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First investigations into the effects of Bt corn crop on Nematofauna^(*)

Abstract - The research, done in 1999, was carried out in 8 north Italian localities in fields planted with isogenic and transgenic corn (Event 176, Novartis). The objective was to find a first indication of the possible effect of transgenic corn on soil fauna and, in particular, on the nematode community, considered a good indicator of the trophic chain state. The nematode community was investigated for general composition, trophic structure and biodiversity, and the findings from Bt and isogenic fields of corn were compared. The classification of 2,000 nematodes belonging to 22 families and 45 different genera was made. The most abundant was *Rhabditis*, which was common to all regions and which often dominated, followed by *Pratylenchus*, *Helicotylenchus* and *Acrobeloides*. These genera made up more than 70% of the total nematodes extracted. The dominant trophic group was the bacteriophagous, followed by the phytophagous. For a synthetic description, and a comparison of the different communities, calculations were made of several biodiversity indexes (Shannon, Hill numbers, Pielou index), the maturity index and the Sørensen similarity index. All the examined sites were found to have quite low biodiversity, the H' values being not more than 1.08. The data, independently of the presence of the transgenic hybrid, indicate a high level of disturbance. The Bt transgenic corn cultivation appears to have no significant influence on the nematofauna, neither at the level of genus composition nor with regard to biodiversity. Nevertheless one region did have a change in trophic group composition: fungal feeders nematodes were more numerous in the soil with Bt corn, while in the field with the isogenic hybrid the bacterial feeders percentages were higher.

Riassunto - *Prime indicazioni riguardanti gli effetti del mais Bt sulla nematofauna.*

La ricerca è stata effettuata nel 1999 in 8 località del Nord Italia in campi seminati con mais isogenico e transgenico (Evento 176, Novartis). Si è voluto fornire una prima indicazione dell'effetto che una coltura di mais transgenico Bt può esercitare sulla fauna edifica ed in particolare sui nematodi considerati buoni indicatori dello stato della catena trofica. Delle comunità di nematodi si sono inve-

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stigate la composizione in generi, la struttura trofica e la diversità comparando quanto riscontrato nei campi di mais Bt e in quelli isogenici. Si sono classificati 2000 nematodi, appartenenti a 22 famiglie e a 45 generi diversi. Il più abbondante è *Rhabditis*, comune a tutte le località, dove spesso è anche dominante, seguito da *Pratylenchus*, *Helicotylenchus* e *Acrobeloides*. Questi generi rappresentano più del 70% del totale dei nematodi estratti. Il gruppo trofico dominante è quello dei batteriofagi, seguito da quello dei fitofagi. Per descrivere in modo sintetico e per confrontare le varie comunità si sono calcolati alcuni indici di biodiversità (Shannon, numeri di Hill, indice di equitabilità di Pielou), l'indice di maturità e quello di similarità di Sørensen. La biodiversità è piuttosto bassa in tutti i siti esaminati: i valori di H' superavano di poco il valore di 1. I dati indicano un elevato grado di disturbanza, indipendentemente dalla presenza dell'ibrido transgenico. La coltura di mais transgenico Bt non sembra essere in grado di influenzare in modo significativo la nematofauna del suolo sia a livello di composizione in generi, che di biodiversità. In una località si è tuttavia registrato un cambiamento nella composizione in gruppi trofici: i nematodi fungivori erano più numerosi nel terreno con mais Bt, mentre nel campo con ibrido isogenico era più alta la percentuale di batteriofagi.

Key words: Nematoda, transgenic corn, biodiversity, non target organisms, Cry IA(b)

INTRODUCTION

The European corn borer, *Ostrinia nubilalis* Hübner, is one of the major pests of corn *Zea mays* L. in Europe and in the USA. European corn borer (ECB) larvae cause average crops losses in Europe of 5-10% depending on the intensity of the infestation (Bressan & Coppolino, 1992).

Recently *Z. mays* was engineered genetically to express the Cry IA(b) protein derived from *Bacillus thuringiensis* var. *kurstaki* (Koziel *et al.*, 1993) with an insecticidal activity against *O. nubilalis*. Genetically engineered crops will most likely offer many valuable benefits, such as increased yields, improved food flavour and nutritional quality, and reduced use of pesticides. On the other hand, transgenic crops could also pose significant risks for the environment. Two are the major potential problems for the environment: the creation of strains of resistant insects and harmful effects on non-target species. Some data are available on the effects of OGMs on non target organisms but the results are contrasting (Pilcher *et al.*, 1997; Hilbeck *et al.*, 1998; Lozzia *et al.*, 1998; Manachini *et al.*, 1999; Lozzia *et al.*, 2000).

Yu *et al.* (1997) carried out an experiment on two different non target soil arthropods: the springtail *Folsomia candida* Willem and the mite *Oppia nitens* Koch. These animals were fed with an extract of two types of Bt cotton leaves, one containing CryIA(b) and the other CryIA(c). No deleterious effects were recorded on the life cycle and mortality of these organisms. However few data are available regarding the potential effect of Bt protein directly in the soil, especially concerning its persistence,

degradation and potential toxic residues. Tapp & Stotzky (1995) observed that Bt toxins could remain in the soil, especially if linked to clay particles. Furthermore Koskella & Stotzky (1997) found that Bt proteins maintain insecticidal activity for even up to 40 days, and also demonstrated that proteins linked to clay are more resistant to bacterial degradation.

The Authors hypothesize that the Bt proteins could remain, and accumulate, in the soil, enhancing the possibility of increasing target resistance and exposure time to non-target organisms.

However no data are available regarding the influence of transgenic corn on soil fauna and on the soil trophic chain. Soil is an excellent habitat for nematodes, in fact 100 cc of soil can contain several thousand. Because of their importance to agriculture, much more is known about plant-parasite nematodes than about other kinds of nematodes in agricultural soil (Norton & Niblack, 1991; Bongers & Ferris, 1999). Most kinds of soil nematodes do not parasitize plants and are beneficial to the decomposition of organic matter. These animals are often referred to as free-living nematodes. Although some plant parasites may live within plant roots, most nematodes inhabit the thin film of moisture around soil particles. The rhizosphere soil around small plant roots and root hairs is a particularly rich habitat for many kinds of nematodes (Freckman & Caswell, 1985). Soil-inhabiting nematodes can also be classified according to their feeding habits. This classification is particularly useful to ecologists in understanding the position of nematodes in soil food webs. The composition of the soil nematode community depends on the vegetation present, as well as on soil type, season, soil moisture level, amount of soil organic matter, and many other factors such as xenobiotics (Norton & Niblack, 1991; Yardim & Edwards, 1998). As they are responsive to so many different factors, it is believed that nematodes could be useful bio-indicators of the condition of the soil environment (Niblack, 1989; Bongers & Ferris, 1999). For this reason the aim of our research has not been to investigate specifically for phytophagous nematodes of corn, but to search for an overall effect of transgenic corn by studying nematodes communities, their biodiversity and trophic structure.

MATERIALS AND METHODS

The study was carried out in 8 different localities in the of Po rift Valley (Italy) from 7 May (sowing) to 25 September 1998 (harvest) (Fig. 1). In each locality there were two fields, one sown with transgenic hybrid Bt 176 corn (Trade Mark Novartis, 125 days rising) and the other with the same hybrid Tempra (isogenic). Each field was planted at a density of 95,000 plants per hectare and 0.8 m inter-row spacing. No insecticides, nematocides or fungicides were used in any of the corn fields. The fertiliser and irrigation regimes were left unchanged with respect to the common agricultural practices. Each field was divided into eight plots of 1.25 hectares. Samples of soil and roots segments from the transgenic and isogenic corn crops were collected with a soil sampling probe (length 50 cm and diameter 4 cm). Fifty sub-samples were

randomly collected from transgenic and isogenic plots and then mixed together to make up a composite sample.



Fig. 1 - Sampling localities with the experimental fields of Bt corn: 1) Sillavengo (Novara); 2) Santhià (Vercelli); 3) Brignano (Bergamo); 4) Lobia (Verona); 5) Belvedere (Padova); 6) Bertessina (Vicenza); 7) Stretti di Eraclea (Venezia); 8) Torre (Venezia).

Soil was processed immediately after collection. For each sample, nematodes were extracted from 200g of soil using a Baermann Funnel and the centrifugation-Ludox flotation method. All nematodes recovered were killed and fixed using 4% formalin at 80°C, successively they were counted and from each sample 100 nematodes were randomly picked out. The specimens were prepared using the De Grisse's method (1969) and placed on Cobb's mounts (De Grisse, 1969). The nematodes were identified at genus level.

Data analysis

The total abundance of the nematodes in isogenic and transgenic corn was compared. Genus composition, percentage and genus dominance was also estimated. A statistical analysis was made, after Log (x+1) transformation, for the total abundance of nematodes and for the abundance of major genera, using the statistical software SPSS version 9.0.

The data of the collections of nematodes has been analysed also using biodiversity and similarity indices as: the Shannon-Weaver index $H' = \sum P_i \log P_i$ where P_i = relative frequency of the single species; the numbers of Hill (1979): N_0 : number of species, $N_1 = e$ where $H' = \text{index of Shannon-Weaver}$, $N_2 = 1/\sum (P_i)^2$, $N_{00} = 1/P \text{ max}$

where P_{max} = absolute frequency of the most representative species, index of equitability of Pielou $J' = H'/\log N_0$; index of similarity of Sørensen $QS = 2c \times 100/(a+b)$ where a and b are the number of species found respectively in the environments A and B while c is the number of those common to both biotypes.

The trophic structure of the nematode communities in the Bt corn crop and isogenic field was also compared. The nematode feeding group was identified according to Yeates *et al.* (1993) as reported below:

Phytophagous: this group includes many members of the order Tylenchida, as well as a few genera in the orders Aphelenchida and Dorylaimida. **Bacterivores:** many kinds of free-living nematodes feed only on bacteria, which are always extremely abundant in soil, including many members of the order Rhabditida as well as several other orders, which are encountered less often. These nematodes are beneficial to the decomposition of organic matter; **Fungivores:** many members of the order Aphelenchida are in this group. Like the bacterivores, fungivores are very important to decomposition; **Predators:** these nematodes feed on other soil nematodes and on other animals of comparable size (Mononchida, a few predators are also found in the Dorylaimida and some other orders); **Omnivores:** The food habits of most nematodes in soil are relatively specific, a few kinds of nematodes can feed on more than one type of food material, and are therefore considered omnivores (some Dorylaimida).

RESULTS AND DISCUSSION

Nematode abundance

Table 1 shows the number of nematodes per 200g of soil in each locality. The total abundance was 1,813 nematodes/200g soil for the transgenic corn crops and 1,353/200g

Table 1 - Abundance of nematodes in 200 g of soil and number of genera collected from transgenic and isogenic corn crops in all localities selected.

Localities	Transgenic		Isogenic	
	Nematodes/200 g	N° of genus	Nematodes/200 g	N° of genus
Sillavengo (Novara)	718	8	273	10
Santhià (Vercelli)	49	15	79	15
Brignano (Bergamo)	183	20	127	23
Lobia (Verona)	45	16	36	18
Belvedere (Padova)	198	14	278	5
Bertesina (Vicenza)	167	18	120	15
Stretti di Eraclea (Venezia)	292	14	291	16
Torre (Venezia)	161	14	149	12
Total	1813		1353	

soil for the isogenic ones. This difference was due to the high number of nematodes recorded in the Bt corn of Sillavengo (Novara) (718 nematodes/200g) compared to the isogenic one (273 nematodes/200g). The locality with the lowest number of nematodes was Lobia (Vicenza), with only 45 nematodes/200g in the Bt field and 79 in the other. However, except that for Sillavengo (Novara), no statistical significant differences were recorded in the total abundance of nematodes ($F = 1.004$; $p = 0.34$; mean for transgenic = 226.63, mean for isogenic = 169.13).

Genus composition

Forty-five genera were identified in all, specifically 37 genera were caught in Bt corn fields and 37 in the isogenic ones, and 29 genera were common to both hybrid corn crops.

Table 2 shows the percent abundance of each nematode genus in the two types of corn fields.

The family of Rhabditidae was the most abundant in both crop types, being respectively 42.3% in the transgenic crops and 40.6% in the isogenic ones.

The genera *Pratylenchus*, *Helicotylenchus* and *Acrobeloides* were present in all localities and together represented about the 50% of the total nematodes. The firsts are phytophagous that have been indicated as being damaging in our latitudes, especially in light soils (Rivoal & Cook, 1993). Nevertheless it is interesting to observe that the abundance of the phytophagous genera is never greater than the damage threshold indicated in the literature.

Table 2 - Abundance of nematode genera in transgenic and isogenic corn crops.

Taxa	Relative percentage	
	Transgenic	Isogenic
<i>Rhabditis 1</i>	24.14	23.34
<i>Rhabditis 2</i>	18.27	17.34
<i>Acrobeloides</i>	6.76	13.52
<i>Pseudoacrobeles</i>	2.66	2.84
<i>Cephalobus</i>	1.77	1.42
Others Cephalobidae	1.55	2.07
<i>Filenchus</i>	2.55	6.76
<i>Helicotylenchus</i>	8.64	6.32
<i>Rotylenchulus</i>	2.10	1.64
<i>Aphelenchus</i>	1.33	1.55
<i>Pratylenchus</i>	13.40	12.43
<i>Aphelencooides</i>	8.19	2.94
Other genera < 1%	8.64	7.85

Some genera were recorded only in the transgenic corn crops as: *Alaimus*, *Mylonchulus*, *Iotonchus*, *Mononchus*, *Thornia*, *Discolaimium*, *Pelodera*, *Basiria*. Instead the genera *Sclerolabia*, *Willinema*, *Eudorylaimus*, *Nygolaimus*, *Monochromadora*, *Cylindrolaimus*, *Diploscapter* and *Tylenchorhynchus* are present only in the isogenic corn crop, the majority of them being present in low percentage but they are of quite relevant ecological importance in that they are predators or omnivores.

No statistical differences were recorded in the abundance of the major genera collected in the transgenic and isogenic corn fields (Table 3). Thus the presence of Bt corn does not seem to affect the abundance of the dominant genera.

However significant statistical differences were found in the abundance of the genera *Acrobeloides* ($F = 4,379$; $p = 0,03$) and *Helicotylenchus* ($F = 9,680$; $p = 0,003$) with respect to locality. In fact the first genus was collected mainly at Lobia (Verona), Stretti di Eraclea (Venezia) and Torre (Venezia); while the second one was dominant in Brignano (Bergamo).

The genera collected are typical of an agricultural environmental and confirm data of other earlier studies carried out in the USA regarding phytophagous nematodes in agro-ecosystems (Norton & Edwards, 1988; Yeates & Hughes, 1990; Rivoal & Cook, 1993; Yeates, 1994). The most abundant genera belong to the family of Rhabditidae, and it seem to be *Rhabditis*, however the classification of this genus is under review so an accurate identification of these two specimens is actually impossible (De Ley personal communication); in addition only juveniles were present. For this reason we have called them *Rhabditis* 1 and *Rhabditis* 2. Like all members of this family, they are able to reproduce quickly and live in highly disturbed environments where there is a great input of nutrients and high abundance of bacteria (Hunt & De Ley, 1996). An increment in their abundance could lead to alterations in ecological succession.

Another well represented family of bacterivores is the Cephalobidae, the greatest abundance being *Acrobeloides*. This taxon is more selective and exigent than Rhabditidae and could have an important role in regulating the bacterial community in soil (Niblack, 1989; Hunt & De Ley, 1996).

The trophic structure was not affected negatively by the presence of Bt corn, in fact no statistical differences were recorded in the percent abundance of any of the

Table 3 - Average abundance of major genera collected from transgenic and isogenic corn fields, and results of ANOVA (df = degree of freedom, F = Fisher, p = level of significant).

	Transgenic	Isogenic	df .	F	p
Rhabditidae	121.65	82.60	1,7	0.765	0.41
<i>Acrobeloides</i>	11.60	18.89	1,7	1.185	0.31
<i>Pratylenchus</i>	37.58	22.00	1,7	0.897	0.37
<i>Helicotylenchus</i>	13.58	7.82	1,7	3.024	0.12
<i>Filenchus</i>	4.64	8.58	1,7	1.235	0.30
<i>Aphelencooides</i>	7.76	4.83	1,7	1.147	0.32

Table 4 - Abundance of different nematode groups collected from transgenic and isogenic corn fields.

	Average %				
	Transgenic	Isogenic	df	F	p
Bacterivores	57.46	61.80	1,7	1.075	0.33
Phytophagous	28.71	30.56	1,7	0.162	0.70
Fungivores	9.42	4.72	1,7	0.652	0.44
Omnivores+Predators	4.41	2.92	1,7	1.893	0.21

trophic groups in the two types of hybrid corn fields (Table 4). The bacterial feeders are the most abundant group, being present in almost 61.08% in the isogenic corn crop and 57.46% in the transgenic one (Table 4), followed by the phytophagous, respectively 30.56% and 28.71%. The percentage of fungal feeders was higher in the transgenic corn crops (9.42%) than in the isogenic one (4.72%). The omnivores and predators were in low percentages.

Figure 2 shows the relative abundance of the trophic groups in all the fields sampled.

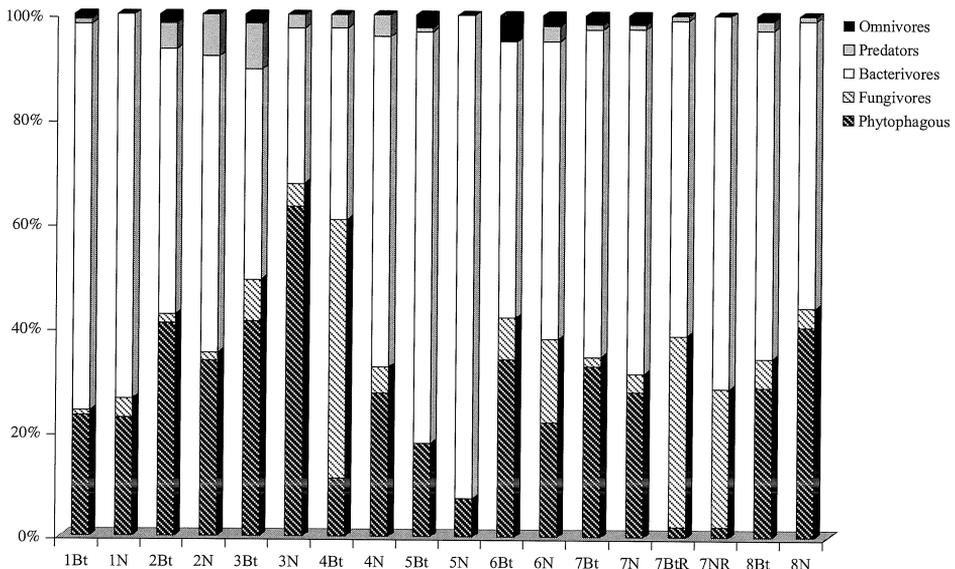


Fig. 2 - Trophic composition of nematode communities from isogenic (N) and transgenic (Bt) corn fields: 1) Santhià (Vercelli); 2) Sillavengo (Novara); 3) Brignano (Bergamo); 4) Lobbia (Verona); 5) Belvedere (Padova); 6) Bertesina (Vicenza); 7) Stretti di Eraclea (Venezia) ; 8) Torre (Venezia).

Although the nematofauna belonging to the transgenic and isogenic corn crops showed similar trophic composition, in Lobbia (Verona) the abundance of the trophic groups in the transgenic corn field differed from that in the isogenic one. In fact the fungal feeder nematodes (especially *Aphelencooides*) were more numerous in the transgenic corn field than in the other one where the dominant trophic group was represented by the bacterivores. It is important to note these changes as both trophic groups are important to the structuring of the soil micro flora and in regulating organic matter decomposition (Niblack, 1989).

The reduction of bacterial feeder nematodes in the transgenic corn field could be due to a direct effect of Bt protein on nematodes or an indirect effect on other trophic structure rings (bacteria, fungi, predators). In fact such protein could be released through root waste, pollen or leaves fallen down on the soil or other vegetal residuals. In fact some authors have demonstrated that Bt protein can link to particles of clay in the soil and become more resistant to microbial degradation (Koskella & Stotzky, 1997; Stotzky, 2000). Although the soil of Lobbia (Verona) has a quite high percentage of clay (35.3%), the highest of the investigated localities, it is important to note that also the fields in Stretti di Eraclea (Venezia) had a good percentage (30.3%), but in this case no change in trophic structure was recorded.

The calculated indices show (Table 5) that the degree of biodiversity and the evenness are almost the same for the communities in the two types of hybrid corn crop. These results are also confirmed by the similarity index (QS= 79.45). The biodiversity was low in both types of corn field, H' being near the value of 1. The nematode communities had a similar evenness (J' = 0.68) indicating that individuals were not well spread over the nematode community. However the value of N_∞ shows that the dominant genus is of less importance in structuring transgenic corn nematode communities, than is the dominating genus in the nematode community of isogenic fields.

The value of the Maturity Index (MI) calculated for the two communities, for the

Table 5 - Biodiversity indices (H' , D , N_0 , N_1 , N_2 , N_∞ , J'), Maturity Index (MI) and similarity index of Sørensen (QS), evaluated on the total nematodes sampled in the transgenic and isogenic corn crops.

Indices	Transgenic	Isogenic
H'	1.068	1.067
D	0.131	0.13
N_0	37	37
N_1	1.309	1.6
N_2	7.634	7.692
N_∞	2.381	2.5
J'	0.681	0.686
MI	1.909	1.878
QS	79.452	

nematodes in the fields of Bt corn and those in isogenic corn fields, are very low and are comparable (Table 5). This confirms earlier observations on the degree of disturbance of intensive agro-ecosystems (Yeates, 1994; Bongers, 1990).

CONCLUSIONS

This work has highlighted how nematofauna composition appears to be little affected by the presence of Bt corn. Nevertheless deeper analyses, spanning several years to cover a possible effect of accumulation, appear necessary, especially in soils rich in clay.

In the still-open debate concerning the appropriateness of using genetically modified plants, evaluating the risk tied to the use of transgenic Bt plants, also the organisms and processes in the soil must be taken into account. Thus a further investigation possibility could be the use of nematodes as bio-indicators.

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