CHEMICAL AND NONCHEMICAL OPTIONS FOR THE MANAGEMENT OF *HETERODERA SCHACHTII* IN NORTHERN SPAIN

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Summary. The susceptible sugarbeet cultivar Acord and the resistant cultivar Nemadie were sown in a field infested with *Heterodera schachtii*. The nematicide fenamiphos was applied to the susceptible cultivar in three different ways: at sowing, 11 weeks after sowing or both times. Differences in eggs and second stage juveniles per cyst at harvest were very marked between the different treatments. Nematode multiplication rates varied from 0.3 to 0.6, in the treatment with two applications of nematicide and for resistant cultivar respectively, to 2.1 and 2.2 for the untreated susceptible cultivar and the nematicide application at sowing. The post-emergence nematicide application led to an intermediate multiplication rate of 1.2. Regression analyses of log-transformed final and initial infestation data showed that the relative efficacy of the treatments changed depending on the initial infestation, the double application of nematicide being the only treatment that prevented nematode multiplication. Yields between the treatments from the susceptible cultivar varied significantly depending on the application of granular nematicide.

Surveys throughout the sugarbeet production areas in the Basque Country have been carried out since 1991 to ascertain the distribution and incidence of the sugarbeet cyst nematode *Heterodera schachtii* in the cultivation area.

Nematode control strategies in the sugarbeet areas include long rotations, chemical treatments and resistant cultivars. For the management of sugarbeet cyst nematode, trap crops have proved to be very useful following cereal crops in the winter season (Müller and Steudel, 1983; Moens *et al.*, 1990; Hafez and Sundararaj, 1998, 1999 and 2000). This applies under northern Spain conditions (López Robles, 1989; Redondo and Villarias, 1991; Iturritxa *et al.,* 1994)

Many attempts to breed resistant sugarbeet cultivars have been made for more than 50 years (Yu, 1984; Jung, 1998). So far, some problems have been reported depending on the source of resistance and on the method of transferring it into the cultivated *Beta vulgaris*. Most importantly is the loss of sugar yield due to nematode feeding hypersensitivity reactions (Heijbroek, 1991) and/or partial resistance responses (Yu, 1981; Mahfoud *et al.*, 1996). On the other hand, chemical control against *H. schachtii* is quite common, resulting in noticeable yield increases, although nematicidal efficiency depends on environmental factors (Hague and Gowen, 1987; Caubel and Muchembled, 1991; Betodlaren, 1998).

The present study was conducted to compare a resistant cultivar with applications of a nematicide for *H. schachtii* control under the particular agroclimatical conditions of the Basque Country in Northern Spain.

MATERIALS AND METHODS

The trial was conducted in a sandy loam field moderately infested with sugarbeet cyst nematode, *Heterodera schachtii* Schmidt at Ullivarri-viña (Alava). The choosen area was moderately infested averaging 3 eggs + second stage juveniles (J_2) of *H. schachtii* per gram of soil. The experimental design was in a completely randomised blocks with 20 replications of the five treatments as indicated in tables I-IV with the susceptible cultivar "Accord" of *Beta vulgaris* L., and the resistant cultivar "Nemadie". The experimental units were plots of 9 m^2. The nematicide fenamiphos was applied in the furrows at the rate of 5 kg a.i./ha when granular (at sowing) and at the rate of 10 kg a.i/ha when liquid (post-emergence). Liquid fenamiphos was applied 11 weeks after sowing, when the plants had at least ten leaves. The growing season extended from the end of March until the middle of November of 1994. The lowest average of monthly minimun temperature was recorded in November (0.7 °C) and the highest of maximun temperature in August (25.2 °C). The accumulated rainfall from March to November was 484 mm.

Soil samples were collected from each plot at sowing and at harvesting. They consisted of 30 cores taken throughout the whole plot with a 2 cm diameter probe. Cysts were recovered from soil samples by the Fenwick method and counted. The number of viable eggs and second stage juveniles inside the cyst was assessed after crushing a random sample of 20 cysts in 20 ml of water. Eggs and second stage juveniles were counted in at least three aliquots of 1 ml.

After harvesting, four samples of 100 plants per treatment were weighed and their sugar content assessed.

Statistical analysis was carried out using SAS software. In order to examine the relationship between egg population density after harvest (Pf) and at sowing (Pi), regression analysis of logPf on logPi was performed for each treatment. Confidence limits of regressors were assessed according to Sokal and Rohlf (1981). Analysis of variance was performed for yield and multiplication rates.

A second experiment was carried out in the Neiker centre in a glasshouse to evaluate two sugar beet cultivars with potential resistance to sugarbeet cyst nematode: Nemadie (Strube) pilled seed and a bare seed of the Maribo Company, and they were compared to the susceptible cultivar Eva.

The cultivars were sown in plastic transparent beakers with a capacity of 250 cc with perforated base filled with a steamed mixture of soil and sand. Cysts were enclosed in muslin bags (buried in the soil just before sowing), at the approximate inoculation rate of 10 eggs and second stage juveniles/g of soil (Salazar, 1991). The beakers were inserted inside opaque pots to avoid light and to allow observations of the nematodes development on the roots .

RESULTS

Infestation densities expressed as cysts/100 g of soil, and eggs and J_2/cyst, are shown in Table I. Cyst numbers were similar for each of the treatments, but there were significant differences between the mean number of eggs per cyst. Eggs and J_2 decreased very noticeably with the resistant cultivar and with the susceptible cultivar with the fenamiphos applications at sowing and at post emergence with respect to the initial populations. New cysts were scarse in plots with both resistant and susceptible cultivars and with two applications of nematicide. Multiplication rates of eggs and juveniles were less than the unity. On the other hand, a single nematicide application at sowing and no nematicide application at sowing gave similar results (Table II). The 89% of the variability in Pf was explained by Pi and treatments (Fig. 1). All the regressions (Fig. 2) between the log-transformed final and initial infestation densities were highly linear ($p<0.01$). Regression coefficients varied between the log-transformed final and initial infestation densities and were highly linear ($p<0.01$). Regression coefficients varied between 0.54 for no nematicide application and 0.89 for the treatment of nematicide application at sowing.

Table I. Population densities *of Heterodera schachtii* in the field at sowing and at harvest.

Treatment	Cyst number per 100 g soil		Eggs + J_2 per cyst	
	At sowing	At harvesting	At sowing	At harvesting
Accord (Susceptible)	6.6 ± 1.91 a	9.3 ± 2.1 a	34 ± 0.13 a	44.7 ± 0.10 b
Accord + fenamiphos at sowing	7 ± 1.35 a	9.1 ± 2.6 a	40 ± 0.19 a	62.3 ± 0.10 a
Accord + fenamiphos at post-emergence	8.7 ± 2.2 a	8.5 ± 1.85 a	37 ± 0.12 a	11.7 ± 0.15 e
Accord + fenamiphos at sowing and post-emergence	7.1 ± 1.32 a	8.8 ± 1.75 a	39 ± 0.16 a	33.3 ± 0.10 c
Nemadie (Resistant)	10.7 ± 1.06 a	11.2 ± 5.20 a	41 ± 0.18 a	19.8 ± 1.12 d

Means in each column followed by the same letters do not differ significantly ($p<0.05$) according to Waller-Duncan test (k=100).

Table II. Mean values of multiplication rate of cyst and eggs + J_2 of *H. schachtii*.

Treatment	Multiplication rate of cysts	Multiplication rate of eggs + J_2
Accord (Susceptible)	1.77 a	2.08 a
Accord + fenamiphos at sowing	1.35 ab	2.21 a
Accord + fenamiphos at post-emergence	1.08 b	0.33 c
Accord + fenamiphos at sowing and post-emergence	1.35 ab	1.16 b
Nemadie (Resistant)	1.17 b	0.61 c

Means in each column followed by the same letters do not differ significantly ($p<0.05$) according to Waller-Duncan test (k=100).

Regression lines corresponding to these two treatments crossed at Pi values of about 1 egg per gram of soil. Line from the resistant cultivar and the corresponding to the theoretical logPf = logPi crossed at about the same initial infestation density (logPi = 0). The regression line corresponding to the double nematicide application lies below this last one, while the post-emergence nematicide application and control crossed only from Pi values of about 3 and 10 eggs per gram of soil, respectively.

Root weight and sugar yield per plant are presented in Table III. The percentages of sugar content in the roots of the susceptible cultivar were independent of the application of nematicide and higher than the sugar content of the resistant cultivar. Nevertheless, root weights varied with respect to the application of nematicide. The raw yield from the treatments that contained nematicide at sowing were significantly higher than that from the untreated susceptible cultivar.

Differences in percentages of sugar content between the resistant and the susceptible cultivar were not high enough to alter the differences in raw yield and therefore sugar content per plant varied in the same way as the root weight did.

In the glasshouse study, the rate of development of the plants was similar in the susceptible and two resistant cultivars, but thereafter the greatest rate of growth occurred in the susceptible cultivar.

The first females appeared one month after sowing at the five-eight leaf stage and the first mature cysts appeared 50 days after sowing and inoculation of the plants (Fig. 3). The number of cysts of the new generation and their viability stage are given in Table IV.

Very high cyst numbers developed in the susceptible host but with a lower viable content in relation to the Strube-hybrid. The number of cysts that developed on this cultivar is notably low but with a high viable content (twice the viable content generated in the susceptible cultivar). Notably is the high rate of multiplication of the nematode in the susceptible cultivar and the Maribo-hybrid compared to the resistant cultivar Nemadie.

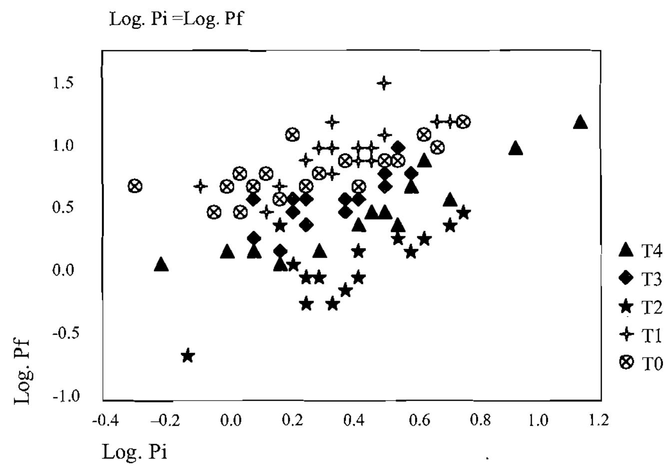

Fig. 1. Relationship among the transformed values of Pi and Pf: T0, susceptible "Accord"; T1 "Accord" + fenamiphos at sowing; T2, "Accord" + fenamiphos at post-emergence; T3 = T1 + T2, "Accord" + Fenamiphos at sowing and at post-emergence; T4, resistant "Nemadie"

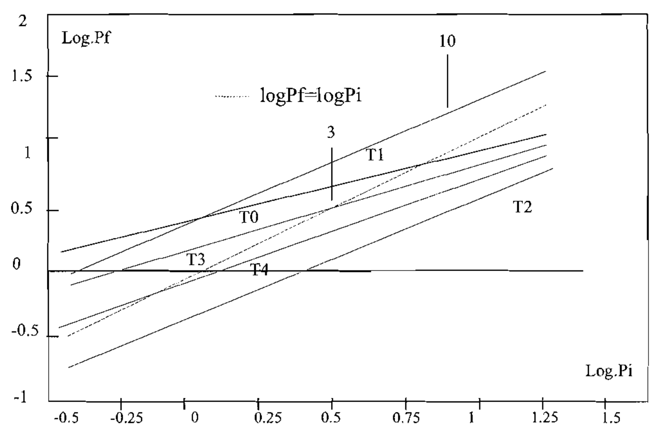

Fig. 2. Regression lines between the log transformed final and initial infestation densities: T0, susceptible "Accord"; T1, "Accord" + fenamiphos at sowing; T2, "Accord" + fenamiphos at post-emergence; T3 = T1 + T2, "Accord" + fenamiphos at sowing and at post-emergence; T4, resistant "Nemadie"

Table III. Yield of sugarbeet in soil infested by *H. schachtii.*

Treatment	Root weight[1] (g)	Sugar weight[1] (g)	Sugar content[1] (%)
Accord (Susceptible)	974 b	166 a	17 a
Accord + fenamiphos at sowing	1225 a	205 a	16.8 a
Accord + fenamiphos at post-emergence	1016 b	173 a	17 a
Accord +fenamiphos at sowing and post-emergence	1229 a	206 a	16.8 a
Nemadie (Resistant)	1012 b	158 b	15.6 a

[1] Average of four samples of 100 plants/treatment.
Values with same letters are not significantly different according to Duncan's multiple range test.(K=100, alfa = 0.05).

Table IV. Variability of final cyst nematode populations in the glasshouse test.

Cultivar	Cyst number	Eggs + J_2 per cyst
Nemadie	73.5 ± 21.09	112.5 ± 7.24 b
Maribo	23.4 ± 18.26	46.86 ± 2.72 a
Eva	173.5 ± 67.23	37.30 ± 1.25 a
Signification	0.52	0.000

Means in each column followed by the same letters do not differ significantly (p<0.05) according to Waller-Duncan test (k=100).

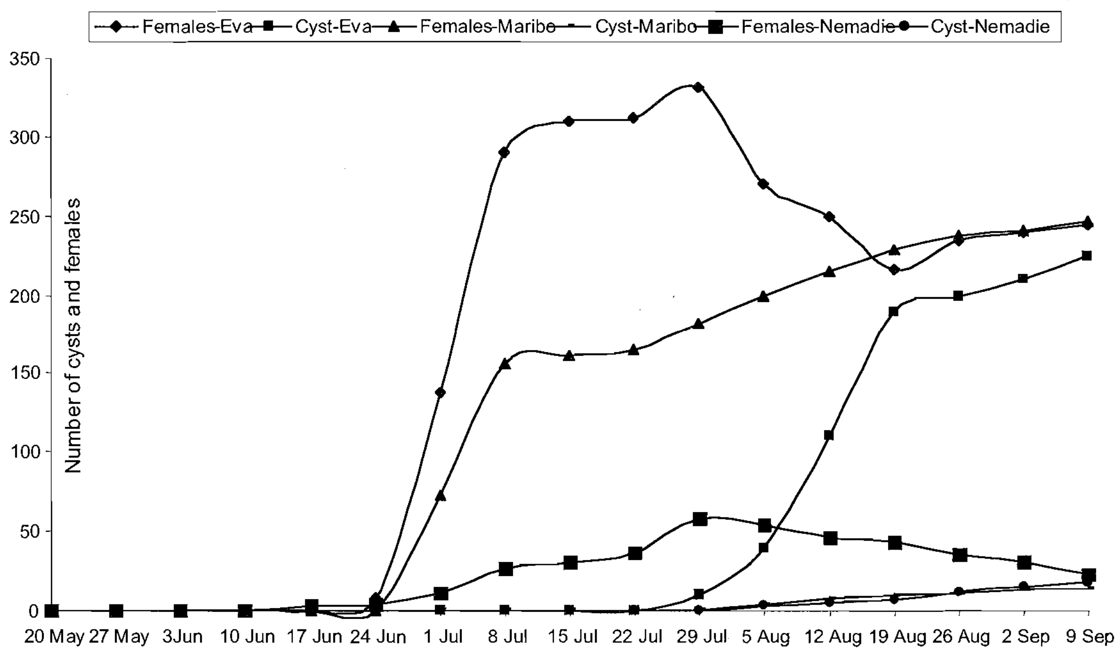

Fig. 3. Development of females and cysts growing in glasshouse conditions in cultivars of sugarbeet: susceptible "Eva" and resistants "Maribo-hybrid" and "Nemadie".

DISCUSSION

Suppression of nematode multiplication (multiplication rate<1) was only observed after application of fenamiphos at post-emergence or after growing a resistant cultivar. Final populations were strongly influenced by their corresponding initial values. At Pi values smaller than 1 egg and J_2/g of soil (-0.1<log Pi<0.1), all the treatments except the susceptible cultivar + fenamiphos at post-emergence, and the treatment with a susceptible cultivar allowed nematode multiplication. On the other hand, the regression lines for the treatments with a susceptible cultivar and this cv. + nematicide at sowing crossed at around the critical Pi value of 1. However, at relatively high Pi values (more then 10 eggs + J_2/g of soil), only the treatment of susceptible cv. + nematicide application at sowing led to a nematode density increase with Pf value greater than that corresponding to the untreated control. Its biological significance must be related to nematode competition for food inside the roots (Trudgill, 1967; Seinhorst, 1986), which was not triggered off in the case of the susceptible cultivar + nematicide at sowing treatment, because of a lesser or delayed penetration of J_2 into the roots (Steele, 1983). Multiplication rates for these two treatments did not differ significantly, but cyst contents recovered from the plots where fenamiphos was applied at sowing were more than those from the plots without fenamiphos, and almost twice the mean contents of the cyst at sowing time in the field. The early and short term effect of the nematicide protected the plants at the first stage of development by inhibiting or delaying hatching (Wauters, 1993). After the hatching of most of the juveniles the plants were in better condition to tolerate nematode attack, as the figure of the yield per plant indicate. In such a situation, a high viable content in the cyst can be expected (Griffin, 1988).

The opposite was the case for the post-sowing nematicide treatment which had an intermediate effect on the final population density. Although the multiplication rate of cysts did not differ if fenamiphos was applied either at sowing or after sowing, the multiplication rate of eggs and juveniles was less when the nematicide was applied later which was due to a reduction in viable cyst contents. Whether such a difference came from the death of some of the nematodes inside the roots by the systemic effect of the nematicide or due to a shortened period for development which could influence on the embryogenesis, is still unknown. Because of the earlier invasion of nematodes into the roots, this treatment was not effective in reducing the nematode damage to the plants but it was efficient in diminishing nematode density at high Pi values. This is in accordance with results obtained by Griffin (1987) who found that the efficacy of postplant application of aldicarb in controlling *H. schachtii* was poor and depended on the initial nematode density.

The growing of a resistant cultivar caused a reduction in egg population density (multiplication rate <1). Taking into account results by Müller (1985) who observed that the number of J_2 of *H. schachtii* that penetrated the roots of a resistant or susceptible host were similar, a reduction in the contents of the "old" cysts can be expected. Nevertheless, the results of the regression analysis in this experiment (multiplication rate >1 at low Pi) indicates that total resistance was not achieved. This is frequently cited (Yu, 1984; Lelivelt and Hoogendoorn, 1993) and agrees with Heijbroek *et al.* (1988) who also reported cyst formation on resistant sugar-beet cultivars, but with a decrease in their egg contents with respect to those in the susceptible plants.

The double application of fenamiphos was the sole treatment that prevented nematode multiplication independently of the initial nematode density, the regression line lying below the theoretical logPf = logPi. It can be stated that the additive effect of the early and late nematicide application of fenamiphos affected the nematode population. On the contrary, such an effect was not recognizable on the sugarbeet yield, showing that the late application of the nematicide was ineffective in protecting the plants even though there was a previous application at sowing.

Taking into account the criteria of an acceptable yield and nematode reduction, two applications of fenamiphos may be considered as an acceptable method for controlling *H. schachtii*. Likewise, when sugarbeet has to be sown in a field infested with sugarbeet cyst nematode at moderate to high infestation levels, growing a resistant cultivar is worth including as part of an integrated pest management. In such cases, the nematicide application after emergence could be recommended, but not just a single application at sowing, for this method can be effective in reducing nematode multiplication and increasing yield, but only at low infestation densities.

It is evident that the studied genotypes were not completely resistant to the cyst nematode populations tested.

The hybrid-Maribo tested in the glasshouse developed fewer cysts than the susceptible cultivar in terms of newly generated eggs and J_2.

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