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Behaviour of the European Corn Borer, *Ostrinia nubilalis* Hb., on transgenic corn (*)

Abstract - The results of test carried out against the European Corn Borer are described. The latter tests was carried out in a confined environment with the aid of artificial infestations. The influence of transgenic corn on infestation, activity and development of the species is underlined. A very good protection rate of transgenic corn was noticed. An increase of the survival rate could be observed while the plants were approaching maturity without less yield. The reduction in insecticidal efficacy can be attributed more to the phenology of the plant and its correlated larval distribution than to a lower self protection of the hybrid. The time course of the survival of the larvae explains why the transgenic plants are very well protected during the stages of development during which the insects would otherwise severely impact on yield. It was also noticed that larvae that could survive had their development delayed, though this delay did not prevent the completion of their development cycle.

Riassunto - *Comportamento della Piralide del mais,* Ostrinia nubilalis *Hb., su mais transgenico.*

Vengono descritti i risultati di prove effettuate contro la Piralide del mais, una delle quali condotta in ambiente confinato, con l'ausilio di infestazioni artificiali. Si evidenzia l'influenza di un mais transgenico su infestazione, attività e sviluppo del Lepidottero, con un ottimo livello di protezione. Quando le piante si avvicinano alla maturità si può osservare un incremento del tasso di sopravvivenza, senza però provocare un calo di produzione. Ciò è attribuibile più alla fenologia della pianta e alla correlata distribuzione larvale, che a una minore autoprotezione dell'ibrido. L'andamento della sopravvivenza delle larve evidenzia come le piante transgeniche siano molto ben protette durante le fasi di sviluppo in cui gli insetti potrebbero altrimenti avere gravi effetti sulla produzione. Si è registrato infine un rallentamento nella velocità di sviluppo delle larve che sopravvivono, ma che comunque sono riuscite a raggiungere la maturità.

Key words: Transgenic corn, corn self protection, *Ostrinia nubilalis.*

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INTRODUCTION

The European Corn Borer (E.C.B.), *Ostrinia nubilalis* Hb., is the major corn pest in many European, American and Asiatic Countries (Anglade, 1985; Umeozor et al., 1985; Calvin et al., 1988). Crop damages are function of population density, of phenological stage of the crop at oviposition and of the length of the damage period (Stengel, 1969; Lynch, 1980). In France, yield losses were estimated as high as 20 q/ha. The control of E.C.B. is still an unsolved problem. Studies performed up to now clarified several aspects of the biology of this insect (Murbach et al., 1973; Süss et al., 1983) and were capable to indicate damage thresholds and suitable pesticides. Nevertheless technical and economic problems in control are still present. Genetic research, that seems to be capable to determine a production increase of 50% (Duvick, 1984; Russel, 1986), can also produce new hybrids capable to a further reduction of damages due to this moths (Motto & Lorenzoni, 1995). At the first step, hybrids characterized by the sturdiness of the culm and the peduncle were used in order to reduce losses during harvest. Finally, ingegnerized hybrids were used, capable to kill larvae by means of a protein, obtained by *Bacillus thuringiensis* Berliner, contained in green tissues (Rigamonti et al., 1996). The results which could be obtained through those hybrids are very promising. According to recent studies, they are more effective against E.C.B. than traditional insecticides or microbial products (Labatte et al., in press).

Table 1 - Principal phenological and agronomic characteristics of the crops. "Resistance" experiment.

	Gorino Veneto	Zimella	Morsano al T.
Sowing	5 May	3 May	24 May
Germination		10 May	
50% of flowering	8 July	23 July	28 July
Harvesting	30 October	23 October	21 November
Previous crop	Soya bean	Corn	Corn
Principal tillage	Tilling	Tilling 50 cm	Tilling
Other tillage		Hoeing	
Seeding density	6,5 plants/m²	6,3 plants/m²	6,5 plants/m²
Soil disinfection	No	No	No
Weeding data		5 May	
Weeding active ingredients	Metolachlor + terbuthylazine	Metolachlor + terbuthylazine	Alachlor + terbuthylazine + pendichlor
Fertilizing	N 300 Kg/Ha P 130 Kg/Ha	15-15-15 10 Q/Ha Urea 2,5 Q/Ha	Poultry-dung 100 Q/Ha N 300 Kg/Ha; P 90 Kg/Ha; K 240 Kg/Ha
Irrigation	2 spray irrigations	3 spray irrigations	1 spray irrigation
Sampling	10-12 October	17-19 October	25-26 October

The present work is aimed to verify the effectiveness in field of those new ingegnerized varieties. In the framework of the agreement entered into between the

Institute of Agricultural Entomology of Milan University and CIBA Seeds, the experiments were carried out during 1995, on the insecticide efficacy of transgenic corn with respect to the E.C.B. in corn farming in northern Italy. The aim of the first experiment was to evaluate the resistance of transformed hybrids. The second served to describe the insecticide efficacy in relation to the phenology of the plant and of the distribution of the parasite.

For the trials hybrids of corn were used in whose genome the gene that codifies the δ-endotoxin cryIA (b) from the *Bacillus thuringiensis* was inserted. All the material that was examined from transgenic plants has been kept and destroyed together with that left in the field. The ears were gathered apart and incinerated, while the rest of the plants was ground and buried.

MATERIALS AND METHOD

Resistance of transgenic hybrids

Environment of the experiment and characteristics of the crop

This experiment was carried out in 3 sites: Gorino Veneto in the Po Delta in the province of Rovigo; Zimella, in the Venetian plain in the province of Verona; and Morsano al Tagliamento, in the lowland of Friuli in the province of Pordenone.

Gorino Veneto area is characterised by an exclusive presence of herbaceous crops, mostly cereals; in first place there is corn but also rice and wheat, followed by alfalfa and by sugar-beet, sunflower and soya bean. Zimella is instead a mixed agricultural area and with a vast range of produce, cereals, vegetables, industrial crops, orchards and grapes, among which corn is clearly the most important. Morsano al Tagliamento is located in a typically corn district with a lesser important presence of vineyards and industrial cultivations.

The experiments were carried out following the normal crop procedures for corn in each of the three environments. The main agronomic operations and phenological characteristics are shown in table 1.

Experimental plot

The experiment used 3 transgenic hybrids, the same 3 hybrids non manipulated and 2 fill-ins (table 2). Each thesis was repeated 3 times in plots of 200 plants lined up in 4 rows with 75 cm. between each row. Between each line and along the external edges an empty space of about 1 m. was left. The isolation of the experiment was guaranteed by the presence of 10 rows of corn around the plots.

Table 2 - Hybrids on tiral and theirs initial

Hybrid	Initial
1 Transgenic	1T
2 Transgenic	2T
3 Transgenic	3T
Fill-in 1	F1
1 Normal	1N
2 Normal	2N
3 Normal	3N
Fill-in 2	F2

Table 3 - Main agronomic and phenological characteristics of the crop. "Insecticidal efficacy" experiment.

	Zimella
Sowing	23 May
Germination	30 May
50% of flowering	27 July
Harvesting	23 October
Previous crop	Corn
Principal tillage	Tilling at 50 cm
Other tillage	Hoeing
Seeding density	6,3 plants/m²
Soil disinfection	No
Weeding data	5 May
Weeding active ingredients	Metolachlor + terbuthylazine
Basal fertilizing	15-15-15 10 Q/Ha
Top fertilizing	Urea 2,5 Q/Ha
Sampling	18 between 6 August and 28 September

For this experiment only one sample was taken, at harvest, made of 10 plants for each plot chosen at random among the 100 in the 2 central rows. Each plant was dissected in the field, subject to the elimination of the terminal internodes, starting from the third above the ear, subdividing the rest of the plant in 3 parts: a) leaves; b) culm; c) ear. This last one comprises the peduncle, corn-cob, grains, styles and bracts. 3 parameters were noticed: 1) larval infestation; 2) the number of galleries; 3) their length. The data was collected separately for each organ.

All the galleries, empty and occupied, were considered for the length (3rd parameter), including therefore both those successful and those incomplete or that were aborted at an initial stage. As far as the number of galleries is concerned only the successful ones were considered. Those that were aborted were counted only when they reached such a dimension to host a larva. In the case of the larvae collected while they were beginning to burrow, the trial gallery was not considered. The galleries in the ear are those of the corn-cob and of the peduncle. The larvae were attributed to the culm only when they were collected inside it while those outside or in the stage of entering were calculated to the leaves.

The larvae placed in the external bracts of the ear and the blade of the leaf around them are assigned to the leaves.

Analysis of the data

The data collected was put under the Analysis of the Variance (Anova) and later, to the test of the Least Significant Difference (LSD), with a probability of error of 5%, in order to differentiate the individual thesis. For the elaboration, the total value of the parameters was used for each plot, both all together and subdivided in organs.

INSECTICIDAL EFFICACY IN RELATION TO THE PHENOLOGY OF THE PLANT.

Environment of the experiment and characteristics of the crop

The experiment on insecticidal efficacy was carried out in Zimella. To obtain the best conditions of homogeneity necessary for a correct evaluation of the insecticidal activity, a hybrid in the transgenic and traditional forms was used, protected by natural infestations and artificially inoculated. To avoid infestations from autochthonous population, the plot was protected by the installation of a tubular structure in aluminium covered by a shading net to make a «cage» approximately 35X15X3.5 meters, containing about 2700 plants and put into use at the end of June.

For the particular characteristics of the experiment it was necessary to modify in part some operations of the crops with respect to the normal cultivation of the crop in the area. The sowing was postponed with a later delay of the other phenological phases, while the presence of the cage obstructed irrigation. The main characteristics of the crop are mentioned in table 3.

Experimental model

About 1350 plants of each type of corn, transgenic and traditional, were sowed along 45 rows. The 2 thesis were next to each other and separated by an empty space of about 1 meter. Two artificial inoculations were carried out, approximately 1 and 3 weeks after the full flowering, on the 5 and 19 August, both of them simulating infestations of the second generation of the E.C.B..

Each insemination included 20 rows, about 600 plants, excluding those poor, damaged or anyway those different from the norm and distributing approximately 45 larvae just born per plant using a «bazooka». It is a bottle, filled with

the mixture of grit and larvae, fitted up with a dispenser distributing a fixed amount of mixture (fig. 1). The material was supplied by the Laboratory I.N.R.A. of Le Magneraud (France), as eggs, put in incubation in a climatic cell and transported to the field at the time of hatching. Some corn grit was added to the larvae and the mixture that was obtained was put in the «bazooka». It was then distributed as a test regulating the quantity of grit until the desired density was obtained for 15 larvae at each «shot». At this point there was the inoculation distributing 3 shots per plant, 2 at the axil of the leaf around the superior ear and 1 at the axil of the immediately inferior leaf. Periodically, some tests to verify that the density of the larvae stayed constant, were carried out.

The sampling, 9 per inoculation, were carried out 2, 4, 6, 9, 12, 17, 24, 33 and 41 days after infestation. The first three times 15 plants per thesis were controlled and the following six times 25, always chosen at random. The dissection took place directly in the field, and interested the whole plant following the modalities already explained in the experiment on resistance, as it happened for the parameters that were taken.

RESULTS AND DISCUSSION

Resistance of transgenic hybrids

Density of the infestation and damages

The average data per plant for each thesis, both all together and for each organ, is written in the tables 4-6. The presence of the E.C.B. was very different in the three environments. In Gorino Veneto it reached the total average value per plant decisively consistant, to be precise 3.39 larvae and 4.82 galleries with a length of 21.83 cm. This has underlined a clear differentiation between the different thesis for all the parameters considered. The transgenic hybrids were clearly less affected than the normal ones, approximately a third in the total values and between 0.16 and 0.28 times for the data relative to the culm (table 7). In Zimella and in Morsano al Tagliamento infestation was clearly lower, 1.72 and 1.07 larvae, 2.27 and 1.06 galleries, 13.40 and 8.01 cm. respectively. The differences between transgenic and traditional hybrids are less apparent and with different aspects in the 2 localities. In Morsano (table 7), if the thesis 2 is excluded, the gap was clear with a relation transgenic/traditional for the three factors of 0.40, 0.36 and 0.30 for the total and 0.24, 0.28 and 0.22 for the culm. In Zimella, instead, very high values were taken on manipulated corn, similar to those observed in Gorino, where, though, the average density on normal corn was more than double. The relations increased to much higher levels of those of the two preceding localities and they evened out at

Table 4 - Density of infestation and larval activity in Gorino Veneto. "Resistance" experiment.

Thesis	Larval localization				Galleries localization and length					
	leaf	culm	ear	total	culm		ear		total	
1T	0.100	0.433	1.000	1.533	N°	1.233	N°	0.900	N°	2.133
					cm	5.370	cm	4.233	cm	9.603
2T	0.233	0.600	0.533	1.367	N°	1.333	N°	0.667	N°	2.000
					cm	7.323	cm	2.850	cm	10.173
3T	0.100	0.500	0.967	1.567	N°	1.300	N°	1.067	N°	2.367
					cm	5.810	cm	4.283	cm	10.093
F1	0.167	4.033	1.233	5.433	N°	5.700	N°	1.900	N°	7.600
					cm	27.333	cm	8.273	cm	35.607
1N	0.233	3.433	1.600	5.267	N°	5.467	N°	1.833	N°	7.300
					cm	22.610	cm	8.250	cm	30.860
2N	0.233	1.900	0.733	2.867	N°	3.433	N°	1.033	N°	3.467
					cm	16.770	cm	5.133	cm	21.903
3N	0.267	2.367	1.000	3.633	N°	3.933	N°	1.700	N°	5.633
					cm	19.103	cm	7.083	cm	26.187
F2	0.200	4.067	1.167	5.433	N°	5.467	N°	1.567	N°	7.033
					cm	25.073	cm	5.167	cm	30.240

0.63, 0.71 and 0.72 in total and 0.74, 0.81 and 0.86 for the culm that thus resulted more attached to the rest of the plant (table 7).

In the case of the hybrids 1 and 2 in Zimella and of only 2 in Morsano al Tagliamento, values higher in the transgenic corn with respect to the traditional were noted. This was due to the total length of the galleries and, especially, to

Table 5 - Density of infestation and larval activity in Zimella. "Resistance" experiment.

Thesis	Larval localization				Galleries localization and length					
	leaf	culm	ear	total	culm		ear		total	
1T	0.067	0.733	0.367	1.167	N°	1.433	N°	0.400	N°	1.833
					cm	10.390	cm	1.883	cm	12.273
2T	0.133	0.733	0.600	1.467	N°	1.267	N°	0.667	N°	1.933
					cm	7.147	cm	4.250	cm	11.397
3T	0.067	0.667	0.433	1.167	N°	1.233	N°	0.433	N°	1.667
					cm	6.593	cm	2.033	cm	8.627
F1	0.300	1.067	0.933	2.300	N°	1.500	N°	1.600	N°	3.100
					cm	12.693	cm	9.083	cm	21.777
1N	0.067	0.567	0.800	1.433	N°	1.400	N°	0.667	N°	2.067
					cm	7.233	cm	4.500	cm	11.733
2N	0.133	0.767	0.700	1.600	N°	1.433	N°	0.700	N°	2.133
					cm	7.017	cm	3.983	cm	11.000
3N	0.167	1.033	1.100	2.300	N°	1.633	N°	0.700	N°	2.333
					cm	9.993	cm	5.233	cm	15.227
F2	0.100	1.367	0.900	2.367	N°	2.133	N°	1.000	N°	3.133
					cm	9.760	cm	5.433	cm	15.203

Table 6 - Density of infestation and larval activity in Morsano al Tagliamento. "Resistance" experiment.

Thesis	Larval localization				Galleries localization and length					
	leaf	culm	ear	total	culm		ear		total	
1T	0.033	0.033	0.333	0.400	N°	0.033	N°	0.233	N°	0.267
					cm	0.167	cm	1.700	cm	1.867
2T	0.000	0.300	0.733	1.033	N°	0.400	N°	0.600	N°	1.000
					cm	2.993	cm	6.933	cm	9.927
3T	0.067	0.200	0.367	0.633	N°	0.333	N°	0.333	N°	0.667
					cm	1.717	cm	2.167	cm	3.883
F1	0.100	0.633	0.833	1.567	N°	0.733	N°	0.600	N°	1.333
					cm	4.913	cm	4.767	cm	9.680
1N	0.033	0.467	0.667	1.167	N°	0.667	N°	0.633	N°	1.300
					cm	3.767	cm	5.200	cm	8.967
2N	0.000	0.267	0.933	1.200	N°	0.367	N°	0.600	N°	0.967
					cm	2.190	cm	6.607	cm	8.797
3N	0.067	0.467	0.733	1.267	N°	0.800	N°	0.800	N°	1.600
					cm	6.267	cm	6.167	cm	12.433
F2	0.000	0.633	0.700	1.333	N°	0.733	N°	0.633	N°	1.367
					cm	4.033	cm	4.467	cm	8.500

the generality of the data relative to the culm, where the difference should have been very sensitive in favour of transgenic corn since there is bacterial toxin in this organ. A similar event could be explained by the natural decrease of the content of toxin that can be noticed with the passing of time in the green parts and with less sensitivity of the older larvae with respect to the younger ones. Almost mature individuals could have managed to penetrate successfully in the culm in

Table 7 - Proportional infestation and activity of O. nubilalis *on transgenic corn compared to traditional hybrids. "Resistance" experiment.*

	Larvae number			Galleries number			Galleries length		
	culm	ear	total	culm	ear	total	culm	ear	total
Gorino									
Total	0,16	0,88	0,33	0,27	0,55	0,35	0,28	0,55	0,34
1	0,13	0,62	0,29	0,23	0,49	0,29	0,24	0,51	0,31
2	0,32	0,73	0,48	0,39	0,65	0,58	0,44	0,56	0,46
3	0,21	0,97	0,43	0,33	0,63	0,42	0,30	0,60	0,39
Zimella									
Total	0,74	0,53	0,63	0,81	0,54	0,71	0,86	0,48	0,72
1	1,29	0,46	0,81	1,02	0,60	0,89	1,44	0,42	1,05
2	0,96	0,86	0,92	0,88	0,95	0,91	1,02	1,07	1,04
3	0,65	0,39	0,51	0,76	0,62	0,71	0,66	0,39	0,57
Morsano									
Total	0,36	0,62	0,53	0,39	0,60	0,49	0,38	0,66	0,54
1	0,07	0,50	0,34	0,05	0,37	0,21	0,04	0,33	0,21
2	1,12	0,79	0,86	1,09	1,00	1,03	1,37	1,05	1,13
3	0,43	0,50	0,50	0,42	0,42	0,42	0,27	0,35	0,31

the terminal stage of the crop cycle. Nonetheless, the attribution of this phenomenon to the presence of resistant specimen cannot be excluded because there is a lack of data on the content of toxin in the external tissues of the culm, especially since this behaviour did not occur in Gorino.

The damages too, here intended as breaks at the level of the peduncle or of the internodes, have followed a similar result as the one reported previously for larval infestation (table 8). The highest values were obtained in Gorino where for the F1 thesis 0.89 breaks were riported per plant. At the level of the peduncle no great differences were noticed between transgenic and traditional hybrids, while for the culm there were no fractures in the transgenic hybrids while there were in all the normal corn, with values ranging among 0.11 and 0.44 per plant. In Zimella, if the F1 thesis where breaks per plant at the peduncle were 0.5 is excluded, transgenic hybrids showed a greater sensitivity instead, even if they did not differ

Table 8 - E.C.B. damage in "resistance" experiment. Total of the points of breaking.

Thesis	Plants Controlled	Peduncle	Culm above the ear	Culm below the ear
Gorino				
1T	11	1	0	0
2T	8	0	0	0
3T	9	4	0	0
F1	9	4	1	3
1N	10	3	2	0
2N	10	2	1	1
3N	9	1	2	0
F2	9	2	1	0
Zimella				
1T	30	1	1	4
2T	30	1	0	2
3T	30	1	1	0
F1	30	15	1	2
1N	30	1	0	0
2N	30	0	1	0
3N	30	0	0	0
F2	30	0	2	0
Morsano				
1T	30	1	0	0
2T	30	1	0	1
3T	30	1	0	0
F1	30	0	0	1
1N	30	1	0	0
2N	30	1	0	1
3N	30	1	0	0
F2	30	0	0	0

Table 9 - Results of the Anova test and of the L.S.D. (P=0.05). "Resistance" experiment.

Parameter	Larvae total	Larvae in the culm	Larvae in the ear	Galleries total	Galleries in the culm	Galleries in the ear
Gorino						
F ratio	23,4256	23,0404	2,2474	20,6040	21,5299	3,0009
F prob.	0,0000	0,0000	0,0852	0,0000	0,0000	0,0326
1T	a	a	a b c	a	a	a b
2T	a	a	a	a	a	a
3T	a	a	a b c	a	a	a b
F1	c	c	b c	d	d	c
1N	c	c	c	c d	c d	c
2N	b	b	a b	b	b	a b c
3N	b	b	a b c	b c	b c	b c
F2	c	c	a b c	c d	d	a b c
Zimella						
F ratio	1,5637	1,9076	0,8118	4,8685	2,1447	3,5297
F prob.	0,2166	0,1348	0,5905	0,0042	0,0978	0,0174
1T	N.S.D.	a	N.S.D.	a b	a b	a
2T		a		a b	a	a
3T		a		a	a	a
F1		a b		c	b	b
1N		a		a b	a	a
2N		a		a b	a	a
3N		a b		b	a b	a
F2		b		b	a b	a
Morsano						
F ratio	6,0394	4,3200	2,2929	6,4040	3,0532	3,2727
F prob.	0,0014	0,0073	0,0803	0,0010	0,0305	0,0235
1T	a	a	a	a	a	a
2T	b c	a b	a b	b	a b c	c
3T	a b	a b	a	a	a b	a b
F1	d	c	b	b	b c	a b c
1N	c d	b c	a b	b	b c	b c
2N	c d	a b	b	b	a b	c
3N	c d	b c	a b	b	c	c
F2	c d	c	a b	b	b c	a b c

N.S.D. = no significant difference

much from traditional ones. In particular, numerous fractures of the culm below the ear were noticed, at a point where it can lead to the loss of the harvest, and they reached the highest in the 1T thesis with 0.17 breaks per plant. In Morsano, as a consequence of the low infestation, even the damages reach a modest total, with 0.07 breaks at the most per plant in thesis 2T and 2N, and so it was not possible to underline the differences.

Analysis of the results

The Anova was calculated for larval infestation and the length of the galleries. The application of the Anova test to the number of larvae present on the leaves

was not considered of any use since these were represented by erring individuals probably going from the ear to the corn-stalk, which did not have any close trophic links with the leaves. On the other hand, the number of galleries was not analysed due to their similarity with their length. The results have been written in table 9.

Table 10 - Infestation and larval activity on transgenic corn, 1st inoculation. "Insecticidal efficacy" experiment.

Contr.	Larval localization					Galleries localization and length					
+ days	grit	leaf	culm	ear	total	culm		ear		total	
1 + 2	1.833	0.083	0.000	2.000	3.917	N°	0.000	N°	0.000	N°	0.000
						cm	0.000	cm	0.000	cm	0.000
2 + 4	0.533	0.067	0.000	2.600	3.200	N°	0.000	N°	0.000	N°	0.000
						cm	0.000	cm	0.000	cm	0.000
3 + 6	0.000	0.467	0.000	2.267	2.733	N°	0.000	N°	0.000	N°	0.000
						cm	0.000	cm	0.000	cm	0.000
4 + 9	0.000	0.080	0.000	2.760	2.840	N°	0.000	N°	0.000	N°	0.000
						cm	0.000	cm	0.000	cm	0.000
5 + 12	0.000	0.050	0.000	1.950	2.000	N°	0.000	N°	0.000	N°	0.000
						cm	0.000	cm	0.000	cm	0.000
6 + 17	0.000	0.000	0.000	2.160	2.160	N°	0.000	N°	0.000	N°	0.000
						cm	0.000	cm	0.000	cm	0.000
7 + 24	0.000	0.200	0.040	1.600	1.840	N°	0.080	N°	0.080	N°	0.160
						cm	0.360	cm	0.220	cm	0.580
8 + 33	0.000	0.040	0.200	1.240	1.480	N°	0.240	N°	0.400	N°	0.640
						cm	0.500	cm	1.680	cm	2.180
9 + 41	0.000	0.040	0.240	0.760	1.040	N°	0.320	N°	0.280	N°	0.600
						cm	1.260	cm	1.440	cm	2.700

The Anova test confirmed what was already known. In Gorino the manipulated hybrids resulted similar to each other and they differed significantly from the common ones both for the total data and for the data relative to the culm. The analysis relative to the ear has shown instead a substantial similarity between the thesis, in particular between homologous transgenic-traditional couples. This data corresponds fully to the expectations in relation to the distribution of the toxin, which is concentrated in the green parts of the plant and in the pollen while it is lacking in the ear, and they witness a complete efficacy in relation to the E.C.B. larvae. The results in Morsano too, notwithstanding the low infestation that helps to hide the eventual differences, agree with those in Gorino, with the exception of thesis 2, that can never be differentiated from its correspondent and not even from the other corn not manipulated. The framework that has been obtained in Zimella is clearly different. It has not been possible in fact to notice any difference between the thesis that have been put forward. This confirms what was said previously relatively to infestation and damages.

INSECTICIDAL EFFICACY IN RELATION TO THE PHENOLOGY OF THE PLANT.

Infestations and damages

The results relative to the infestation and to the activity of the larvae are in the tables 10-13. The total average efficacy of transgenic plants decreases with the passing of maturity of the host plant (fig. 2). The efficacy goes from about 80% for the first inoculation to 55% for the second. At the same time the length

Table 11 - Infestation and larval activity on traditional corn, 1st inoculation. "Insecticidal efficacy" experiment.

Contr.	Larval localization					Galleries localization and length					
+ days	grit	leaf	culm	ear	total	culm		ear		total	
1 + 2	7.667	0.833	0.000	2.250	10.750	N°	0.000	N°	0.000	N°	0.000
						cm	0.000	cm	0.000	cm	0.000
2 + 4	5.267	0.933	0.000	2.533	8.733	N°	0.000	N°	0.000	N°	0.000
						cm	0.000	cm	0.000	cm	0.000
3 + 6	1.467	4.733	0.067	5.600	11.867	N°	0.067	N°	0.000	N°	0.067
						cm	0.053	cm	0.000	cm	0.053
4 + 9	0.360	3.280	0.000	5.200	8.840	N°	0.000	N°	0.040	N°	0.040
						cm	0.000	cm	0.040	cm	0.040
5 + 12	0.600	4.500	0.000	5.650	10.750	N°	0.000	N°	0.450	N°	0.450
						cm	0.000	cm	0.925	cm	0.925
6 + 17	0.320	3.000	0.000	9.640	12.960	N°	0.000	N°	0.440	N°	0.440
						cm	0.000	cm	1.372	cm	1.372
7 + 24	0.000	2.720	0.800	4.880	8.400	N°	0.920	N°	1.240	N°	2.160
						cm	2.368	cm	5.860	cm	8.228
8 + 33	0.000	1.720	2.680	2.400	6.800	N°	2.880	N°	1.000	N°	3.880
						cm	10.216	cm	3.960	cm	14.176
9 + 41	0.000	1.360	2.320	1.920	5.600	N°	2.840	N°	1.160	N°	4.000
						cm	12.264	cm	5.860	cm	18.124

of time necessary before mortality becomes evident increases. These differences, could be attributed to the biology of the larvae of the E.C.B. rather than to a lower effective efficacy of the plants. At the beginning a notable proportion of the larvae remains in the grit, especially in the case of the normal corn (fig. 3). Later, these larvae move onto the leaves and ears, preferring much more the latter as the grains develop (fig. 3). At the time of the first infestation, which took place 9 days after flowering, the grains are in a very precocious phase of development. Almost 50% of the individuals settles on the leaves with a consequent mortality rate almost total on the transgenic hosts.

In the second infestation, the grains are well developed and the leaves less appetizing. Only a minimal part of the specimen chooses these last ones and so there is a higher total survival rate (fig. 2). In the following days the larvae grow in the

Table 12 - Infestation and larval activity on transgenic corn, 2nd inoculation. "Insecticidal efficacy" experiment.

Contr.	Larval localization					Galleries localization and length					
+ days	grit	leaf	culm	ear	total	culm		ear		total	
1 + 2	4.333	1.267	0.000	7.600	13.200	N°	0.000	N°	0.000	N°	0.000
						cm	0.000	cm	0.000	cm	0.000
2 + 4	0.600	0.267	0.000	7.467	8.333	N°	0.000	N°	0.000	N°	0.000
						cm	0.000	cm	0.000	cm	0.000
3 + 6	0.200	0.000	0.000	7.200	7.400	N°	0.000	N°	0.000	N°	0.000
						cm	0.000	cm	0.000	cm	0.000
4 + 9	0.160	0.000	0.000	5.400	5.560	N°	0.000	N°	0.000	N°	0.000
						cm	0.000	cm	0.000	cm	0.000
5 + 12	0.040	0.000	0.000	6.040	6.080	N°	0.000	N°	0.040	N°	0.040
						cm	0.000	cm	0.048	cm	0.048
6 + 17	0.000	0.120	0.000	6.000	6.120	N°	0.000	N°	0.040	N°	0.040
						cm	0.000	cm	0.120	cm	0.120
7 + 24	0.000	0.120	0.000	4.080	4.200	N°	0.040	N°	0.640	N°	0.680
						cm	0.132	cm	1.424	cm	1.556
8 + 33	0.000	0.560	1.000	3.840	5.400	N°	1.080	N°	1.360	N°	2.440
						cm	2.780	cm	3.820	cm	6.600
9 + 41	0.000	0.600	1.440	1.400	3.440	N°	1.560	N°	0.680	N°	2.240
						cm	6.396	cm	3.360	cm	9.756

Table 13 - Infestation and larval activity on traditional corn, 2nd inoculation. "Insecticidal efficacy" experiment.

Contr.	Larval localization					Galleries localization and length					
+ days	grit	leaf	culm	ear	total	culm		ear		total	
1 + 2	7.467	0.333	0.000	6.800	14.600	N°	0.000	N°	0.000	N°	0.000
						cm	0.000	cm	0.000	cm	0.000
2 + 4	3.200	0.333	0.000	8.800	12.333	N°	0.000	N°	0.000	N°	0.000
						cm	0.000	cm	0.000	cm	0.000
3 + 6	1.333	0.800	0.000	8.400	10.533	N°	0.000	N°	0.000	N°	0.000
						cm	0.000	cm	0.000	cm	0.000
4 + 9	0.480	0.520	0.000	12.200	13.200	N°	0.000	N°	0.440	N°	0.440
						cm	0.000	cm	0.520	cm	0.520
5 + 12	0.280	0.560	0.000	12.480	13.320	N°	0.000	N°	1.400	N°	1.400
						cm	0.012	cm	1.680	cm	1.692
6 + 17	0.000	1.360	0.200	9.880	11.440	N°	0.240	N°	1.600	N°	1.840
						cm	0.600	cm	2.972	cm	3.572
7 + 24	0.000	1.240	2.320	7.640	11.200	N°	2.440	N°	1.800	N°	4.240
						cm	7.128	cm	5.444	cm	12.572
8 + 33	0.000	1.200	2.960	4.200	8.360	N°	3.040	N°	2.880	N°	5.920
						cm	11.860	cm	10.624	cm	22.484
9 + 41	0.000	0.800	4.520	3.120	8.440	N°	4.840	N°	2.240	N°	7.080
						cm	22.428	cm	10.120	cm	32.548

Table 14 - E.C.B. damages in the "insecticidal efficacy" experiment. Total points of breaking.

	Plants Controlled	Peduncle		Culm above the ear		Culm below the ear	
1st inoculation		Tran.	Trad.	Tran.	Trad.	Tran.	Trad.
7 + 24	25	0	0	0	1	0	0
8 + 33	25	0	0	1	4	0	2
9 + 41	25	0	0	0	5	0	0
2nd inoculation		Tran.	Trad.	Tran.	Trad.	Tran.	Trad.
7 + 24	25	0	0	0	1	0	0
8 + 33	25	0	0	0	4	0	1
9 + 41	25	0	1	0	8	2	3

organs where they settled, passing with time in the more internal parts. In the ears the move takes place from the styles to the grains and to the bracts and, finally to the corn-stalk and to the peduncle.

The mortality rate in the ears, at first low, later becomes quite high and constant, about 60% and 50% respectively in the 1st and 2nd infestation (fig. 2). This fact can be explained by a small presence of toxin, which would need more time to kill the E.C.B.. The last hypothesis could also explain the delay in the larvae growth seen on the improved corn and which is underlined in a delayed move of the larvae towards the culm (fig. 3). They reached identical dimensions to those found on normal plants but they took a week more to complete their growth.

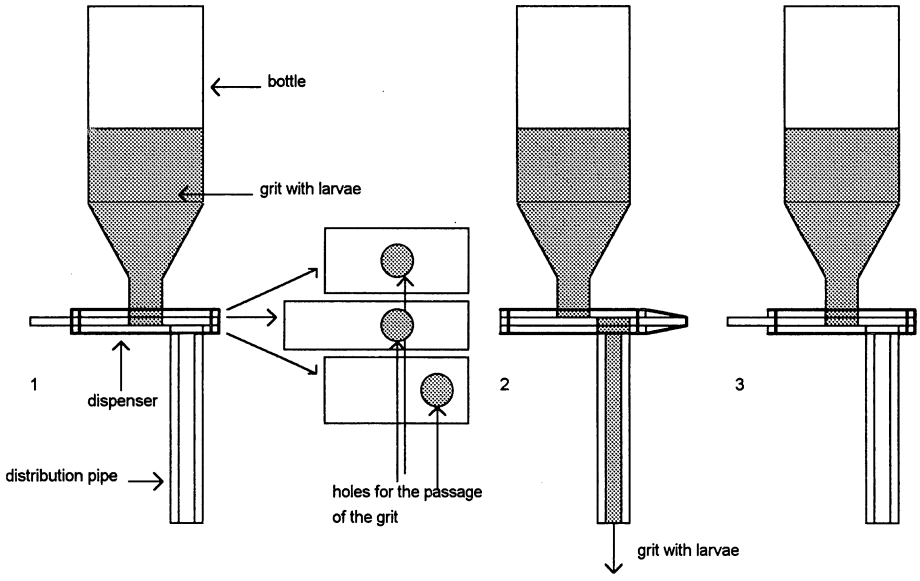

Fig. 1 - Drawing of the "bazooka" and its functioning.

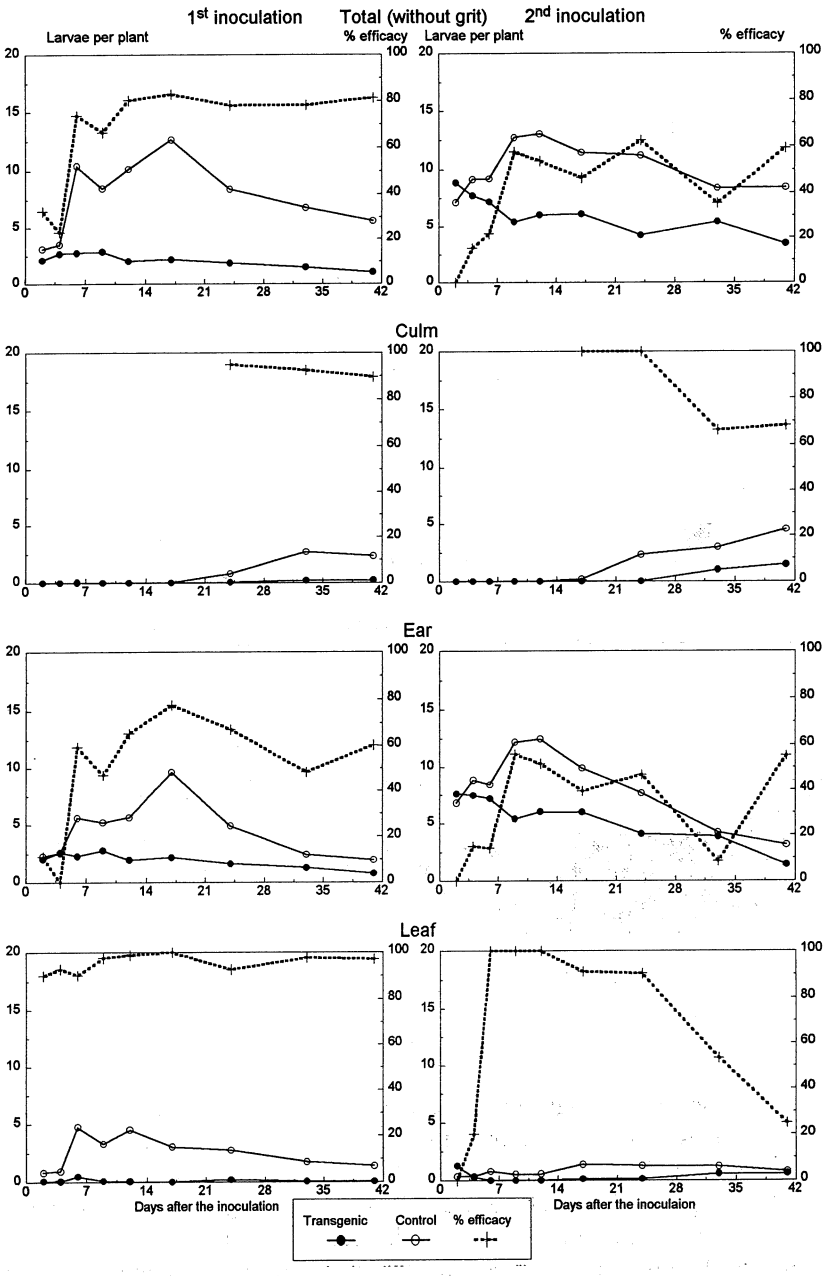

Fig. 2 - Larval density and efficacy in the different organs. "Insecticidal efficacy" experiment.

When they have reached at least the 4th age, starting from about 20 days after inoculation, the larvae begin to migrate towards the culm (fig. 3). The youngest burrow long galleries of alimentation, those already mature only build short tunnels where to spend the winter. If in the 1st infestation the insecticide activity was almost total, always superior to 90%, for the 2nd it settled at about 70% (fig. 2). For the explanation of this phenomenon the experiment on resistance should be

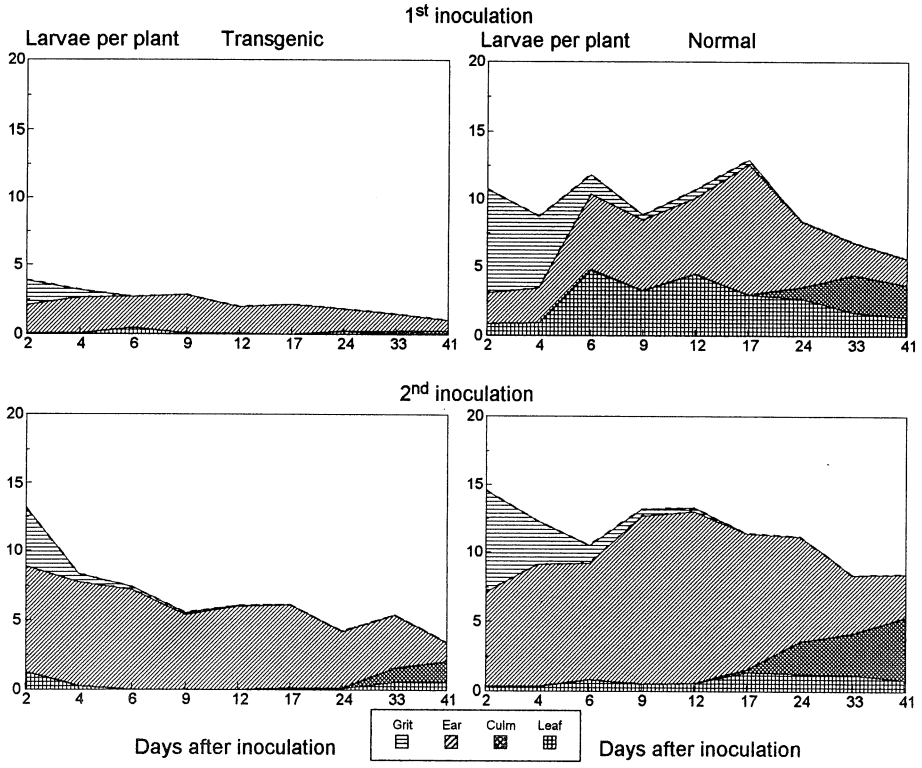

Fig. 3 - Larval distribution on transgenic and normal corn. "Insecticidal efficacy" experiment.

looked at. Due to this migration, in the last sampling numerous individuals between the leaves and the culm or erring along the corn-stalk were collected. This has been attributed to the «leaves» and thus a renewal of the presence on this organ and an apparent lower efficacy can be observed (fig. 2). This, though, is due to the event in question and not to a reduction of toxin.

The larval activity feels strongly of this behaviour; The reduction of the galleries in the culm is almost similar to the reduction in the number of larvae, while it is a little higher in the ear and, consequently, in the total value (fig.

4). Therefore there does not seem to be a substantial reduction of the average larval activity in transgenic corn, considered as cm. of galleries per individual. The damages are lower, considered as breaks, (table 14) which are practically

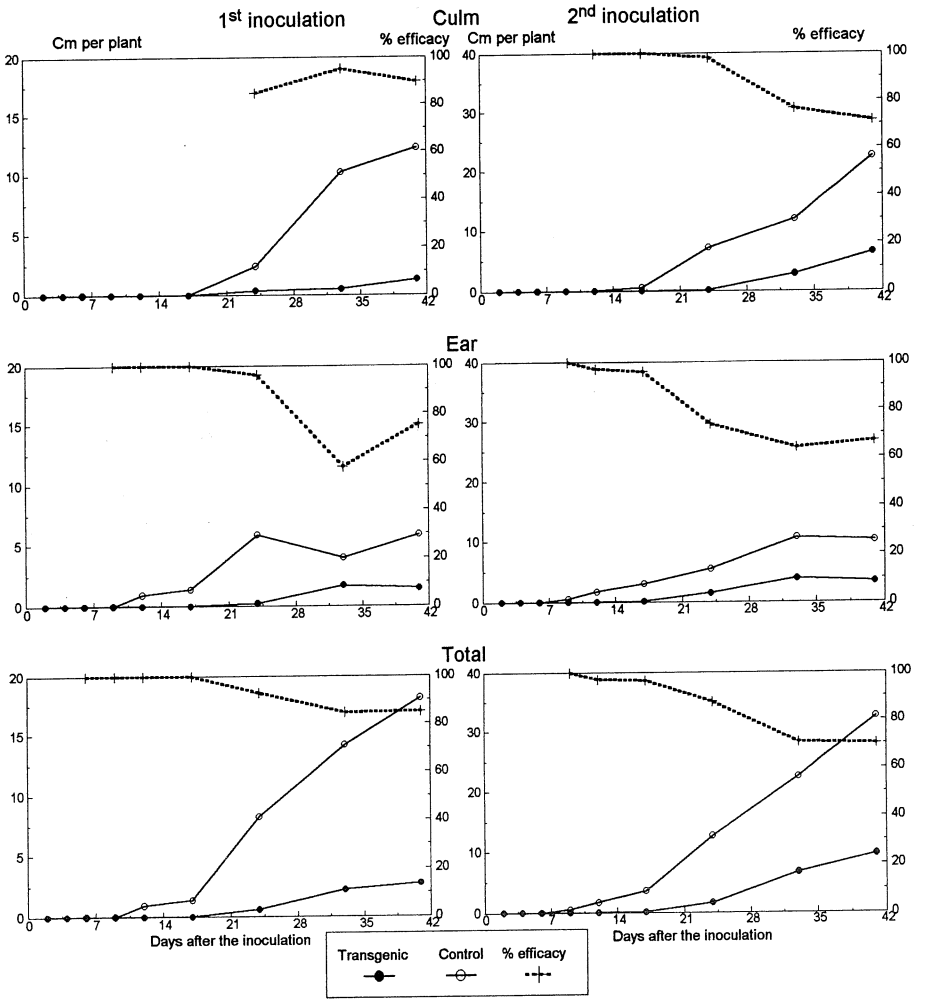

Fig. 4 - Larval activity and efficacy in different organs. "Insecticidal efficacy" experiment.

absent in manipulated corn and grow progressively in normal, until they reach a value of 0.24 and 0.48 breaks per plant in the 1st and 2nd infestation, taking into consideration both the peduncle, very rarely, and the culm above and below the ear.

CONCLUSIONS

Resistance of transgenic hybrids

Notwithstanding the scarce presence of E.C.B. in 2 environments out of 3 has obstructed the complete success of the experiment, the data that was obtained agree almost completely with those expected. The presence and activity of *O. nubilalis* are significantly reduced in transgenic hybrids, in particular in the organs where the endotoxin of the *Bacillus thuringiensis* is present. Manipulated hybrids were significantly different from the homologues and from the traditional hybrids in Gorino, the only place where larvae infestation has been consistant. Hybrid 1 was the most interesting. Hybrid 2 showed some anomalies. The data of Zimella, which disagreed with all the rest and could not be attributed to a proclaimed cause, requires further studies.

Insecticidal efficacy in relation to the phenology of the plant.

The transgenic corn has shown a good insecticidal activity, at its highest in the leaves, a little less in the culm and relatively average in the ear. Overall, it decreases and slows down with the passing of the season, remaining constant at the level of the leaves and ear and slightly decreasing in the culm. The lower efficacy can be attributed more to the phenology of the plant and the correlated larvae distribution, than to a reduction of the toxic level of the hybrid. Besides the mortality rate, a delay in the development of the larvae can be underlined. The larvae manage anyhow to complete their life cycle with success before harvesting. The reduction induced in the larval activity is only a little higher than the activity of infestation, moreover, the average consumption per individual seems to stay almost constant. The reduction of the damages, always in relation to the density of the larvae, is instead clearly more sensitive.

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