crop. *Ann. Appl. Biol.* 136: 229–238.
- Scholte, K. (2000b) Screening of non-tuber bearing Solanaceae for resistance to and induction of juvenile hatch of potato cyst nematodes and their potential for trap cropping. *Ann. Appl. Biol.* 136: 239–246.
- Scholte, K. (2000c) Growth and development of plants with potential for use as trap crops for potato cyst nematodes and their effects on the numbers of juveniles in cysts. *Ann. Appl. Biol.* 137: 31–42.
- Scholte, K. and J. Vos (2000) Effects of potential trap crops and planting date on soil infestation with potato cyst nematodes and root-knot nematodes. *Ann. Appl. Biol.* 137: 153–164.
- Turner, S. J. and A. R. Stone (1981) Hatching of potato cyst nematodes (*Globodera rostochiensis*, *G. pallida*) in root exudates of *Solanum vernei* hybrids. *Nematologica* 27: 315–318.
- Yoshihara, T. and K. Kegasawa (1989) A method for the extraction of cyst of soybean cyst nematode from large volumes of wet soil. *Jpn. J. Nematol.* 19: 52–55 (in Japanese).