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GIOVANNA CURTO

SUSTAINABLE METHODS FOR MANAGEMENT OF CYST NEMATODES

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Abstract. The cyst nematode, *Heterodera schachtii* Schmidt, is the most dangerous sugar beet pest. It causes serious stands and yield decreases wherever sugar beet is grown. The adoption of wide crop rotations and the cultivation of *Brassicaceae* nematicidal plants and sugar beet tolerant varieties, concur to maintain good yields in infested soils. The history in the last 25 years regarding the progress in applied researches on agronomical, biological and genetic cyst nematode control, and the recommended practical techniques for the North-Italian farmers are reported.

1. INTRODUCTION

Sugar beet cyst nematode *Heterodera schachtii* Schmidt, one of the most dangerous and widespread pest of sugar beet (*Beta vulgaris* L. ssp. *saccharifera*), causes several plant damages. Changes in the absorbent cells, with subsequent nutritional imbalance and reduction of the root weight, may induce yield losses higher than 50% with an infestation of 300–400 eggs-2nd stage juveniles (J2) in 100 g of dry soil (Tacconi, 1987b).

Even if the recent restructuring of sacchariferous industrial sector caused a drastic reduction in the sugar beet crop surface in Italy, the cyst nematode infestations continue to represent a serious problem, since sugar beet crops are localized in areas close to the sugar refineries, with the aim of reducing the costs of taproot transportation. As a consequence, the choice of inserting sugar beet crops in a medium-long rotation scheme, results from the factory distance and not from the level of soil infestation.

Heterodera schachtii is widespread in the European sugar beet areas. In Italy, the infestations may reduce especially the weight of sugar beet roots and are strictly related to local climatic conditions: soil temperatures higher than 10°C for a long time increase the pest generation number and consequently the larval infestation level in soil. In addition, high temperatures stress infested sugar beet plants, reducing the roots ability to accumulate sucrose reserves.

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The nematode activity stops in autumn and winter, after the sugar beet harvest and with soil temperature lower than 8–10°C, to start again in spring. The completion of one *H. schachtii* generation is reached at the thermal sum of 465°C, that is the sum of the daily mean temperatures higher than 10°C. Therefore, while in Central-Northern Europe *H. schachtii* completes 3 generations per year, currently in Northern Italy it may complete at least 3–4 generations (BETA, 2006), because of the general temperature increase. Consequently since the ‘80th, the damage threshold has been fixed as 100 eggs-J2 in 100 g of dry soil, significantly lower than in Central-Northern Europe.

Table 1. Field trial results on Heterodera schachtii *chemical control on sugar beet during 1974–1975 (Tacconi & Saretto, 1975).*

Active ingredient	*Living cysts before treatment (IP)*	*Living cysts at harvest (FP)*	*FP/IP*	*Root weight (ton/ha)**	*Polarization (%)*	*Sucrose (ton/ha)**
Methyl Bromide	8.25	7.75	0.93	61.83 a	14.67	9.07 a
(1.2-Dichloropropane + 1.3-Dichloropropene) 80% + Meth lisothioc anate 20%	8.75	6.75	0.77	47.53 b	14.50	6.91 b
Aldicarb 10%	8.50	6.75	0.79	43.72 bc	14.31	6.28 bc
Oxamyl 10%	7.75	12.25	1.58	38.25 bc	14.06	5.40 bc
Phenamiphos 10%	14.00	11.00	0.79	36.07 bc	14.30	5.20 bc
Phorate 10%	8.50	10.50	1.24	40.16 bc	13.63	5.45 bc
Carbofuran 5%	6.25	7.25	1.16	40.78 bc	13.51	5.51 bc
Untreated Control	7.75	9.50	1.23	31.57 c	13.87	4.47 c

**Values significantly different for P = 0.05 (Duncan Test).*

2. SUGAR BEET CYST NEMATODE IN NORTHERN ITALY

The distribution of H. schachtii in Italy was ascertained by surveys carried out in 1990 and in 2004. In 1990 the most affected Italian regions were those with largest sugar beet crop surfaces and sugar refinery densities: Emilia-Romagna, Veneto, Lombardy and Apulia (Tacconi, 1993a). In 2004, the *H. schachtii* most damaged areas, in Northern Italy, were: Emilia-Romagna (48% of sugar beet crop surface), Piedmont (14%), Lombardy (11%) and Veneto (11%), with the most of infestations between light (less than 100 eggs-J2 in 100 g of dry soil) and medium (100–200 eggs-J2) levels. The highest infested areas were identified both in the eastern part of Emilia-Romagna and in the provinces of Rovigo (Veneto), Pavia (Lombardy), Alexandria and Asti (both in Piedmont) (Beltrami, Zavanella, & Curto, 2006b) (Fig. 1).

Currently, infested areas exceed 10% of the sugar beet Italian surface in Abruzzo and Emilia-Romagna, and are lower than 5% in Piedmont, Lombardy, Veneto, Tuscany, Apulia and 1% in Latium and Basilicata (source Cooperative Sugar beet Producers – Co.PRO.B.). Therefore, pest control is crucial for maintaining the crop productivity and ensuring an adequate income to farmers.

Today, farmers may choose among a series of agronomical techniques, which may warrant a success in controlling cyst nematodes, if correctly and punctually applied.

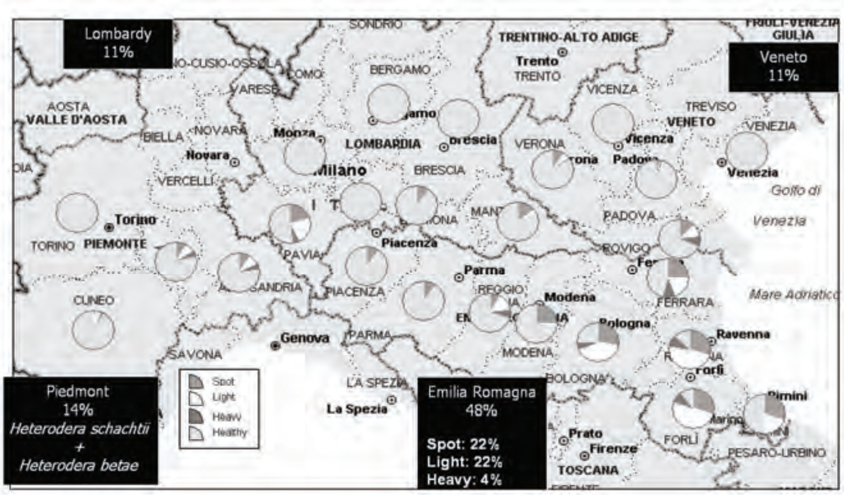

Figure 1. Spreading of Heterodera schachtii *in Northern Italy showing prevalence classes on sugar beet crop surface (from Beltrami* et al.*, 2006b).*

3. *HETERODERA SCHACHTII* BIOCONTROL IN NORTHERN ITALY

3.1. Chemical and Agronomic Control

From the ‘60th to the ‘70th, chemical control of cyst nematodes was investigated in several sugar beet field trials in Northern Italy, particularly in Emilia-Romagna and Veneto areas. The results were clear: chemical nematicidal applications were in most cases ineffective (Table 1), both in increasing sugar beet yields (Tacconi & Grasselli, 1978; Tacconi & Olimpieri, 1981; Bongiovanni, 1963; Tacconi & Ugolini, 1967; Greco, Lamberti, De Marinis, & Brandonisio, 1978) and in controlling the nematodes population (Zambelli & De Leonardis, 1974; Tacconi & Saretto, 1975). Furthermore, they appeared very expensive and toxic for the environment.

About 25 years ago, *H. schachtii* life cycle was investigated in greenhouse studies and in field trials (Tacconi, 1979, 1982), fixing the economic damage threshold of 100 eggs-J2 in 100 g of dry soil (Tacconi & Trentini, 1978; Tacconi &

Casarini, 1978; Greco, Brandonisio, & De Marinis, 1982a; Greco, Brandonisio, & De Marinis,1982b; Tacconi, 1987a). Researches effectively addressed the definition of appropriate four-years or six-years crop rotations, including *H. schachtii* non host crops, since these methods appeared more suitable for the environment, climate, soil and crops of Northern Italy plains (Table 2). Results showed that wide rotations always decreased the nematode population below the damage threshold, in moderately infested soils, and increased root yields (Tacconi & Olimpieri, 1985; Tacconi & Santi, 1991; Tacconi & Venturi, 1991).

Table 2. Effect of sugar beet crop rotations with non host crops of Heterodera schachtii *(from Tacconi & Venturi,1991)*

Rotations	*Crops in rotations **	*Nematode stages · g^{-1} before last crop*	*Root weight (ton/ha)*	*Polarization (%)*	*Sucrose (ton/ha)**
1981		Egg-J2			
Biennial	(B-O) + (B-W) + B	2.69	27.30	14.31	3.90
Quadriennal	(B-O-M-W) + B	1.34	34.60	13.95	4.81
1983		Cysts			
Biennial	(B-O) + (B-W) + (B-W) + B	15.75	52.70	13.23	7.01
Sexennial	(B-M-M-M-M-W) + B	5.00	73.30	13.79	10.05
Sexennial	(B-A-A-A-M-W) + B	3.25	77.60	12.35	9.59
Sexennial	(B-O-M-W-M-W) + B	6.50	77.10	14.14	10.86
1986		Egg-J2			
Biennial	(B-S) + (B-S) + B	6.80	10.60	13.15	1.38
Quadriennal	(B-S-W-M) + B	1.85	43.40	13.53	5.81
1988		Egg-J2			
Triennial	(B-S-W) + (B-S-W) + B	8.25	50.40	12.57	6.34
Sexennial	(B-S-W-M-M-W) + B	0.28	61.60	11.47	7.01

**B = sugar beet; W = wheat; M = maize; S = soybean; A = alfa-alfa; O = oats.*

In Northern Italy, in the same years, the susceptibility of some cultivated plant species towards indigenous populations of *H. schachtii* was screened in bioassays, in order to define the best rotations for agronomic control. They were: sunflower (*Helianthus annuus* L.), soybean (*Glycine max* L.), broad bean (*Vicia faba* L.), white clover (*Trifolium repens* L.), alfa-alfa (*Medicago sativa* L.), wheat (*Triticum* spp.), barley (*Hordeum vulgare* L.), maize (*Zea mays* L.), sorghum (*Sorghum vulgare* Pers.), potato (*Solanum tuberosum* L.) and tobacco (*Nicotiana tabacum* L.), which were classified as non host crops; reversed clover (*Trifolium resupinatum* L.), red clover (*Trifolium pratense* L.), eggplant (*Solanum melongena* L.), chickpea (*Cicer arietinum* L.), sweet pea (*Lathyrus odoratus* L.), hairy vetch (*Vicia villosa*

Roth.), classified as poor hosts (less than 1 adult female on the root); tomato (*Solanum lycopersicum* L.) classified as light host (1–4 females on the root); bean (*Phaseolus vulgaris* L.) classified as host (4.1–7 females on the root); carnation (*Dianthus caryophyllus* L.), pea (*Pisum sativum* L.) cv. Perfection, red radish (*Raphanus sativus* L. ssp. *major*), rape (*Brassica napus* L. var. *oleifera*), bird rape (*Brassica campestris* L. var. *oleifera*), cabbage (*Brassica oleracea* L.), white mustard (*Sinapis alba* L.), charlock mustard (*Sinapis arvensis* L.), spinach (*Spinacia oleracea* L.) and common buchwheat (*Polygonum fagopyrum* L.) classified as very good hosts (more than 10 adult females on the root) (Tacconi, 1993b, 1996, 1997).

Agronomic control represents even today one of the most effective methods for cyst nematodes management, together with a correct weeds management during rotation, since most widespread weeds are hosts of *H. schachtii* too. They are: redroot amaranth (*Amaranthus retroflexus* L.), bishop's weed (*Ammi majus* L.), scarlet pimpernel (*Anagallis arvensis* L.), shepherd's purse (*Capsella bursa pastoris* (L.) Medic.), fat-hen (*Chenopodium album* L.), black bind weed (*Fallopia convolvolus* (L.) A. Löve), willow weed (*Polygonum persicaria* L.), purslane (*Portulaca oleracea* L.), wild radish (*Raphanus raphanistrum* L.), sheep’s sorrel (*Rumex acetosella* L.), black nightshade (*Solanum nigrum* L.) and common chickweed (*Stellaria media* L.) (Tacconi & Santi, 1981), while velvetleaf (*Abutilon theophrasti* Medic.) is non host of *H. schachtii* (Tacconi & De Vincentis, 1996).

Other cultural practices, which can help farmers to control cyst nematodes, are: efficient hydraulic layout; clean equipment; earlier sowing. The latter procedure aims at staggering both the sugar beet and the cyst nematode cycles and obtaining sturdier plants, able to resist to the nematode infestation. Further procedures include harvesting of susceptible varieties within August, in order to avoid the damage increase and the parasite development (BETA, 2006).

3.2. Biological Control

3.2.1. Brassicaceae *Nematicidal Intercrops*

The quality improvement of sugar beet crop, decreasing nematode infestations below the threshold value and increasing both taproot weight and sucrose, was effectively achieved through the study of rotations effects, including intercrops of *Brassicaceae* species, selected for high glucosinolate content. The cells of these plants, in fact, contain the glucosinolate-myrosinase system, which, following cell lesions and enzymatic hydrolysis, produces a number of biologically active compounds including isothiocyanates, nitriles, epithionitriles and thiocyanates (Fahey, Zalcmann., & Talalay, 2001).

Nematicidal *Brassicaceae* can accumulate the majority of glucosinolates either in the root system (catch effect) or in the stems and leaves (biofumigant effect). The first process is the most suitable to control cyst nematodes.

Brassicaceae catch crops attract the juvenile stages of endoparasitic nematodes working as a trap, since these, after root penetration, are poisoned by hydrolysis products and are not successful in completing their developmental cycle in 10–12 weeks, that is the intercropping time. Consequently, the nematode population in soil progressively decreases. At full flowering the plants are chopped and immediately incorporated at around 20 cm depth by means of a stalk cutter and a miller, working at some meters distance from each other. A light irrigation sprinkled after incorporation in soil, aims at promoting the glucosinolate hydrolysis and the subsequent isothiocyanate release (Lazzeri, Leoni, Bernardi, Malaguti, & Cinti, 2004b).

The nematicidal effect of a catch crop is produced during the whole cultivation time, while its incorporation as green manure shows an overall ammendant effect, increasing the organic matter amount and improving soil fertility, being the biofumigant effect during incorporation only secondary.

3.2.2. Application of Heterodera schachtii *Biocontrol in Northern Italy*

In Italy, the first researches regarding the control of cyst nematodes by means of nematicidal plants, go back to 1983 and continued with high impulse for all the '90th. These studies concerned the life cycle of *H. schachtii* in the roots of either cultivated or biocidal plants, through in vitro and in vivo experiments carrid out both in laboratory and in glasshouse, with the purpose of achieving the most effective rotation schemes for sugar beet crops, including nematicidal intercrops.

The first in vitro tests were performed in 5 cm diameter Petri dishes, soaking *H. schachtii* J2 in a *Brassicaceae* glucosinolate solution, at different concentrations, after glucosinolate hydrolysis by means of myrosinase. The nematodes were observed after 24, 48, 72 and 96 hrs, screening the percent mortality of J2. The allyl isothiocyanate, resulting by the hydrolysis of sinigrin, showed the highest J2 mortality after 24 hrs at an initial glucosinolate concentration of 0.5%, while at the same concentration other rapeseed glucosinolates (gluconapin, glucotropeolin, dehydroerucin) caused the J2 death after 48 hrs (Lazzeri, Tacconi, & Palmieri, 1993).

The in vivo studies were developed in subsequent steps, at first in glasshouse in either 5 l pots each containing 7–8 plants (Tacconi, Mambelli, Menichetti, & Pola, 1989) or 54 ml plastic microcells (units) each containing 1 plant (Tacconi & Pola, 1996). All the biocidal selections were cultivated in sterilised soil and inoculated with a known number of *H. schachtii* J2.

Results were checked in semifield conditions, in 1 m^2 plots each containing 1 m^3 of infested soil (Tacconi, De Vincentis, Lazzeri, & Malaguti, 1998; Tacconi, Lazzeri, & Palmieri, 2000) and in field trials, concerning the study of rotation schemes including either non host or biocidal catch crops (Tacconi & Olimpieri, 1983; Tacconi, Biancardi, & Olimpieri, 1990; Tacconi & Regazzi, 1990; Tacconi, Mambelli, & Venturi, 1991; Tacconi, Biancardi, & Olimpieri, 1995; Tacconi et al., 2000).

In glasshouse experiments, the development of juveniles (J3 and J4) and adults (males and females) in roots was examined after root homogenisation (Stemerding, 1964) in periodical checks. These studies showed the biocidal plant ability in interrupting the *H. schachtii* life cycle to J3 or J4 female, without any formation of adult female and cysts. On the contrary, juvenile males developed to adults, changing the sex-ratio of the nematode population. The effectiveness of biocidal plants in reducing *H. schachtii* population was described either by the biotest (Behringer, Heinicke, Von Kries., Müller, & Schmidt, 1984) as percent ratio between the number of adult white females on biocidal plant roots and on sugar beet ones, or by the reproduction factor (R) (Ferris et al., 1993), that is the ratio between nematode population in soil after the catch crop incorporation (FP) and before the catch crop sowing (IP), (R = FP/IP).

In 2006 in vitro tests were performed according to the method described in Lazzeri, Curto, Leoni, and Dallavalle (2004a). The in vitro experiments were carried out in glass cavity blocks, soaking the J2 in a glucosinolate solution and adding myrosinase which reacted directly in the block. The blocks were sealed to preserve the volatile compounds, and the nematicidal and nematistatic effects were observed either after 24 or 48 hrs. Gluconasturtiin, glucoerucin and sinigrin were tested at different concentrations for the definition of LC50 towards *H. schachtii* J2 (Table 3).

Table 3. Glucosinolate concentrations checked in vitro *bioessays towards* Heterodera schachtii *second stage juveniles.*

Glucosinolate	*Concentration (mM)*								
Gluconasturtiin	0.013	0.026	0.05	0.1	0.125	0.15	0.25	0.5	1
Glucoerucin	0.0625	0.0125	0.15	0.2	0.25	0.5			
Sinigrin	1								

The J2 mortality was the same in gluconasturtiin either after 24 or 48 hrs (0.125<LC50<0.25): the nematicidal action was very fast (already after 24 h) while the immobilisation effect resulted poor. In glucoerucin, the nematicidal action resulted slower than in gluconasturtiin: the highest J2 mortality was reached after 48 hrs (0.15<LC50<0.20 mM), while a strong immobilisation effect was observed after 24 hrs (0.20<LC50<0.25 mM). In general, the toxic effect towards *H. schachtii* J2 is achieved by highest glucosinolate concentrations (Lazzeri et al., 2004a).

3.2.3. Nematicidal Plant Species in Heterodera schachtii *Control*

Main glucosinolates effective against *H. schachtii* in Northern Italy agronomic conditions derive either from radish (*Raphanus sativus* L. ssp. *oleiformis*) or white mustard (*Sinapis alba* L.) varieties. *Raphanus sativus* ssp. *oleiformis* cv. Nemex and cv. Pegletta, *S. alba* cv. Maxi and other varieties with high nematicidal power were at first tested as catch crops and used as intercrops in quadriennial rotation schemes (Tables 4, 5) (Tacconi et al., 1989; Tacconi & Venturi, 1991).

Table 4. Reproduction factor of Heterodera schachtii *population, between rotation end and beginning, with and without nematicidal catch crops (Tacconi & Venturi, 1991).*

Rotations (1983–1989)[*]	*FP/IP without catch crop*	*FP/IP with catch crop*
Biennial (B-Ba) + (B-W) + (B-W) + B	3.78	2.38
Triennial (B-Ba-W) + (B-S-W) + B	3.17	1.58
Quadriennal (O-M-W-B)	0.11	0.07
Quadriennal + Biennial (B-Ba-S-W) + (B-W) + B	2.25	2.35

[*] *B = sugar beet; Ba = barley; M = maize; O = oats; S = soybean; W = wheat.*

In studies carried out in the ‘90th, some selections of other plant genera such as *Cleome spinosa* Jacq. (family *Capparaceae)*, *Eruca sativa* Mill. cv. Prisca and *Reseda luteola* L. (family *Resedaceae*) resulted effective against *H. schachtii* (Table 6) (Tacconi et al., 1998), and recently also against the southern root-knot nematode, *Meloidogyne incognita* (Curto, Dallavalle, & Lazzeri, 2005).

Since 2004 both the main *Brassicaceae* varieties, marketed as nematicidal plants for control of *H. schachtii*, and other selections previously evaluated as effective in control of *M. incognita* (Curto et al., 2005; Curto, Lazzeri, Dallavalle, Santi, & Malaguti, 2006a; Curto, Lazzeri, Santi, & Dallavalle, 2006b), were tested in Northern Italy (Emilia-Romagna), checking their effectiveness on the indigenous population of *H. schachtii* in the local, environmental conditions.

Results showed a good genetic stability of the old varieties and generally a satisfying effectiveness in the newest selections, with a decrease in nematode population higher than 80% (Beltrami, Curto, & Zavanella, 2006a; Beltrami et al., 2006b). Only a brassica blend between white mustard (*S. alba* L.) and oriental mustard (*Brassica juncea* L.) allowed *H. schachtii* to multiply more than on sugar beet, while *R. sativus* L. ssp. *oleiformis* cv. Carlos, did not keep its performance in time, decreasing in two following years its ability in interrupting the cyst nematode cycle (R>1).

Eruca sativa cv. Nemat, very efficient as catch crop against *M. incognita*, did not confirm its biocidal effects on *H. schachtii* (Table 7). The *H. schachtii* life cycle in the roots was interrupted generally at the J3 stage, but several male adults were observed (Beltrami et al., 2006b). The green matter released in soil by biocidal varieties was always conspicuous, varying from 5 to 10.1 kg/m^2 (Beltrami, Zavanella, & Curto, 2007).

Table 5. Host status of fodder radish and white mustard biocidal selections vs. sugar beet cyst nematode Heterodera schachtii, *in a field test in Northern Italy (Tacconi* et al.*, 1989)*

Plant species	*Female specimens/10 g roots*				*Male specimens/10 g roots*		*Host* status*
	J2	*J3–J4*	*Adult*	*Cyst*	*J3–J4*	*Adult*	
Beta vulgaris L. ssp. *saccharifera* cv. Sigma	6.5	12.0	14.0	2.6	3.0	2.0	5
Raphanus sativus L. ssp. *oleiformis* cv. Sereno	1.9	1.8	0.7	0.1	2.8	4.0	3
R. sativus L. ssp. *oleiformis* cv. Pegletta	3.2	1.4	0.0	0.0	3.9	2.9	1
R. sativus L. ssp. *oleiformis* cv. Levana	5.4	5.5	0.8	1.1	6.4	4.1	3
R. sativus L. ssp. *oleiformis* cv. Nemex	2.7	0.8	0.0	0.0	2.5	1.9	1
Sinapis alba L. cv. Emergo	1.7	1.5	0.2	0.0	2.4	2.3	2
S. alba L. cv. Maxi	1.9	0.9	0.0	0.0	0.1	0.6	1

**Based on BIOTEST (Behringer* et al.*, 1984): a catch crop shows nematicidal effects, at a host status included from 1 to 3.*

3.2.4. Management of Nematicidal Intercrops in Northern Italy

In Northern Italy, two periods are recommended for the cultivation of nematicidal intercrops: a spring time on set-aside fields and a summer period, after the harvest of winter cereals. Currently, the spring intercropping is the most practised because of set-aside spreading, highest effectiveness in cyst nematode control and very low costs. In this case, the *Brassicaceae* catch crops must be kept far from red radish seed crops (*Raphanus sativus* L. ssp. *major*), which could be polluted by unwished crosses with nematicidal plants, since both crops flower at the same time.

Management of spring intercrops includes (BETA, 2006): a glyphosate-based herbicide treatment, 3–4 days before sowing; sowing of nematicidal varieties on unbroken soil at the end of March; either mowing or plant cutting and incorporation in soil at full flowering, and a deep ploughing in August, to prepare soil for the sugar beet crop in the following spring.

Table 6. Host status of biocidal selections vs. sugar beet cyst nematode Heterodera schachtii *(Tacconi* et al.*, 1998).*

Plant species		*Female/g roots*			*Males/g roots*		*Host* status*
	J2	*J3–J4*	*Adult*	*Cyst*	*J3–J4*	*Adult*	
Beta vulgaris L. ssp. *saccharifera* cv. Dima	2.6	10.4	19.9	0.8	11.5	8.3	5
Raphanus sativus L. ssp. *oleiformis* cv. Pegletta	5.6	3.3	0.0	0.0	3.1	0.3	1
Cleome spinosa Jacq. Italian ecotype	3.7	3.5	0.2	0.0	1.3	1.1	1
Eruca sativa Mill. cv. Prisca	4.4	1.6	0.1	0.0	1.6	0.6	1
Reseda luteola L.	0.1	0.0	0.0	0.1	0.0	0.0	0
Sinapis arvensis L. Sri Lanka ecotype	1.4	1.5	3.2	0.2	2.8	1.3	5

** Based on BIOTEST (Behringer* et al.*, 1984): a catch crop shows nematicidal effects, at a host status included from 1 to 3.*

Management of a summer intercrop in quadriennal rotations requires more inputs than the spring one: at the end of August the biocidal variety must be sown on unbroken soil after the cereal harvesting. Its cultivation time lasts from September to November, and could necessitate an irrigation aid and an insecticidal application against *Altica* sp. At the end of November, the nematicidal intercrop must be dried up by glyphosate, then the soil tilled in winter and sown with maize, sorghum or soybean, in the following March–April.

The fall cultivation was initially studied to allow small farms to grow biocidal intercrops, but the results were inconsistent and in 35% of cases either indifference or increase in cyst nematode infestations were recorded. Late sowing delays the biocidal crop cycle, while the decrease in soil temperatures reduces the glucosinolate store into their roots. The thermal sum in soil remains below the nematode optimum, with a progressive cyst dormancy (biological minimum at 8–10°C). Therefore, in the autumnal cultivation some cover crop effects, such as the supply of organic matter and the limitation of nitrate leaching, become predominant.

Table 7. Host status of biocidal selections vs. a North Italian population of Heterodera schachtii *(Curto* et al.*, unpublished data).*

Plant specie	*Variety*	R^a
Raphanus sativus L. ssp. *oleiformis*	Terranova*	0.00
	Comet**	0.05
	Corporal*	0.06
	Adios**	0.06
	Regresso*	0.08
	Diabolo***	0.14
	Arena***	0.15
	Remonta**	0.18
	Colonel***	0.25
	Pegletta**	0.38
	Karakter**	0.61
	Carlos**	1.61
Sinapis alba L. + *Brassica juncea* L.	Terraprotect*	2.85
Sinapis alba L.	Accent***	0.24
	Concerta*	0.34
Sinapis arvensis L.	*	12.30
Rapistrum rugosum L.	*	0.00
Eruca sativa Mill.	Nemat**	1.02
Sorghum vulgare	Triumph*	0.04
Crotolaria juncea L.	*	0.09
Beta vulgaris L. ssp. *saccharifera*	Orion*	1.50
	Gea**	3.50

R^a = eggs/J2 ratio in 100 g of dry soil at the beginning and the end of each cycle.
= varieties checked only one year; **= mean of two years; *= mean of three years.*

3.2.5. Promotion of Heterodera schachtii *Biocontrol in Northern Italy*

Since the ‘80th end, the biological management of *H. schachtii* with *Brassicaceae* nematicidal intercrops was effectively promoted both by sugar companies and sugar beet farmer associations (Co.PRO.B.), spreading this technique to the most of Northern Italy (Emilia-Romagna, Lombardy, Veneto) with innovative mind.

Emilia-Romagna regional administration and sugar beet farmer national associations supported the insertion of nematicidal intercrops in the rotation schemes, with the objective of reclaiming heavily infested soils. Grants to sugar beet farmers were warranted, both for purchasing biocidal radish seeds and getting technical assistance in the rotation planning and the biocidal crop cultivation.

From 1994 to 2001 more than 10,000 ha (on a total surface of 76,000 ha of sugar beet crops), were sown in Emilia-Romagna with biocidal *Brassicaceae* intercrops, within the “Sugar beet Cyst Nematode Project” promoted by Co.PRO.B. in the sugar beet districts of Bologna and Ferrara provinces, with an annual trend in continuous development. Regarding the sowing time, most of biocidal intercrops were cultivated in spring, on set-aside fields. Sowings within April 30th were 72%

in 1999 and 70% in 2000, the residual 30% being represented by September sowings and only the lowest part by Summer ones, after harvesting of either cereals or other crops (i.e. onion).

Currently in Italy, the contraction of sugar beet surface and the closure of sugar refineries induced farmers to abandon most infested fields, moving the sugar beet cultivation towards areas with low *H. schachtii* infestation, or closer to the sugar refineries. In the last years the insertion of a biocidal intercrop in the rotation schemes was considered as a possible way to increase the efficacy of sugar beet tolerant varieties, when cyst nematode infestation are higher than 400 egg-J2 in 100 g of dry soil.

3.*2.6. Resistance and Tolerane*

The selection of sugar beet genotypes tolerant to *H. schachtii*, achieved only recently interesting productive performances. The new genotypes derive from crosses between cultivated selections of sugar beet (*Beta vulgaris* L. ssp. *saccharifera*) and spontaneous species, such as *Beta maritima* and *Beta procumbens*, both carriers of resistance genes to the cyst nematode.

In 2003 the Italian National Technical Commission (CTN) performed the first trials concerning some new tolerant lines and in 2004 the commercialisation of resistant cv Paulina and tolerant cv Pauletta (both by KWS) started.

The definition of either resistant or tolerant sugar beet variety was recently described. A resistant variety is able to limit the nematode reproduction, while a tolerant variety is able to decrease the productive losses, if compared with a susceptible one (Plantard et al., 2006). On the contrary, results obtained in Italy showed that the tolerant variety Pauletta, grown on *H. schachtii* infested soil, had much higher yields than the resistant one and was equally able to limit the nematode reproduction. Currently, it is the only one variety marketed in a consistent number of unities in Italy. Trials carried out in Emilia-Romagna both in full field and pots demonstrated that the productivity of the resistant cv Paulina was lower than susceptible control (cv. Gea) with poor yields, in sugar and root weights, either in healthy or infested soils (Beltrami et al., 2006b). For this reason, it was no more commercialised in Italy, since 2006.

Other new varieties defined as tolerant both to rhizomania (Beet necrotic yellow vein virus) and sugar beet cyst nematode, were introduced on the Italian market in the last three years: Fenice and Flex (Delitzsch), Colorado and Florida (Betaseeds), Piera (KWS). Results (Table 8) of several trials (Beltrami et al., 2007) showed no relevant differences in root yields between the susceptible variety cv. Gea and the tolerant ones (cvs. Pauletta, Colorado, Fenice, Piera and Flex) when grown in healthy soil. However, a higher root yield was recorded in tolerant varieties when they were cultivated either in lightly infested soil (<100 eggs-J2) with a 20% increase, or in infested ones with a 50% increase, compared with the *H. schachtii* susceptible sugar beet cultivars.

Regarding polarization values, the susceptible variety always evidenced a heavy decrease in its polarization, coinciding with an increase in *H. schachtii* population density, while in the tolerant varieties and particularly in Piera and Flex, this reduction was lower. High levels of both thick juice and invert sugar reveal a poor quality of sugar beets, stressed by the cyst nematode. These unfavourable values were sensibly higher in the susceptible variety than in the cvs. Pauletta, Colorado, Fenice, Piera, Flex and Florida.

The Gross Sealable Production (GSP) in infested soil was much higher in tolerant varieties (cvs. Pauletta, Colorado, Fenice, Piera and Flex) than either susceptible beet cultivars or the “resistant” cv. Paulina. Both Piera and Flex, because of their higher polarimetric title, evidenced a higher GSP compared with Pauletta, Colorado and Fenice. In healthy soil, the results of the susceptible variety (cv. Gea) did not differ statistically from the effectiveness of the tolerant ones. But other traditional varieties, also susceptible to *H. schachtii* but more productive than cv. Gea, could be appropriately cultivated in soil where the cyst nematode was not recorded. It is worth to nota that all the current tolerant varieties are not tolerant to sugar beet leaf spots (*Cercospora baeticola* L.).

The ability of sugar beet tolerant varieties to lower the cyst nematode population was checked in Northern Italy fields and in pots. Results show R values between 2 and 4 in the tolerant varieties and between 16 and 20 in the susceptible ones (Beltrami et al., 2006b). In a soil with a *H. schachtii* infestation of 100 eggs-J2 in 100 g of dry soil, the cultivation of a tolerant variety allowed a nematode population density of 200–400 eggs-J2 100 g^{-1} of dry soil, whereas the susceptible variety reached a nematode population of 1600–2000 eggs-J2 100 g^{-1} of dry soil.

On the basis of further observations, tolerant sugar beet varieties seem to decrease their ability to control the *H. schachtii* population when the initial infestation is higher than 400 eggs-J2·100 g^{-1} of dry soil (Beltrami et al., 2007).

4. OUTLOOK OF BIOCONTROL IN NORTHERN ITALY

Even if the Italian sugar beet crop surface was sensibly reduced in these last years, the crop productivity is still threatened by *H. schachtii*. Therefore, sowing of tolerant sugar beet varieties, even in soils with a very low cyst nematode infestation, is strongly advised. The most recommended tolerant cultivars are Pauletta, Colorado, Fenice, Piera, Flex and Florida, the last three ones being more suitable for fall harvestings than the others.

In soils free from *H. schachtii*, the use of best traditional sugar beet varieties (both rhizomania and sugar beet leaf spots tolerant) is strongly suggested, because they allow the best productive results.

In soil characterised by very heavy cyst nematode infestations (more than 300 eggs-J2·100 g^{-1} of dry soil) the cultivation of nematicidal *Brassicaceae* intercrops is always recommended, since are able to quickly and effectively improve soil, releasing large amounts of organic matter.

Table 8. Productive results of tolerant sugar beet varieties either on healthy or lightly infested soil (normalized with average data 2005–2006) (Beltrami et al.*, 2007).*

Variety	*Healthy soil*				
	Root	*Polarization*	*Sucrose*	*Thick juice*	*GSP*
Gea**	100.0	100.0	100.0	100.0	100.0
Flex	94.6	102.9	97.5	100.3	98.4
Piera	96.4	101.4	97.5	100.1	97.5
Fenice	107.2	92.9	99.6	99.0	96.3
Colorado	105.3	93.0	98.0	99.0	94.8
Pauletta	103.4	93.9	97.2	99.1	94.3
Paulina	96.4	92.3	88.8	97.3	85.5
DMS 0.05	8.5	2.5	7.7	0.5	8.0
	*Lightly infested soil**				
	Root	*Polarization*	*Sucrose*	*Thick juice*	*GSP*
Gea**	100.0	100.0	100.0	100.0	100.0
Flex	117.0	104.8	123.1	100.1	125.5
Piera	117.7	106.0	125.4	100.1	128.9
Fenice	125.8	97.0	122.1	98.4	120.0
Colorado	132.8	95.1	126.7	98.1	122.9
Pauletta	130.2	95.9	124.5	98.1	120.5
Paulina	112.7	94.4	106.4	96.7	103.4
DMS 0.05	8.6	2.6	9.1	0.8	9.8

** <100 eggs-J2 in 100 g of dried soil*
*** Commercial standard*

In Northern Italy, technical services are organised at the regional level, with the aim of supporting farmers in sugar beet crop decisions, according to regional guidelines of integrated crop management. These guidelines are updated every year on the basis of the results achieved by private and public research institutes and companies. One of the main investigation company in sugar beet is BETA ITALIA S.c.a.r.l., whose partners are Finbieticola, gathering the main sugar beet farmer associations (ANB, CNB, and ABI) and Assozucchero, which includes the whole sugar industry compartment (Italia Zuccheri, Eridania-Sadam, SFIR, COPROB and Zuccherificio del Molise). Public institutes involved in sugar beet research are the Research Institute for Industrial Crops – Council for Research in Agriculture (CRA-ISCI) Rovigo section, the Phytosanitary Service of Emilia-Romagna Region in Bologna and some Italian Universities.

The effective control of *H. schachtii*, linked to high productive levels in sugar beet crops, are currently achieved by the integration of agronomical and biological strategies. Either the soil health or the cyst infestation level, ascertained through nematological analysis, represent the factors for choosing the most suitable strategy. Most productive varieties, susceptible to *H. schachtii*, must be grown on healthy soil, while the tolerant ones, suitable for early or late harvests, must be cultivated on infested soil. Moreover, nematological analyses represent the only method suitable to reveal heavy nematode infestations with more than 300–400 eggs-J2, corresponding to the threshold excluding the sugar beet cultivation and recommends the sowing of biocidal *Brassicaceae* intercrops for soil recovery.

Anyway, even if few farmers of some sugar beet districts still follow short rotations, the technical support service recommends four year rotations in healthy soil and five year rotations with ascertained nematode infestation, as crucial cultural care for achieving best effectiveness of whatever pest and disease control strategy.

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