Boll. Zool. agr. Bachic.
Ser. II, 32 (1): 35-47

28 April 2000

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Laboratory studies on the effects of transgenic corn on the spider mite *Tetranychus urticae* Koch

Abstract - The possible influence of transgenic Event 176 corn on the development of the mite *Tetranychus urticae* Koch was evaluated for the first and the second generation. Laboratory studies were carried out on transgenic and isogenic corn using this phytophagous mite, measuring the time of development of the different stages, the survival of both sexes and the abundance of eggs laid and hatched. The results do not highlight significant differences between the parameters studied.

Riassunto - *Studi di laboratorio sugli effetti di mais transgenico su* Tetranychus urticae *Koch.*

La possibile influenza di mais transgenico Evento 176 sullo sviluppo dell'acaro *Tetranychus urticae* Koch è stata valutata sia in prima che in seconda generazione. Studi di laboratorio sono stati condotti con allevamenti del fitofago su mais transgenico e isogenico, misurando i tempi di sviluppo dei differenti stadi, la sopravvivenza di entrambi i sessi e l'abbondanza di uova deposte e schiuse. I risultati non evidenziano differenze significative tra i parametri indagati.

Key words: Bt corn, *Tetranychus urticae,* non target species, side effects.

INTRODUCTION

One of the main problems regarding the use of Bt transgenic plants for the control of *Ostrinia nubilalis* (Hb.), *Leptinotarsa decemlineata* Say, *Helicoverpa zea* (Boddie), *Heliothis virescens* (F.), lies in the fact that very little is known about their possible effects on the environment and, particularly, on non-targeted entomofauna, despite such plants having been grown in some countries since as long ago as 1996. The few studies already made are, themselves, conflicting; Sims (1995), Dougan *et al.* (1996), Pilcher *et al.* (1997) and Lozzia *et al.* (1998) have reported that the Bt plants they used do not create problems for the tested insects, on the other hand Hilbeck *et al.* (1998) detected some negative effects.

Sims (1995) has carried out tests on transgenic cotton that expresses the protein

CryIA(c) active against *H. zea*, a key insect for this crop in comparison with some auxiliary species as *Coleomegilla maculata* (De Geer), *Apis mellifera* L., *Nasonia vitripennis* (Walker) and *Hippodamia convergens* (Guérin-Méneville); carrying out tests on both the young and adults stages has verified that Bt cotton has no negative effect on such species.

Dougan *et al.* (1996) tried to determine whether there was any possible effect on the biological cycle of *H. convergens* fed with the aphid *Myzus persicae* (Sulzer), fed in its turn on transgenic potato leaves that express the δ-endotoxin CryIIIA. Analysing different parameters, such as the time of the larval development, the number of preyed upon aphids, nymph weight, fertility, longevity, and found no differences that would demonstrate possible toxic effects of a transgenic plant through the prey of a predator like *H. convergens*.

Instead Pilcher *et al.* (1997) carried out laboratory studies to determine the possible effects of Bt Event 176 corn pollens, capable of expressing CryIA(b), on three predators *C. maculata, Orius insidiosus* Say and *Chrysoperla carnea* Stephens, this last was indicated by the same Authors as a possible predator of *O. nubilalis*. The study failed to highlight any significant differences between the individuals fed with normal pollen and those fed the Bt.

Lozzia *et al.* (1998) verified that Bt corn plants do not negatively influence either the biological cycle or the fertility of the aphid *Rhopalosiphum padi* L.. Also the biological cycle of *C. carnea* fed aphids grown on transgenic corn showed the same results.

On the other hand, work carried out by Hilbeck *et al.* (1998) indicated possible negative effects on green lacewing *C. carnea.* Compared with predators raised on larvae fed isogenic leaves it was found that there was a possible effect of CryIA(b) on green lacewing fed *O. nubilalis* and *Spodoptera littoralis* (Boisd.) larvae that had been grown for 24-72 hours on leaves of transgenic corn. As *S. littoralis* was not a target it was used as a yardstick, results from various experiments carried out on it having shown it not to be influenced by the protein CryIA(b); this is probably due to the absence of specific receptors at the epithelium level of the medium intestine.

The analysis took account of the overall mortality during larval development, the development time of the individual larval and that pre-imaginal. Green lacewings fed European Corn Borer larvae grown on Bt corn showed all the analysed parameters to be significantly different from those of larvae fed other diets. Hilbeck *et al.* (1998) came to the conclusion that the effect on the lacewings could probably be associated with the ingestion of the CryIA(b) protein acquired from prey fed on transgenic leaves.

Nevertheless there have been few studies directly evaluating the plant to assess the effects of transgenic corn on non-targeted entomofauna. This prompted us to verify, through laboratory trials, the effects of Bt corn plants on a non-target arthropod. Particular attention was paid to analysing the various phases of the biological cycle of *Tetranychus urticae* Koch. Such a mite was chosen not only for its high polyphagia and marked presence on corn (Archer & Bynum, 1993) but also for its manner of nutrition. In fact *T. urticae* sucks the cell content directly, at the same time taking up part of the cytoplasm and cellular bodies contained therein. It is known that the chlo-

roplasts in the mesenteron of the mite remain a long time, digestion being quite slow (Van der Geest, 1985). Thus it is practically certain that *T. urticae* ingests the protein, though its location in the cell is not known with any certainty, whether present in small cellular bodies, in the cytoplasm, on the membrane or a bit all over the place.

Chapman and Hoy (1991) have done laboratory trials to verify the possible effects of using formulations based on *Bacillus thuringiensis* var *tenebrionis*(1) at different concentrations on *T. urticae*; the results have not revealed any significant effect of Bt on the development times, the number of eggs deposited or the percentage of hatching of *T. urticae*. The experiments were carried out using cut pieces of leaves of *Phaseulus vulgaris* L. that had been sprayed with a product based on *B. thuringiensis* var. *tenebrionis* in the quantities indicated on the label. However it is quite likely that the mite does not ingest the product as it sucks up the content of the mesophyll cells and hence does not ingest material present on the leaves surface. Thus our research was aimed at directly evaluating the effects of transgenic corn on the cycle of the said mite.

MATERIALS AND METHODS

The experimentation required the use of corn hybrids: one "transgenic", that is capable of synthesising the protein Cry IA(b) (Event 176[2]) and the other corresponding "isogenic". To carry out the tests it was necessary to use seedlings grown separately without the need to cut the plants at all, as indicated by Gutierrez (1990) for the breeding of *Tetranychidae* on bean leaves. To do this small PVC glasses of the following dimensions were used: diameter of the lower base 5 cm, of the superior base 6 cm, height 8 cm. The bottom was covered with 3 grams of ternary fertiliser, N:P:K 2.6:1.5:2 (where the nitrogen content was 50% in the nitric form and the rest in the ammoniacal), made up by a synthetic insoluble ion exchange resin (cationic-anionic resin ratio 1:2 and cationic exchange capacity on the dry substance of 150 meq/100g). The substratum was made of expanded clay. The glasses were placed inside a climatised chamber at 22.5 ± 0.5°C temperature, relative humidity > 75%. The light was supplied with a lighting system consisting of three fluorescent tubes, Marzafluor Blanc Industries 33 6K 58 W BI, and three Philips TLD 58 W $W/82_7$ New generation able to give the intensity of 200-250 mmol s^{-1} m^{-2} necessary for the development of plants requiring a lot of light, with a 16:8 L:D photoperiod.

For the test second and third leaves were used, according to Fearing *et al.* (1997) at this stage the content of CryIA(b) in the Bt corn leaves is rather high; the value is about 700-1200 ng/g of fresh weight. The leaves of each seedling were used without cutting them, the leaves investigated being placed in a sandwich structure, modified compared with that of Gutierrez (1990) and Munger (1942); inside this was placed the mite, isolating it from the other individuals. The structure was made of three parts of plexiglas:

1 - one part, of rectangular form (2.5 cm x 7 cm) 0.2 cm of thick, to which there

(1) Crown spar-total, trade mark Crown Industrial and M-ONE, of the Mycogen
(2) Trade marker Novartis Seed

adhered a sheath of black rubber;

2 - another, with the same dimensions as the previous one but 0.5 cm thick, at whose centre there was a circular hole of 0.8 cm. This hole had, in its turn, two small lateral holes that were covered internally with a 50 μm nylon net. The 0.8 cm hole was the rearing chamber of the mite and the small lateral holes guaranteed a change of air, avoiding the formation of condensation;

3 - the last part, rectangular (2.5 cm x 4.5 cm) and 0.2 cm thick, served as the lid of the chamber.

The three pieces were assembled as indicated in Fig. 1. The plant was used when the second leaf appeared fully extended; the first part of the structure was placed so

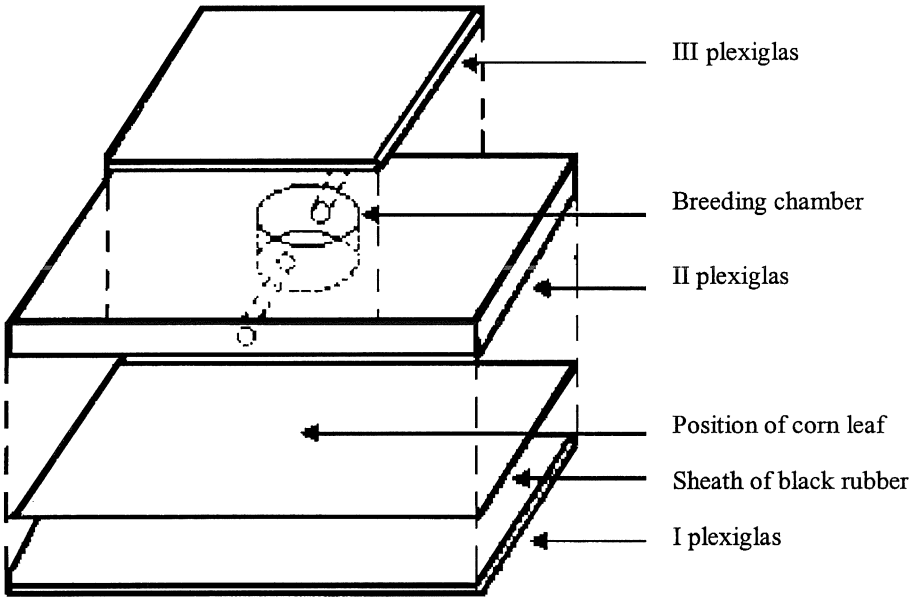

Fig. 1 - Equipment used for the individual rearing of *T. urticae*.

that the sheath was turned toward the lower blade of the leaf, whilst the second was superimposed on the first, the leaf remaining between the two pieces The two parts were temporarily fixed by two laterally placed clips. The mites were then taken from the mass breeding and individually placed in each chamber.

The tests led to the evaluation, for two successive generations, of several parameters of the biological cycle of *T. urticae*. The spider mite were collected in the 1997 from corn fields in Como (North Italy). Observations were made daily, removing the two clips from the glass and positioning the chamber for stereoscopy, careful attention being paid not to damage the leaves. The trials were started by taking 30 female individuals (for each type of corn) from the mass breeding and placing them indi-

vidually inside the cells. Each day for a period of two or three days they were transferred onto new leaves until the desired number of eggs was obtained. It was possible to have more than one egg for each cell. At this point the females were eliminated. The data were collected from the moment the eggs began being deposited. After hatching the larvae were transferred individually onto fresh leaves, generally remaining on the same leaf until becoming adult; only if the leaf yellowed did we intervene, moving them onto a new substrate. Once adults had been obtained couples were formed, and kept isolated until the death of both individuals. Those remaining alone were kept isolated and data collected until their death. Each time the presence of a new egg was observed in a cell the couple was transferred onto a new leaf; in this way we were able to know the exact date of each deposition, allowing us to evaluate the second generation. The small cells with the eggs were maintained with the whole plant for a maximum of 10 days and the cells where there was hatching were eliminated. The larvae born were then transferred individually, during the daily control, to a new cell where they generally remained until they reached the adult stage.

Data analysis

Different parameters were analysed for two generations. For the first generation, those born to females from the mass breeding base, observations were made for all the individuals throughout their development until their death, calculating also their fecundity and fertility. For the second generation, individuals generated by the females of the first generation, only the length of pre-imaginal development was considered. The ANOVA and χ^2 tests were calculated to analyse the data. The percentage were transformed in arcs $\sqrt{\%}$.

RESULTS

Tests on the first generation

Biological cycle

The analysis of the first generation was carried out on the number eggs hatched and not hatched, and on larval mortality throughout post-embryo development. The

Table 1 – Data and results of the χ^2 test on the first generation egg of T. urticae.

	Observed			Expected	
	Isogenic	Transgenic	Total	Isogenic	Transgenic
Non hatched	10	8	18	9.103	8.897
Hatched	78	78	156	78.897	77.103
Total	88	86	174		
$\chi^2 = 0.199$					
P = 0.655					

Table 2 – Data and results of the χ^2 test on mortality during post-embryonic development of the first generation of T. urticae.

	Observed			Expected	
	Isogenic	Transgenic	Total	Isogenic	Transgenic
Dead	9	8	17	8.273	8.727
Alive	64	69	133	64.727	68.273
Total	73	77	150		
$\chi^2 = 0.140$					
P = 0.708					

analysis foresaw the use of the χ^2 test that in both cases resulted non significant (Tables 1 and 2 respectively)

Analysing the parameters of male development (Table 3) revealed the embryo phase to last an average of 5 ± 0.2 days for those grown on normal corn, with a minimum of 4 days and a maximum of 6 days, whereas for the individuals grown on transgenic Bt corn the average length was 5.6 ± 0.2 days, with a minimum of 5 days and a maximum of 7 days, and a highly significant value of P = 0.009. The ANOVA results were significant only for the male development time, not for the female. The other stages showed no big differences, also demonstrated by ANOVA results that were not significant.

Instead the females of *T. urticae* show (table 3), as the only significant data, the time of pre-imaginal development (P = 0.022). There was an average of 14.7 ± 0.7 days with extremes of 10 and 25 days for mites grown on normal corn and 13 ± 0.3 days

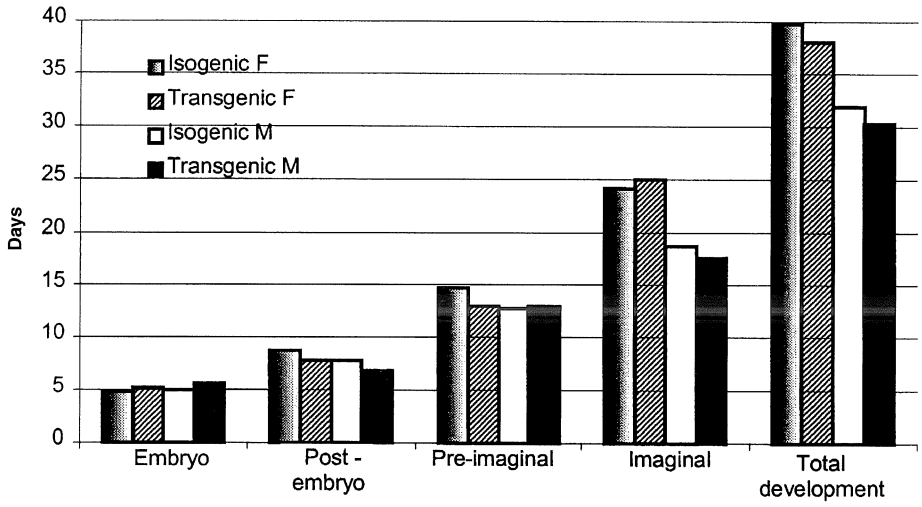

Fig. 2 - Mean time of development of the various stages of the I generation of *T. urticae*.

Table 3 – Time in days of the various phases of development of the I generation divided by sex and substrate.

Type of corn	Isogenic				Transgenic				F	P
Life stage	cases observed	min	max	mean ± S.E.	cases observed	min	max	mean ± S.E.		
Embryo ♀	22	4	7	4.77 ± 0.17	30	4	6	5.16 ± 0.12	3.51	0.067
Post-embryo ♀	22	6	21	8.72 ± 0.77	30	6	16	7.86 ± 0.32	1.28	0.262
Pre-imaginal ♀	29	10	25	14.65 ± 0.70	41	11	19	13.05 ± 0.29	5.45	0.022*
Imaginal ♀	26	5	42	24.15 ± 1.18	36	4	41	25.03 ± 1.61	0.12	0.724
Total life ♀	26	18	56	39.82 ± 2.05	36	20	54	38.11 ± 1.55	0.46	0.499
Embryo ♂	23	4	6	5.00 ± 0.15	16	5	7	5.62 ± 0.15	7.68	0.009**
Post-embryo ♂	23	6	14	7.78 ± 0.41	16	5	9	6.75 ± 0.32	3.29	0.078
Pre-imaginal ♂	35	10	19	12.81 ± 0.32	28	11	16	12.96 ± 0.27	0.12	0.735
Imaginal ♂	30	3	32	18.66 ± 1.20	20	5	30	17.60 ± 1.74	0.27	0.606
Total life ♂	30	17	44	31.85 ± 1.19	20	14	44	30.30 ± 1.88	0.53	0.468

F= calculated value, P= probability of error observed on calculated value.
* = significant ** = highly significant

with values of between 11 and 19 days for those fed on Bt corn leaves. One explanation could lie in the fact that several of the females grown on isogenic corn took longer to develop than the average, although the adult individuals were still normal.

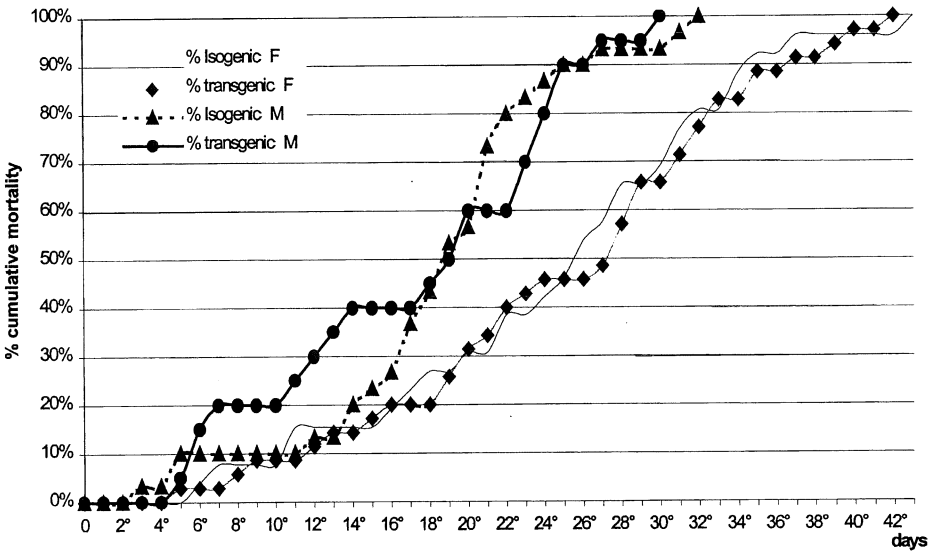

Fig. 3 - Percentage of cumulative mortality of both sexes of *T. urticae*.

This difference relating to these females is also supported by the fact the variance test for homogeneity was highly significant (P = 0.007). In the second generation the ANOVA relative to pre-imaginal development was not significant (Table 6). Figure 2 shows the average of the various life stages of the first generation.

Finally a comparison was made of the course of the cumulative mortality (Fig. 3) of the males and females grown on isogenic corn and transgenic corn and analysed daily with the χ^2 test. In both cases the test was not significant. Such data is verifiable also graphically for the females deriving from isogenic corn and those coming from the transgenic in that the curves tend to be very close to each other. For the males the slight difference observable graphically between the sixth and the seventeenth day is however statistically not significant (P falls between 0.350 and 1).

Laying time

The data in Table 4 are from the observations made during the phase of deposition. The investigation refers to 54 laying females (22 on isogenic, 32 on transgenic), except that for the parameter of eggs laid, where also non-laying females were considered (4 on isogenic, 3 on transgenic), and for that at the beginning of the laying where consideration was also given to those lost later, but that had begun to lay, 2 on both the normal and the Bt.

The females on the normal corn produced 644 eggs (if we add to these the 2 lost that had begun to lay we have 667 eggs), with a mean of 24.8 ± 3.1, a minimum of 0 and a maximum of 51 eggs. The average fertility was 26.8 ± 2.7 eggs hatched and a total of 589 mites born. The percentage of the individual hatching was, in 8 cases, 100%, and in the other cases there was a minimum of 48.6% (17 hatched eggs out of 35 laid). Also analysed was the number of eggs/day, that for those fed normal corn was an average of 1.5 ± 0.2.

The females observed on Bt corn laid 990 eggs (1011 if account is taken of those laid by the 2 females lost), equal to an average of 28.3 ± 2.9 and range values between

Table 4 – Data of the laying period of generation I females of T. urticae. *The data, expressed in days, are for the beginning, the end and the duration of laying.*

Type of corn	Isogenic				Transgenic				F	P
Parameters	cases observed	min	max	mean ± S.E.	cases observed	min	max	mean ± S.E.		
Initial laying	24	1	8	2.92 ± 0.39	34	1	10	3.71 ± 0.49	1.50	0.224
End of laying	22	10	34	22.00 ± 1.43	32	9	35	22.12 ± 1.33	0.00	0.950
Duration of laying	22	7	32	20.00 ± 1.31	32	2	30	19.28 ± 1.27	0.14	0.703
N° eggs laid	26	0	51	24.77 ± 3.06	35	0	69	28.28 ± 2.91	0.67	0.415
N° eggs hatched	22	2	49	26.78 ± 2.65	21	1	68	30.24 ± 3.44	0.64	0.427
% of hatching	22	48.6	100	77.75 ± 3.13	21	48.8	100	77.19 ± 3.07	0.01	0.900
N° eggs/day per ♀	22	0.7	2.6	1.55 ± 0.16	32	0.6	2.8	1.62 ± 0.11	0.13	0.716

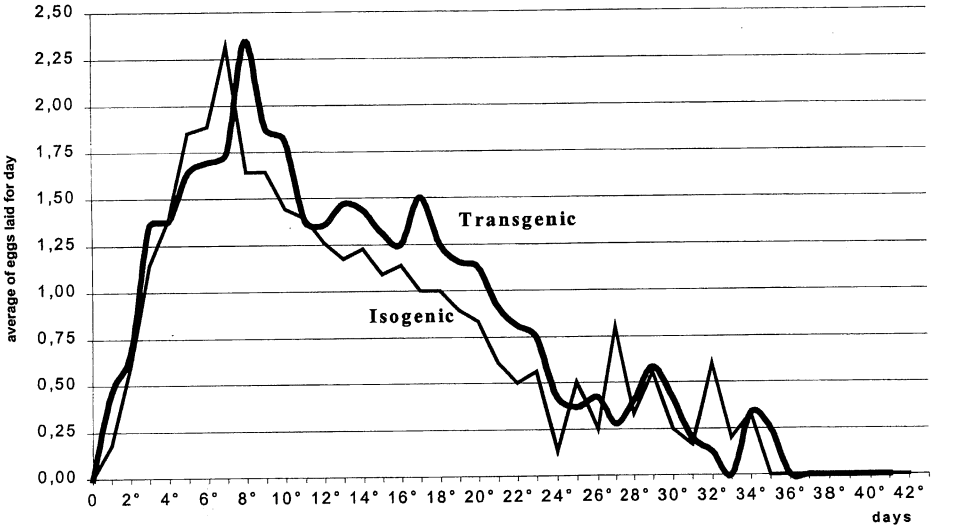

Fig. 4 - Laying curve, expressed as the mean of the daily eggs laid by all the females.

0 and 69. The number of eggs hatched was observed for only 21 females, that in all laid 716, in that the laying space available, the material and the time were not sufficient to evaluate all (1011). The average hatched 30.2 ± 3.4 eggs corresponding to a hatching percentage that reaches 100% in 5 cases and with a minimum of 48.8% (20 hatched on 41 laid). Finally there was the calculation of the average of eggs/day laid by 32 females with values between 0.6 and a maximum of 2.8 and a daily average of 1.6 ± 0.1. The analysis of these data using ANOVA resulted in values classed not significant.

Table 5 - Number of females and eggs laid daily.

	Days	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
Isogenic	Females	28	28	28	28	27	26	25	25	25	25	23	23	23	22	22	21	20	19
	Eggs	2	17	32	39	50	49	58	41	41	36	32	29	27	27	24	25	20	19
Transgenic	Females	37	37	37	37	36	36	35	35	33	32	32	31	30	30	29	28	28	28
	Eggs	17	25	50	51	59	61	61	82	62	58	44	42	44	43	38	35	42	35
	Days	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36
Isogenic	Females	19	18	18	16	16	15	14	12	10	9	9	8	6	5	5	3	2	2
	Eggs	17	15	11	8	9	2	7	3	8	3	5	2	1	3	1	1	0	0
Transgenic	Females	26	24	23	21	20	19	19	19	18	15	12	12	10	8	6	6	4	4
	Eggs	30	27	21	17	15	8	7	8	5	6	7	5	2	1	0	2	1	0

The dynamics of the daily laying of the group of females was studied (Fig. 4); for the isogenic the maximum value was reached on the seventh day, with a daily ave-

Table 6 - Duration in days of the various development phases of the II generation divided by sex.

Type of corn	Isogenic				Transgenic				F	P
Stage	cases observed	min	max	mean ± S.E.	cases observed	min	max	mean ± S.E.		
Embryo ♀	53	4	6	4.92 ± 0.10	43	4	7	4.98 ± 0.11	0.12	0.724
Post-embryo ♀	53	4	24	8.33 ± 0.38	43	6	10	7.76 ± 0.23	1.49	0.225
Pre-imaginal ♀	53	10	29	13.25 ± 0.38	43	10	16	12.73 ± 0.29	1.08	0.300
Embryo ♂	16	4	6	4.81 ± 0.10	13	4	7	5.38 ± 0.31	3.62	0.068
Post-embryo ♂	16	5	8	7.84 ± 0.61	13	5	8	6.85 ± 0.28	1.88	0.182
Pre-imaginal ♂	16	10	14	12.65 ± 0.65	13	10	15	12.23 ± 0.48	0.26	0.615

Table 7 - Data and results of the χ^2 test on mortality during post embryonal development of the II generation of T. urticae.

	Observed			Expected	
	Isogenic	Transgenic	Total	Isogenic	Transgenic
Dead	16	9	25	14.167	10.833
Alive	69	56	125	70.167	54.167
Total	85	65	150		
$\chi^2 = 0.657$					
P = 0.418					

Table 8 - Data and results of χ 2 the test on the number of males and females among individuals grown on transgenic and isogenic corn of the II generation of T. urticae.

	Observed			Expected	
	Isogenic	Transgenic	Total	Isogenic	Transgenic
Males	16	13	29	16.008	12.992
Females	53	43	96	52.992	43.008
Total	69	56	125		
χ2= 0.000					
P = 0.997					

rage of 2.3 eggs and 58 eggs laid in the day, for the transgenic the maximum was on the eighth day, with a daily average of 2.3 eggs and 82 eggs laid. Also observable is the course of the average daily laying from the moment of the appearance of each female adult, indicated as day 0, until the death of the last female. It can also be seen from the graph when the females ceased egg deposition, those on isogenic corn at the 35th day, those on Bt corn at the 36th day. An oscillation in the values can also be seen graphically, from the twenty fourth to the thirty sixth day. This is due to the fact that

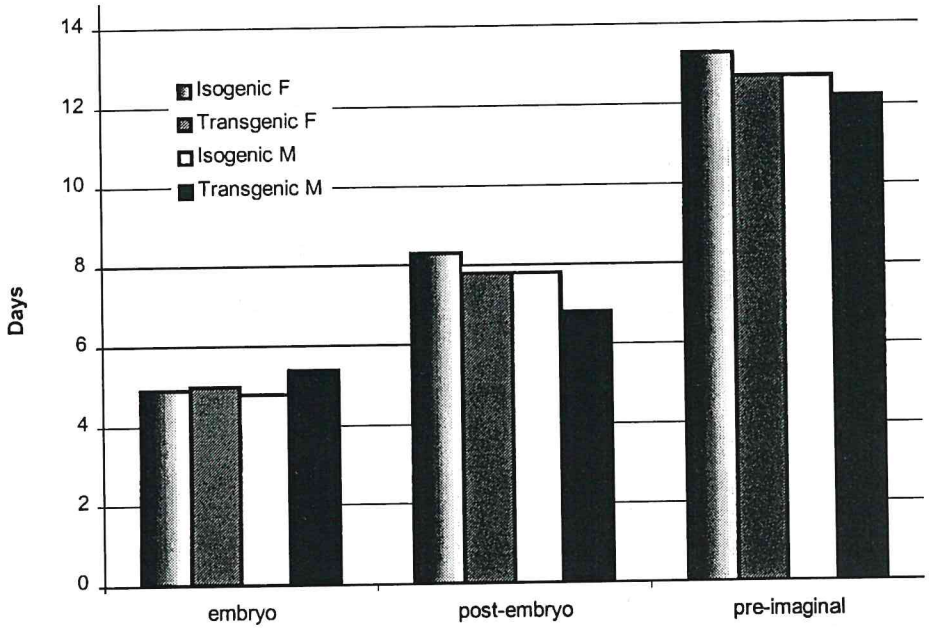

Fig. 5 – Average time of development of the various stages of II generation *T. urticae*.

few females were still alive, thus a small number of eggs laid is enough to bring about a marked oscillation in the average values (Table 5). Analyses using χ^2 of the daily average, day by day, were made and also in this case there were no significant data (being between 1 and 0.054).

TESTS ON THE SECOND GENERATION

Biological cycle

The observations were made on isolated individuals and the object was the larvae from eggs deposited by the females of the first generation and grown on the same type of corn as the female of the first laying generation. The trials involving the II generation revealed the ANOVA not to be significant for any of the phases of the development cycle studied (Table 6), furthermore the two parameters found significant in the I generation were not significant in this case. Note that the number of males analysed is very low (16 on normal e 13 on Bt) and thus of limited statistical relevance. Figure 5 shows the mean development time of the various stages of development. Tables 7 and 8 show the data and results of the χ^2 test calculated on mortality during post-embryo development and on the number of males and females obtained on the normal corn and the transgenic; neither were significant.

CONCLUSIONS

In general there was no evidence of any significant variation that could be attributed to a possible ingestion of the protein CryIA(b) for the various parameters considered on *Tetranychus urticae* Koch in two successive generations.

Analyses of the data of the males of the I generation carried out with ANOVA were not significant for post-embryo, pre-imaginal, imaginal or total development; instead for embryo development they were markedly significant. Nevertheless it is believed that this result does not depend on the possible acquisition of the toxin CryIA(b) by the laying females in that they were taken directly from isogenic leaves and maintained for only 2-3 days on Bt corn leaves, the eggs deposited would thus have already been mature within the mite.

The data of the females of the I generation were non significant for embryo, post-embryo, imaginal and total development. Vice versa in as far as concerns pre-imaginal development P was significant (0.022). Nevertheless it was the females raised on isogenic corn that took longer to develop, while the females fed transgenic corn had a much faster development.

The first generation was analysed with regard to the beginning, the end and the overall laying period expressed in days, number of eggs laid and hatched, percentage of hatching, overall and daily average of the N° of eggs/day per female; none of these values were significant. The χ^2 test was also applied to the number of hatched and not-hatched eggs, larval mortality during post-embryo development, daily egg laying and the cumulative mortality of the adults. In none of the cases were the results significant.

The data of the II generation of *T. urticae,* concerning embryo, post-embryo and pre-imaginal development all gave results that were not significant; also the χ^2 test carried out on the number of dead larvae during post-embryo development and on the number of males and females gave not significant results.

The absence of significant results for almost all the parameters analysed allows it to be said that *T. urticae* is probably not influenced by the CryIA(b) present in the green corn tissue, most certainly ingested by the mite. A possible explanation could be that the mite, though ingesting the protein, does not possess specific receptors at the level of the epithelial cells of the intestine where the toxin acts. However there is a need for a deeper knowledge of the possible effects of transgenic plants in the agro-ecosystem, and this calls for further studies on any possible interaction that can occur between Bt plants and non-target phytophagous and beneficial arthropods.

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CONCLUSIONS

In general there was no evidence of any significant variation that could be attributed to a possible ingestion of the protein CryIA(b) for the various parameters considered on *Tetranychus urticae* Koch in two successive generations.

Analyses of the data of the males of the I generation carried out with ANOVA were not significant for post-embryo, pre-imaginal, imaginal or total development; instead for embryo development they were markedly significant. Nevertheless it is believed that this result does not depend on the possible acquisition of the toxin CryIA(b) by the laying females in that they were taken directly from isogenic leaves and maintained for only 2-3 days on Bt corn leaves, the eggs deposited would thus have already been mature within the mite.

The data of the females of the I generation were non significant for embryo, post-embryo, imaginal and total development. Vice versa in as far as concerns pre-imaginal development P was significant (0.022). Nevertheless it was the females raised on isogenic corn that took longer to develop, while the females fed transgenic corn had a much faster development.

The first generation was analysed with regard to the beginning, the end and the overall laying period expressed in days, number of eggs laid and hatched, percentage of hatching, overall and daily average of the N° of eggs/day per female; none of these values were significant. The χ^2 test was also applied to the number of hatched and not-hatched eggs, larval mortality during post-embryo development, daily egg laying and the cumulative mortality of the adults. In none of the cases were the results significant.

The data of the II generation of *T. urticae,* concerning embryo, post-embryo and pre-imaginal development all gave results that were not significant; also the χ^2 test carried out on the number of dead larvae during post-embryo development and on the number of males and females gave not significant results.

The absence of significant results for almost all the parameters analysed allows it to be said that *T. urticae* is probably not influenced by the CryIA(b) present in the green corn tissue, most certainly ingested by the mite. A possible explanation could be that the mite, though ingesting the protein, does not possess specific receptors at the level of the epithelial cells of the intestine where the toxin acts. However there is a need for a deeper knowledge of the possible effects of transgenic plants in the agro-ecosystem, and this calls for further studies on any possible interaction that can occur between Bt plants and non-target phytophagous and beneficial arthropods.

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Accepted 30 March 2000