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Effects of Bt corn on *Rhopalosiphum padi* L. (Rhynchota Aphididae) and on its predator *Chrysoperla carnea* Stephen (Neuroptera Chrysopidae).

Abstract - Two years of study on the influence of Bt maize on development of aphids and their interaction with natural predators are reported. Two different experiments were conducted to separate the impact of insecticidal plants on sucking insects from the prey-predators interaction. Laboratory studies determined the effects of feeding corn, *Zea mays*, leaves expressing a *Bacillus thuringiensis*-derived CrylAb protein on the aphid *Rhopalosiphum padi.* In turn the predator *Chrysoperla carnea* was reared on aphids that had fed on transgenic and isogenic corn.
No detrimental effects of the transgenic Bt corn on postembryonic developmental time, fecundity or survival of *R. padi* were recorded. Moreover, no influence on preimaginal development or mortality of *C. carnea* were observed when reared on *R. padi* that had fed on Bt corn.

Riassunto - *Effetti di mais transgenico su* Rhopalosiphum padi *L. (Rhynchota Aphididae) e sul suo predatore* Chrysoperla carnea *Stephen (Neuroptera Chrysopidae).*
È stata considerata la possibile influenza del mais transgenico sullo sviluppo dell'afide *Rhopalosiphum padi,* nell'interazione con il predatore *Chrysoperla carnea.* Studi di laboratorio sono stati condotti negli anni 1997 e 1998 con allevamento di afidi su mais transgenico e su mais normale, misurando i tempi di sviluppo dei differenti stadi, la sopravvivenza e la fecondità. Inoltre sono stati osservati lo sviluppo e la mortalità di *C. carnea* allevata su afidi provenienti dai due tipi di mais. I risultati non evidenziano differenze significative tra i parametri indagati.

Key words: Bt corn, *Rhopalosiphum padi, Chrysoperla carnea,* non target effects.

INTRODUCTION

The world-wide increase in the use of transgenic crops against several insect pests, arises the question of whether insecticidal proteins from *Bacillus thuringiensis* var.

kurstaki Berliner could have any negative effects on natural enemies and, in general, on non-target insects (Orr & Landis, 1997; Gould 1998).

MacIntosh *et al.* (1990) reported that several of the Bt-toxins have biological activity specific for insect species belonging to the order Lepidoptera, with little or no effect on insect of others orders. Sims (1995) found, using a different bioassay, that Cry IA(c) toxin from *B. thuringiensis* expressed in cotton did not produce a toxic effect on several beneficial insects (e.g. *Chrysoperla carnea, Apis mellifera, Hippodamia convergens*) all tested as larvae and adults. Laboratory studies (Pilcher *et al.* 1997) on effects of feeding corn pollen expressing a CrylAb protein derived from *B. thuringiensis* subsp. *kurstaki* found no acute detrimental effects on preimaginal development and survival among 3 predatory species: *Coleomegilla maculata* DeGeer (Coleoptera: Coccinellidae), *Orius insidiosus* Say (Heteroptera: Anthocoridae) and *Chrysoperla carnea* Stephens (Neuroptera: Chrysopidae). In contrast, Hilbeck *et al.* (1998) reported that development time of *Chrysoperla carnea* (green lacewing) larvae was prolonged when reared on *Ostrinia nubilalis* that had fed on transgenic corn, suggesting that reduced fitness of chrysopid larvae was indirectly associated with *B. thuringiensis*. However, *C. carnea* use corn plants as a substrate for oviposition and as a host to several prey species, especially the corn leaf aphid *Rhopalosiphum padi* (Diazaranda & Monserrat, 1995; Pilcher *et al.*, 1997), thrips (Jarvils and Guthrie, 1987; Coll & Botrell, 1991) and, occasionally corn borers (Sparks *et al.*, 1966) including *O. nubilalis* (European corn borer). *C. carnea* has limited opportunity to prey on young *O. nubilalis* larvae, because the larvae quickly bore into the corn stalk (Coll & Botrell, 1991). Some authors have also reported that in the laboratory *C. carnea* has a preference for aphids rather than for lepidopterean eggs and larvae (New, 1984; Klingen *et al.*, 1996). Thus the ECB is not the most available and favourite food for *C. carnea,* but in the laboratory, as is the case with many generalist predators, they can be fed with other prey species, particularly when no other choice is provided.

Moreover, no studies until now have been done on the effect of transgenic Bt corn on sucking insects. In the literature it is reported that the Cry1Ab Bt toxin in Bt 176 maize is expressed primarily in pollen and green plant tissues, (Fearing *et al.*, 1996) but no data is actually available on the whether this insecticidal protein is expressed in phloem. Aphids feeding on unsuitable corn can show a reduced body size, delayed development, decreased longevity and reduced fecundity, as when they feed on resistant plants (Unger & Quinsenberry, 1997). Measuring these parameters for aphids and chrysopids, could help determine if there are any influences of Bt corn on specific tritrophic interactions and any evidence of the presence of the Bt-protein in the phloem.

The goal of the present study was to conduct a more in depth evaluation of the influence of genetically modified corn on the biology of non-target phytophagous insects and predators, choosing a typical tritrophic relationship in the corn crop: *Rhopalosiphum padi,* feeding on transgenic Bt and conventional *Zea mays,* as a phytophagous insect, and *C. carnea* as its common predator.

MATERIALS AND METHODS

Development of Rhopalosiphum padi *on transgenic and corn.*

The specimens of *R. padi* L. used for the experiment were field-collected in the spring and subsequently transferred and bred separately in isolation on a transgenic Bt corn, hybrid, derived from transformation Event 176,* or on nontransgenic hybrid corn that was isogenic to the unmodified hybrid. To obtain the data for this study, neonate aphids were bred on two types of corn under laboratory controlled conditions, and stage-specific development times until maturity were recorded.

A cut leaf (3° leaf) from corn, used as a breeding substrate, was fixed into a sponge for cut flowers measuring 10×5×7 cm, in order to maintain it in the desired position. A nutritive solution (Chrysal®) was placed under the sponge. These conditions allowed the cut leaves to be preserved in good condition for a week.

Small cells able to isolate the aphid on the desired part of the leaf were used in order to observe the insect. Each cell was a small, transparent, rubber cylinder (∅ 11 mm; h 10 mm) linked to a peg to maintain adhesion to the leaf, and to a tube, connected to a pump, to refuel the air.

All the cells were placed 50 cm from the mixed light source, which maintained, at leaf level, an intensity of 200–250 μmol s^1 m^2. For lighting, three fluorescent tubes, Marzafluor Blanc Industrie 33 6K TF 58 W BI, and three Philips TLD 58 W/827 New generation were used. The experiment was carried out inside a growth chamber at 25 ±1°C with 75 ±5% relative humidity and a 16:8 hr L:D photoperiod.

Neonate specimens, obtained from females kept in isolation, were placed in individual cells. Observations were made daily, and the dates on which exuviae were found were recorded, as they indicated the moulting of the insect to the following instar. The date of the first birth was also recorded.

Forty aphids for each year, 20 of which bred on leaves of transgenic corn, and 20 on nontransgenic corn, were studied. Aphids bred on genetically modified plants were used for observations on the same type of plants. The same relation between plant, leaves and aphids was followed for traditional corn also.

Fecundity and longevity of Rhopalosiphum padi *(L.)*

For this experiment viviparous females coming from breeding on transgenic and nontransgenic plants were used. The insects were individually isolated on parts of leaves closed inside containers made specifically for this test method. It was thus possible to make daily observations and record the number of births and the number of viviparous females found dead.

The container used for this experiment was made up of 2 glasses on top of each

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* Bt 176 maize is a product of Novartis Seeds.

other in order to create two isolated compartments, one containing the upper part of the leaf with the aphid, and the other the nutritive solution. The glasses were placed in a growth chamber at 25 ±1°C with 75 ±5% relative humidity and a 16:8 hr L:D photoperiod.

Daily activities included the recording and taking off of neonate juvenile stages. Every 5 days the leaves were changed transferring females into a new box. For each year 20 females on leaves of transgenic corn and 20 females on traditional corn were observed.

Mortality of Chrysoperla carnea

This experiment was designed to highlight any differences in development or mortality between lacewings fed with aphids grown on genetically modified corn and others fed with aphids grown on traditional corn. Aphids provided an optimum diet to *C. carnea* larval development (Pilcher *et al.*, 1997).

Lacewings, obtained from the laboratories of Biolab in Cesena (Italy), were used for the initial assay at the second instar, while for the second assay, having eggs, as neonate larvae. In both cases the insects were fed with *R. padi* throughout the experiment. For daily observations the times relative to pupation and emergence of the adult lacewing were recorded, as were any deaths that occurred before the completion of development. The containers and breeding conditions used were the same as those described for the aphid fecundity and mortality tests. In this experiment the colonies of aphids were left to grow freely so that they supplied a continuous and unlimited food source to the carnivore.

Statistical analysis

Each insect was considered as a single unit in the data evaluation. ANOVA was used to evaluate effects on aphids by type of corn (transgenic corn and isogenic corn), replications, type of corn by replication, sampling time, and type of corn by sampling time. Student t-test was used to compare means among sampling times within each type of corn. Standard errors of the means were also calculated. The percentages of *C. carnea* mortality recorded in 1997 were analysed with the χ^2 test. During the 1998 test, fewer deaths of *C. carnea* larvae occurred, and the data available were insufficient-for a statistical analysis.

RESULTS

Development of Rhopalosiphum padi *on transgenic and-nontransgenic corn*

No statistically significant differences were observed for any developmental phases between aphids bred on engineered and on conventional corn in 1997 or 1998

(P> 0.05, Table 1). During the entire experiment period no deaths were observed, and all individuals completed development. In addition there were no differences in developmental time between aphids fed with nontransgenic and transgenic diets during all instars. Fig. 1 shows the mean stage-specific developmental times, calculated for both years. No differences were observed between the two years in developmental time for all stages.

Fecundity and longevity of Rhopalosiphum padi *(L.) on transgenic and nontransgenic corn*

There were no significant differences between *R. padi* fed on transgenic corn and conventional corn in fecundity, lifespan, or mortality or for both years, as measured by the mean number of juveniles born, the mean the number of days between the first and the last birth, and the mean number of days until death (Table 2).

Mean fecundity for 1997 and 1998 combined was 20.74 nymphs/female for individuals grown on nontransgenic corn and 20.95 nymphs/female for individuals coming from transgenic corn, averaged over 1997 and 1998. Mean life spans for both years combined were 19.18 days for aphids reared on transgenic corn and 19.615 days for aphids reared on nontransgenic corn. The mean time from the first instar to the last birth was 11.205 days for females reared on transgenic corn and 11.245 for those reared on isogenic corn.

Fig. 2 shows that the curves of the average of number of aphids born after the

Table 1 - Developmental time (days) of Rhopalosiphum padi *feeding on transgenic Bt and isogenic corn leaves.*

Type of corn	Isogenic	Transgenic		
		1997		
	Mean ± SE	Mean ± SE	F	P
1^a instar nymphs	1.00 ± 0.00	1.00 ± 0.00		
2^a instar nymphs	2.50 ± 0.513	2.60 ± 0.503	0.06	0.87
3^a instar nymphs	4.00 ± 0.795	4.15 ± 0.813	3.20	0.09
4^a instar nymphs	5.65 ± 0.489	5.65 ± 0.489	0.10	0.72
Neonate	7.15 ± 0.366	7.10 ± 0.308	0.16	0.78
		1998		
	Mean ± SE	Mean ± SE	F	P
1^a instar nymphs	1.00 ± 0.00	1.00 ± 0.00		
2^a instar nymphs	2.55 ± 0.69	2.60 ± 0.68	0.05	0.82
3^a instar nymphs	3.95 ± 0.89	4.45 ± 0.88	3.18	0.08
4^a instar nymphs	5.65 ± 0.49	5.70 ± 0.47	0.11	0.74
Neonate	7.25 ± 0.44	7.20 ± 0.41	0.14	0.71

Table 2 - Fecundity and longevity (days) of Rhopalosiphum padi *feeding on transgenic Bt and isogenic corn leaves.*

	1997			
Type of corn	Isogenic Mean ± SE	Transgenic Mean ± SE	F	P
Number of nymphs	20.65 ± 2.78	21.00 ± 3.41	2.48	0.12
Days until the last birth	11.25 ± 1.61	11.20 ± 1.24	1.69	0.20
Mean lifespan (days)	19.60 ± 1.35	19.15 ± 1.56	1.93	0.17
	1998			
Type of corn	Isogenic Mean ± SE	Transgenic Mean ± SE	F	P
Number of nymphs	20.82 ± 2.80	20.90 ± 3.45	2.06	0.82
Days until the last birth	11.24 ± 1.71	11.21 ± 1.33	1.28	0.60
Mean lifespan (days)	19.63 ± 1.34	19.21 ± 1.42	2.9	0.10

first birth from females reared on transgenic and on isogenic corn have a similar trend. A similar tendency is also evident in comparing the graphs for 1997 and 1998 (Fig. 2). Cumulative percent mortality of *R. padi* fed on transgenic Bt and on isogenic corn is shown for both years (Fig. 3).

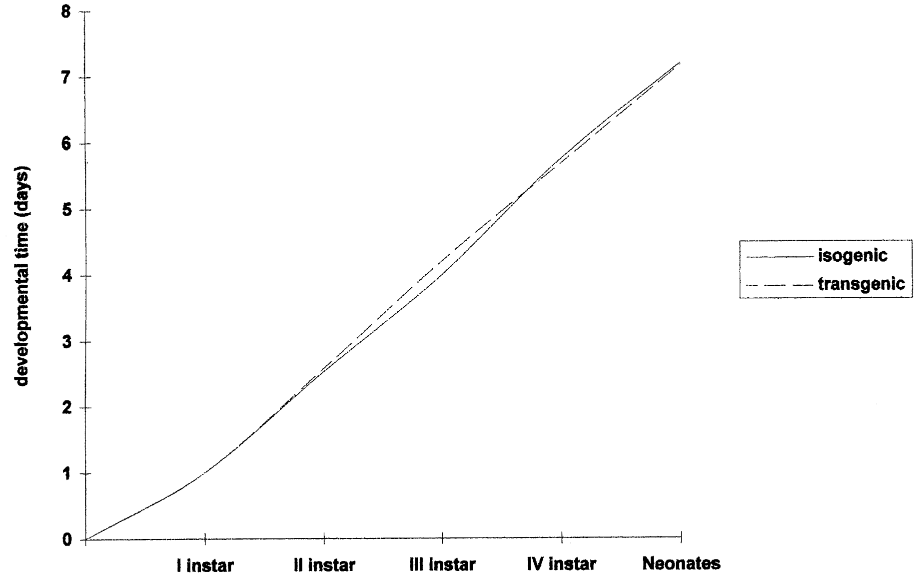

Fig. 1 - Stage-specific development times (days) for *Rhopalosiphum padi* feeding on transgenic and isogenic corn leaves; average of two years (1997-1998).

Development and mortality of Chrysoperla carnea

No significant developmental differences were found between *C. carnea* fed on aphids grown on transgenic and isogenic corn from 1° instar larvae to adults (Table 3, P= 0.74 for 1997 and P= 0.19 for 1998). Consequently, no significant differences were found for the developmental time of the intermediate stages (Table 3). Total immature developmental time (first instar to adult) averaged 17.9 days for both aphid treatments.

The percentages of deaths, analysed with the χ^2 test, are not statistically different between the two groups (Table 4). Thus there were no significant differences in the

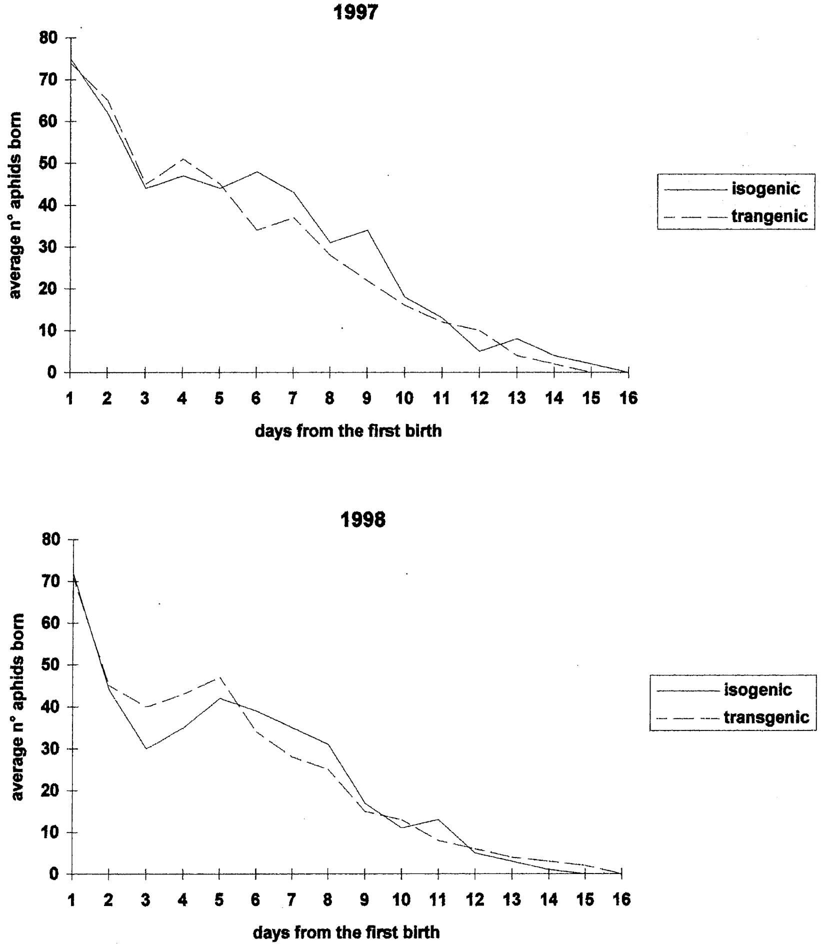

Fig. 2 - Average of number of *Rhopalosiphum padi* (aphids) born after the first birth to females feeding on transgenic (Bt) or isogenic corn.

mortality of *C. carnea* fed with aphids reared on Bt-corn and conventional corn. The number of larval~~e~~ deaths in 1998 was lower, and not sufficient for statistical analysis. This could be due to an improvement of rearing techniques.

Table 3 - Developmental time (days) of different stages of C. carnea *reared on* Rhopalosiphum padi *feeding on isogenic and transgenic Bt corn leaves.*

1997				
Stage of *C. Carnea*	Isogenic Mean ± SE	Transgenic Mean ± SE	F	P
1° instar to pupa	5.26 ± 0.43	4.4 ± 0.22	1.66	0.2
pupa to adult	8.88 ± 0.2	8.41 ± 0.21	1.55	0.21
Total immature development	18 ± 0.3	17.75 ± 0.22	0.1	0.74
1998				
	Isogenic Mean ± SE	Transgenic Mean ± SE	F	P
1° instar to pupa	5.47 ± 0.53	4.87 ± 0.55	0.85	0.37
pupa to adult	8.93 ± 0.41	8.40 ± 0.45	0.43	0.52
Total immature development	18.13 ± 0.45	17.83 ± 0.21	1.79	0.19

Table 4 - χ^2 test for the mortality of C. carnea *reared on* Rhopalosiphum padi *feeding on isogenic or transgenic Bt corn leaves.*

	observed			expected	
	Isogenic	**Bt**	**Total**	**Isogenic**	**Bt**
dead	4	8	12	3,08	2,91
alive	70	62	132	33,91	32,08
Total	74	70	144		
				critical value	
χ^2	0,38	0,4	= **0.854**		
	0,34	0,03			
				p < 5%	3,84
P	0,355			p < 1%	6,64

DISCUSSION

No adverse effects of *Bacillus thuringiensis* corn were observed on developmental time, fecundity and longevity of the aphid *R. padi*. Several studies (Unger & Quisenberry 1997; Farid *et al.* 1998) reported that aphids fed on certain plants or artificial diets demonstrate reduced fecundity, developmental delay and reduced longevity. There were no indications of detrimental effects caused by transgenic Bt corn on *R. padi* in the present study. Survival was similar for individuals reared on Bt corn and nontransgenic corn. In addition, nymphs took a similar length of time to complete

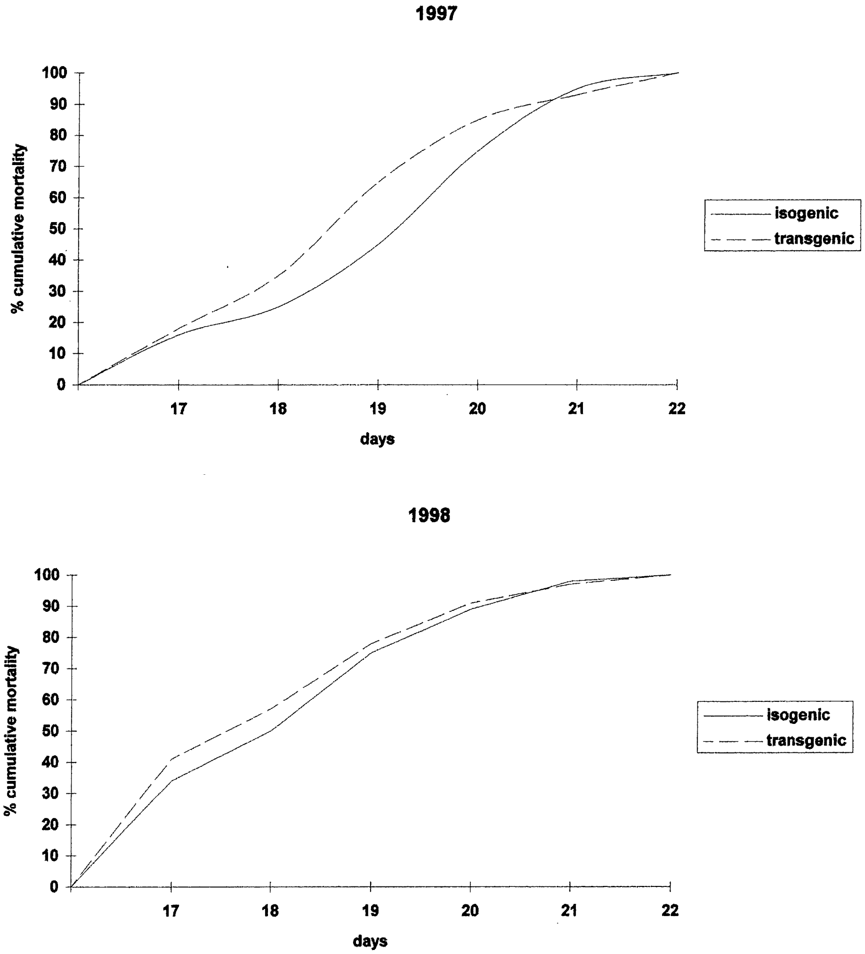

Fig. 3 - Cumulative percentage mortality curves of 40 *Rhopalosiphum padi* females (20 per plant type each year) fed on transgenic (Bt) and isogenic corn.

development. Transgenic corn leaves were as suitable as non-transgenic corn as a diet. This could be explained mainly in two ways: either Bt toxin is not present in the phloem or Bt toxin does not affect *R. padi.* Sims (1995) found that the longevity of the aphid *Myzus persicae* was not affected by Cry IAc protein. However, the effect of this toxin was tested in a bioassay and not directly on the transgenic Bt cotton plant engineered to produce CryIAc.

Developmental time and mortality of *C. carnea* larvae were similar when fed on aphids grown on transgenic or nontransgenic corn. Our results confirm the study of Pilcher *et al.* (1997) where no differences were found in survival between *C carnea* fed with Bt-corn pollen and normal corn pollen. However Hilbeck *et al.* (1998) found that the mortality of immature larvae of *C. carnea* was higher when they were fed with *O. nubilalis* and *Spodoptera littoralis* reared on *B. thuringiensis* corn, while only the chrysopid larvae raised on ECB fed on Bt corn showed prolonged development time throughout their immature stage. A similar result was found by Salama *et al.* (1982) using *B. thuringiensis* var. *entomocidus*, while Sims (1995) in a laboratory bioassay reported that *B. thuringiensis* var. *kurstaki* CryIAc did not have a detrimental effect on *C. carnea.*

In our study aphids reared on transgenic corn did not represent a suboptimal prey for chrysopids. This could be due to the absence of Bt toxin in the phloem, so it is not ingested by aphids, or due to insensitivity of *R. padi* to the effect of the Bt toxin. Lepidopteran-specific Bt proteins are known to exert their toxicity only in a basic pH midgut, such as occurs in several corn borer species (MacIntosh *et al.*, 1990), but not in aphids. In addition, sensitive lepidopteran species have specific binding sites for Bt proteins in their midgut.

Until now, however, no concrete studies have been done to assess the presence of Bt toxin in the phloem of Bt corn (Fearing *et al.*, 1997), as it is difficult to isolate phloem from the rest of the corn plant without contaminating it with Bt protein produced in green plant tissue.

In an actual field situation, it is extremely unlikely that *C. carnea* larvae will develop by feeding exclusively on *O. nubilalis* (Hilbeck *et al.*, 1998), and it is more probable that green lacewing larvae feed on aphids (Klingen *et al.*, 1996; New 1984). To predict the ecological and evolutionary outcome of a particular plant-pest-enemy interaction we need to understand the biology of interacting species (Johnson, 1997).

By studying the compatibility between transgenic corn and non-target insects under laboratory conditions, we can better assess these interactions and improve the knowledge about transgenic-corn agroecosystem and eventually develop pest management tactics, where warranted. This study indicates that transgenic corn leaves do not have toxic effects on one of the most common sucking insects of corn and on its predator, green lacewings. Thus the tritrophic relationships among these transgenic plants, non-target phytophagous insects and their predators are not affected by the presence of Bt-toxin, or this toxin does not pass into the phloem.

Because of the relatively high number of natural enemies of the ECB recorded in different conditions and locations (Martelli, 1938; Jarvils & Guthrie, 1987; Coll & Botrell, 1991), and because of the many different insects which interact with corn or with ECB, further studies are necessary to investigate other tritrophic relationships, especially beneficial, for future integrated pest management systems that employ host plant resistance and other biological methods to control corn pests.

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