Effect of *Heterodera goettingiana* population densities on the yield of pea, broad bean and vetch

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Summary

Three microplot experiments were undertaken in Italy in 1985-1987 to relate yield of pea, broad bean and vetch to population densities of *Heterodera goettingiana*. The microplots were bottomless square (30 × 30 cm) tubes, 50-cm long. They contained 35 dm^3 of soil infested with eggs of *H. goettingiana* at the rate of 0, 0.25, 0.5, 1, 2, 4, 8, 16, 32, 64, 128, 256, or 512 eggs/g soil. The microplots were sown to pea (November 22, 1985), broad bean (November 24, 1986) and vetch (November 25, 1986). Plant growth for pea was measured as green pea pods and plant tops, for broad bean as dry beans and plant tops, and for vetch as hay production. *H. goettingiana* was very damaging to pea and broad bean, while vetch was more tolerant. Tolerance limits *(T)* were 0.5, 0.8, and 2.0 eggs/g soil for pea, broad bean and vetch, while minimum yields *(m)* were 0.0, 0.1-0.2, and 0.4, respectively. Yield losses of 20 and 50 % occurred at 3 and 8 eggs/g soil for pea, 5 and 15 eggs/g soil for broad bean, and 20 and 78 eggs/g soil for vetch. Complete crop failure occurred at 32 and 64 eggs/g soil for pea and broad bean, respectively.

Résumé

Effet du taux de population d'Heterodera goettingiana *sur la récolte de pois, fève et vesce*

Trois expérimentations en microparcelles ont été effectuées en Italie en 1985-1987 pour connaître les relations entre la valeur de la récolte de pois, fève et vesce et la densité des populations d'*Heterodera goettingiana*. Ces microparcelles consistaient en tubes carrés (30 × 30 cm), de 50 cm de haut et dépourvus de fond; ils contenaient 35 dm^3 de sol infesté avec des œufs de *H. goettingiana* aux taux de 0; 0,25; 0,5; 1; 2; 4; 8; 16; 32; 64; 128; 256 et 512 œufs par gramme de sol. Les microparcelles ont été ensemencées en pois (22 novembre 1985), fève (24 novembre 86) et vesce (25 novembre 1986). La croissance des plantes a été mesurée en poids de gousses vertes et hauteur de plants (pois), poids sec des fèves et hauteur des plants (fève) et en production de matière verte (vesce). *H. goettingiana* s'est montré très agressif envers le pois et la fève, tandis que la vesce paraît plus tolérante. Les tolérances limites *(T)* sont de 0,5; 0,8 et 2,0 œufs/g de sol et les récoltes minimales *(m)* de 0,0; 0,1 et 0,4 pour le pois, la fève et la vesce, respectivement. Des diminutions de récolte de 20 et 50 % se produisent à 3 et 8 œufs/g de sol pour le pois, 5 et 15 œufs/g de sol pour la fève et 20 et 78 œufs/g de sol pour la vesce. La suppression totale de la récolte se produit aux taux de 32 et 64 œufs/g de sol pour le pois et la fève, respectivement.

The pea cyst nematode, *Heterodera goettingiana* Liebscher, has been reported to damage garden pea (*Pisum sativum* L.), broad bean (*Vicia faba major* L.) and vetch (*Vicia sativa* L.) in several countries (Di Vito & Greco, 1986). The nematode can also reproduce on other cultivated and wild plant species. In Italy, garden peas are harvested as green pea pods for the fresh or frozen food market, while broad bean is cultivated for both green pods and dry beans. Vetch grown for hay is one of the most important fodder crops in southern Italy.

Attempts have been made to control *H. goettingiana* with nematicide treatments (Di Vito & Lamberti, 1976; Whitehead *et al.*, 1979) and crop rotation (Stemerding, 1960; Di Vito & Greco, 1986), but the profitability of these approaches is not well documented. As a basis for rational management systems, information is needed on the yield of host crops when exposed to a range of nematode population densities. Seinhorst (1965, 1986) developed a model to describe the relationship between yield of the host crop and initial nematode population densities. The model has been used as a basis for calculating economic thresholds of various nematodes on different crops (Ferris, 1981). Earlier investigations on the population dynamics of *H. goettingiana* in England, the Netherlands and Italy have not yielded information on the effect of the nematode on yield of pea pods, broad bean and vetch that could be used for economic threshold calculations in southern Italy (Winslow, 1955; Stemerding, 1960; Jones *et al.*, 1965; Winfield, 1965; Di Vito, Lamberti & Inserra, 1978). The objectives of our research were to relate yield of pea, bean and vetch to population densities of *H. goettingiana;* to estimate tolerance limits for each crop; to provide data for economic threshold calculations for various management options; and to determine the effect of host crops on reproduction of the nematode.

Materials and methods

Experiments were conducted in bottomless concrete tiles, 30 × 30 cm square × 50 cm length. The plots were sunk into the ground so that 5 cm remained above the surface. They were arranged in ten randomized blocks of fourteen microplots each in a field in Bari (southern Italy).

The microplots were filled with red mediterranean soil (37.5 % clay, 32.0 % sand, 30.5 % silt and 2.3 % organic matter, with a pH of 7.8) from a field that had been fumigated with methyl bromide four months previously and that was not infested with *H. goettingiana*. Twenty grammes of phosphate fertilizer (20 % P_2O_5) was added to each microplot at the time of nematode inoculation.

EFFECT OF POPULATION DENSITIES OF *H. GOETTINGIANA* ON PEA YIELD

H. goettingiana inoculum was obtained by collecting field soils from the rhizosphere of infested broad beans after harvest (June, 1985). The soil was stored outdoors in a shaded location until early October. Cysts were extracted from the soil using a modified Fenwick can similar to, but larger than, that described by Caswell, Thomason and McKinney (1985). Extracted cysts and organic debris were dried at room temperature, then thoroughly mixed with 60 kg of the soil used to fill the microplots. Cysts were extracted from 10 g sub-samples of this soil by washing the samples through a 25-mesh (710 µm-pore) sieve over a 60-mesh (250 µm-pore) sieve. The cysts were picked from the debris retained on the 60-mesh sieve, crushed (Seinhorst & Den Ouden, 1966), and their egg content determined. The initial population density was 6930 ± 276 eggs/g soil. An appropriate amount of the infested soil was mixed into the soil of each microplot on November 21, 1985, to create a range of population densities of 0, 0.25, 0.5, 1, 2, 4, 8, 16, 32, 64, 128, 256, and 512 *H. goettingiana* eggs/g soil. There were ten replications of each treatment. Ten microplots were inoculated with 64 eggs/g soil and left fallow to determine the decline of the nematode population in the absence of a host. Fifteen pea seeds (cv. Progress 9) were sown in each microplot on November 22, 1985. The plants were thinned to eight per microplot soon after seedling emergence.

EFFECT OF *H. GOETTINGIANA* POPULATION DENSITIES ON BROAD BEAN AND VETCH YIELD

In 1986, microplots were inoculated with eggs obtained from cysts extracted from the pea microplots used in 1985. The population density of the inoculum was 4091 ± 98 eggs/g soil. The inoculum was mixed into the soil of the microplots on November 23, 1986, to create a range of population densities : 0, 0.125, 0.25, 0.5, 1, 2, 4, 8, 16, 32, 64, 128 and 256 *H. goettingiana* eggs/g soil. Ten microplots were inoculated with 55.5 eggs/g soil and left fallow to determine nematode survival in the absence of a host. The remainder of the microplots were planted with 3.3 g (approx. 55 seeds) of a local vetch variety or three pre-germinated broad bean seeds (cv. Agua dulce), on November 24 and 25, respectively. There were ten replicates of each treatment. Two hundred millilitres of *Rhizobium* suspension were added to each microplot on November 26, and all microplots were lightly irrigated.

CULTURAL DETAILS AND HARVEST PROCEDURES

Insect damage by leaf miner on peas and vetch, and aphids on broad bean, were controlled by a single spray of 200 ml/hl of Methidathion insecticide. A powdery mildew-preventative treatment of Triadimefon fungicide (80 g/hl) was applied in early April. Microplots were irrigated to compensate for low spring rainfall; three times for peas, twice for broad bean, and once for vetch. Microplots were maintained weed-free by hand pulling and shallow hoeing.

In the 1985/86 experiment, pea pods were harvested and weighed on April 28, and May 6, 1986. Green and dry plant-top weights were determined after the second harvest. In the 1986/87 experiment, broad beans were harvested on June 9, 1987, when pods were dry and plants were defoliated. Numbers of pods, seeds, seeds per pod, and dry bean yield per microplot, were determined.

Vetch was cut at ground level on May 4, 1987, dried in a greenhouse maintained at 20-26 °C, and weighed for hay production.

During the 1985/86 pea experiment, environmental conditions were suitable for both pea development and nematode reproduction. During the 1986/87 experiment, air and soil temperatures were unusually low at the end of December and in early March when a snowfall occurred. The broad beans were covered with transparent polyethylene film for a few days during the periods of low temperatures, and no detrimental effect was observed on either broad beans or vetch.

Soon after harvest, 20-core composite samples (1.5-2 kg soil) were collected to a depth of 30 cm from each microplot, using a 2.5 cm diam auger. Soil samples were air-dried, mixed, and 200 g sub-samples removed. Cysts were extracted from the sub-samples with a Fenwick can, dried at 60 °C, and separated from the organic debris by Seinhorst's method (Seinhorst, 1974) in which a 1.25 s.g. $MgSO_4$ solution is used, instead of ethanol, to separate cysts from organic material. Cysts were crushed to determine their egg content (Seinhorst & Den Ouden, 1966).

ANALYSES

Parameter values were selected empirically to describe yield data in relation to *H. goettingiana* inoculum density by the equation

$$y = m + (1 - m)z^{(P-T)} \text{ .... } (eq.\ 1)$$

(Seinhorst, 1965; 1986), in which y is the relative yield ($y = 1$ for $P \leq T$), m is the minimum relative yield (that yield obtained at very large values of P); P is the initial nematode population density (eggs/g soil); T is the tolerance limit (value of P above which yield losses occur); z is a constant < 1, such that $z^{-T} = 1.05$.

Parameter values for the model

$$P_f = rfa\,(1 - q^{P_i})\,(-\ln(q))^{-1} + b\,(1 - ry)\,P_i \text{ .. } (eq.\ 2)$$

(Seinhorst, 1970; 1986, were determined to adequately describe the relationship between initial (P_i) and final (P_f) soil population densities for each crop. In this equation, r is the proportion of eggs (P_i) that hatch when at population levels below the tolerance limit (plant growth not reduced by nematode damage), a is the maximum reproduction rate (P_f/P_i) of the nematode, f is the relative amount of food available to the nematode (here, f in *eq. 2* = y in *eq. 1*), b is the proportion of nematode eggs that do not hatch in the absence of a host, and y is the relative yield as in *eq. 1*. To fit the equation to the data, we assumed that $f = y$, $r = 1$, $b = 1$, and $M = (a/(-\ln q))$ = (the maximum possible numbers of nematodes that may occur at high P_i in the absence of damage).

Results

EFFECT OF POPULATION DENSITIES OF *H. GOETTINGIANA* ON PEA YIELD

Pea seedlings emerged in early January, 1986, and symptoms of nematode infestation (stunted plants and yellow leaves) were evident 60 days later, on March 4, in microplots infested with ≥ 64 eggs/g soil. Symptoms were evident 22 days later in microplots inoculated with 32 eggs/g soil and in a proportion of the plots with 16 and 8 eggs/g soil. By April 29, symptoms were evident in all microplots with ≥ 8 eggs/g soil, and 50 % of those with 4 eggs/g soil. Symptoms never became evident at lower inoculum densities.

Graphical representation of pea yield data, and empirical fitting of the Seinhorst (1965, 1986) model, suggested a tolerance limit (T) of 0.5 *H. geottingiana* eggs/g soil for pea, and minimum yields (m) of 0 and 0.03 for pea pod yield and top plant weight, respectively (Fig. 1). Yield losses were 20 %, 50 %, and nearly 100 % in microplots infested with 4, 8, and 32 eggs/g soil, respectively (Fig. 1).

EFFECT OF *H. GOETTINGIANA* POPULATION DENSITIES ON BROAD BEAN AND VETCH YIELD

Vetch emerged by December 10, 1986, and broad bean, 2 weeks later. There were no symptoms of nematode damage during the winter, but all broad beans in microplots infested with ≥ 32 eggs/g soil, and 60 % of those in microplots with 16 eggs/g soil, were stunted and had yellow leaves by March 24, 1987. Eventually, symptoms were evident in the remainder of the microplots infested with 16 eggs/g soil and in some infested with 8 eggs/g soil. There was no relationship between time of flowering and nematode population density, but plants senesced earlier in microplots that showed symptoms of nematode damage. Weights of plant tops and dry beans, and numbers of pods and seeds per microplot, were negatively affected by *H. goettingiana*. Pods and seeds from microplots infested with ≥ 128 eggs/g soil were too small and too few to be marketable. A tolerance limit of 0.8 *H. goettingiana* eggs/g soil was derived for all plant components by fitting *eq. 1* to the data (Fig. 2).

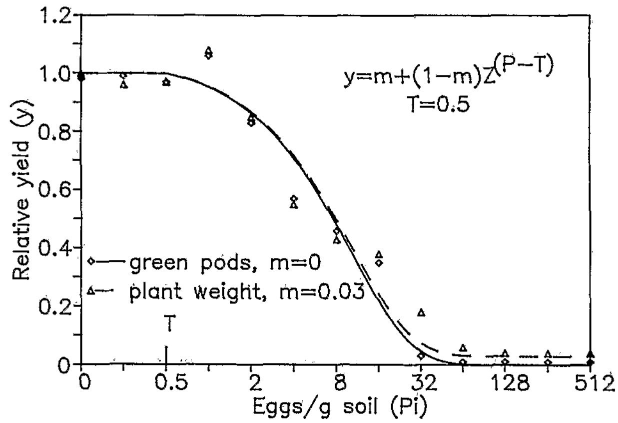

Fig. 1. Relationship between soil population densities of *Heterodera goettingiana* at sowing (P_i) and relative yield (y) of green pods and plant tops of pea grown in microplots in 1985/86.

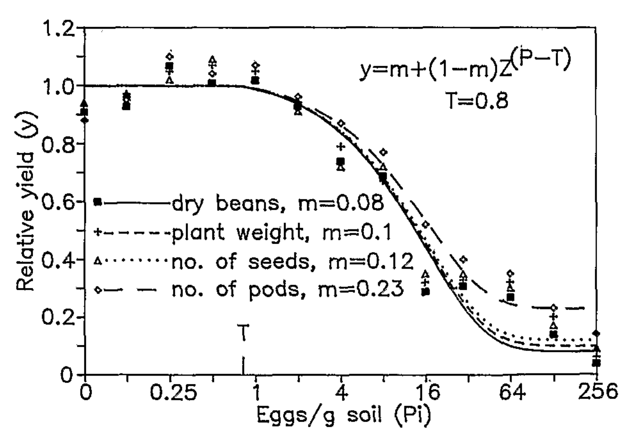

Fig. 2. Relationship between soil population densities of *Heterodera goettingiana* at sowing (P_i) and relative yield (y) of dry beans and plant tops, or relative number (y) of pods and seeds of broad bean grown in microplots in 1986/87.

Minimum yields *(m)* were 0.08, 0.10, 0.12, and 0.23 for dry bean yield, plant-top weight, numbers of seeds and pods per microplot, respectively. Yield losses were 20 % and 50 % at 5 and 15 eggs/g soil, respectively, and there was complete crop failure at ≥ 64 eggs/g soil (Fig. 2).

Symptoms of nematode damage were less evident on vetch; however, plants in microplots infested with ≥ 64 eggs/g soil showed reddish-yellow symptoms by March 27, 1987. The symptoms became more severe after that date when plant growth was suppressed; they were particularly evident in microplots with ≥ 32 eggs/g soil. A tolerance limit of 2 *H. goettingiana* eggs/g soil, and minimum yield of 0.4, were estimated for vetch by fitting *eq. 1* to the data (Fig. 3). Hay yield losses of 20 % and 50 % occurred at 20 and 78 eggs/g soil, respectively. Yield losses of 60 % occurred at the highest nematode population densities (Fig. 3).

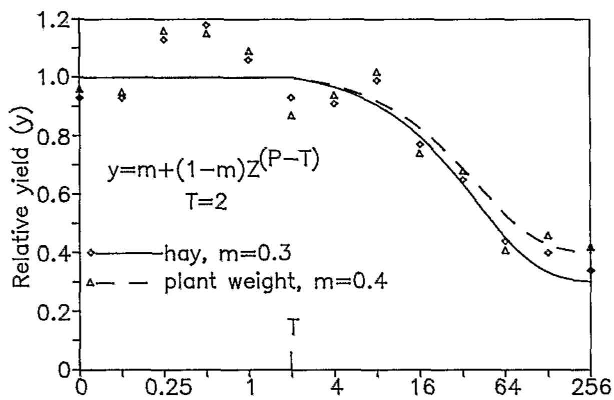

Fig. 3. Relationship between soil population densities of *Heterodera goettingiana* at sowing (P_i) and relative yield *(y)* of green plants and hay of vetch grown in microplots in 1986/87 [Eggs/g soil (P_i)].

Heterodera goettingiana population increase on pea, broad bean, and vetch

Maximum soil population densities of *H. goettingiana* were 573, 391, and 743 eggs/g soil in microplots planted to pea, broad bean and vetch, respectively (Table 1). Final population densities of *H. goettingiana* in microplots planted to broad bean were not significantly different from those in microplots planted with pea (Table 1). However, significantly more cysts were produced at all P_i levels in microplots planted with vetch than with either pea or broad bean (Student's t-test). The percentage of new cysts was similar in all microplots inoculated with the lower initial (< 16 eggs/g soil) population densities. A sharp decline of new cysts occurred in microplots inoculated with population densities ≥ 16 eggs/g soil and planted with pea or broad bean, and ≥ 32 eggs/g soil and planted with vetch. Numbers of eggs/cysts in microplots planted with pea or broad bean did not differ, but there were significantly fewer eggs/cysts in microplots planted with vetch. In general, number of eggs/cyst were lower at population densities of ≤ 1 or ≤ 0.5 eggs/g soil on peas and broad beans, respectively; they also declined at initial population densities around 32 and 64 eggs/g soil, respectively (Table 1). No substantial changes in nematode population densities were observed in the microplots without host plants.

Table 1

Effect of population densities of *Heterodera goettingiana* at sowing on the number of cysts, percent of new cysts, and eggs/cysts, in microplots sown to pea (P) in 1985/86 and broad bean (B) and vetch (V) in 1986/87.

Eggs/g soil at sowing	Cysts/200 g soil			% New cysts			Eggs/cysts		
	P	B	V	P	B	V	P	B	V
0.125		5.7	20.3		97.7	99.3		141	85
0.25	23.5	11.9	33.8	98.9	97.7	99.2	127	141	101
0.50	43	24.7	38.6	98.8	97.8	98.6	115	155	155
1	47.6	65.2	76.3	97.8	98.4	98.6	146	212	176
2	120	91	141.5	98.2	97.7	98.5	206	195	115
4	204	186.4	267.5	98.0	97.7	98.4	247	190	158
8	431	322	613.9	98.1	97.3	98.6	243	206	44
16	487	338	924	96.6	95.0	98.2	235	185	79
32	461	415	1 253	92.8	91.8	97.3	209	189	52
64	301	356	1 333	77.9	80.9	94.9	148	198	71
128	306	462	1 360	56.5	70.6	90.0	132	170	109
256	464	524	1 201	42.6	48.1	77.4	149	111	112
512	790			32.6			143		

Data of initial and final population densities fitted *eq. 2* reasonably well, especially at initial population densities ≤ 4-8 eggs/g soil (Fig. 4). In determining parameter values for *eq. 2*, estimates of *M* (the number of nematodes that can occur on each crop in the absence of damage) were 2000, 2500, and 1000 for pea, broad bean, and vetch, respectively. Maximum reproduction rates were 90, 46 and 69, and equilibrium densities were approximately 500, 260, and 400 for pea, broad bean and vetch, respectively (Fig. 4).

Discussion

Previous studies on the pathogenicity of *H. goettingiana* have produced conflicting results. Jones and Moriarty (1956) found no clear evidence of a relationship between population densities of pea cyst nematode and yield of peas, beans and vetch. However, Stemerding (1960) and Moriarty (1962, 1963*a*) reported that yield of pea was negatively correlated with initial population density of *H. goettingiana*. Later, Winfield (1965) found that complete crop failure occurred only at 331 eggs/g soil. The negative effect of *H. goettingiana* on pea yield was confirmed by Jones *et al.* (1965) who reported that a sigmoid curve best described the relationship of yield to a wide range of population densities (4 - 359 eggs/g soil).

In 1978, Di Vito, Lamberti and Inserra clearly demonstrated that the Seinhorst equation (Seinhorst, 1965; 1986) adequately described the relationship between pea tops and a range of initial population densities of *H. goettingiana*. They derived a tolerance limit of pea tops to the nematode of 4.4 eggs/cm^3 soil, but did not measure the effect of density on pod yield. The present studies demonstrate that *H. goettingiana* is very damaging to pea and broad bean in southern Italy. The damage is much greater than that reported in England (Winfield, 1965; Jones *et al.*, 1965). The differences are probably attributable to differences in cultivars and climate; the spring in southern Italy is rather dry.

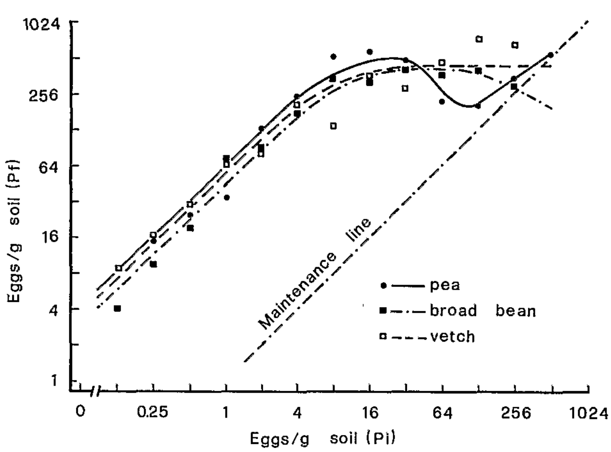

Fig. 4. Relationship between population densities of *Heterodera goettingiana* at sowing *(P_i) and after harvest (P_f)* on pea, broad bean and vetch grown in microplots in 1985-1987.

In our studies, vetch germinated more rapidly than peas and broad beans. Plants grew during the winter months when *H. goettingiana* development is slow (Greco, Di Vito & Lamberti, 1986), which may have prevented nematode damage to young plants. Consequently, the tolerance limit and minimum yield were higher for vetch than for pea and broad bean. In contrast to the results of Moriarty (1963*a*) in England, broad beans sustained about the same damage as pea from *H. goettingiana* in southern Italy.

Italian populations of *H. goettingiana* reproduced well on pea, broad bean and vetch. Differences in the reproduction of the nematode between the crops probably reflect seasonal effects and agronomic practices, as well as crop susceptibility. Data from Di Vito, Greco and Lamberti (1980) indicate that, under the same conditions, reproduction of *H. goettingiana* on pea, broad bean and vetch is similar. However, in our studies, there were differences in growing seasons, season length, and plant growth, all of which may influence population increase of the nematode. Root weight was not determined in these experiments, but root systems were visually larger in plots planted with vetch, and smaller in those planted to broad bean, correlating with the significantly higher number of cysts/200 g soil found in the vetch microplots. However, the number of eggs/cyst was smaller in the vetch microplots, as most of the nematodes were still white females at harvest in early May. In microplots with larger population densities, plants matured earlier. This may have contributed to the lower number of eggs/cyst observed in pea and broad bean microplots with high initial population densities.

Our studies confirmed previous observations that the decline of the *H. goettingiana* population in the absence of a host is very slow (Brown, 1958; Stemerding, 1960; Moriarty, 1963*b*; Di Vito & Greco, 1986). Extended periods of rotation to non-host crops are necessary for management of the nematode. Our data on the effect of *H. goettingiana* on pea, broad bean and vetch, and on the reproduction of the nematode on these crops, provide a basis for development of optimal management strategies for the nematode.

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