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B. MANACHINI

Effects of transgenic corn on *Lydella thompsoni* Herting (Diptera Tachinidae) parasitoid of *Ostrinia nubilalis* Hb. (Lepidoptera Crambidae)(*)

Abstract - Field trials and laboratory bioassay were used to evaluate the effects of transgenic corn, that produces the CryIAb toxin of *Bacillus thuringiensis*, on non the target species *Lydella thompsoni* Herting, parasitoid of *Ostrinia nubilalis* Hb.. In 1998 and 1999 the larvae of *O. nubilalis* were collected from Bt corn and non Bt-corn at 9 geographical sites. Considering the total larva amount, the European corn borer (ECB) from transgenic corn showed lower parasitism levels in terms of both percentage and absolute parasitoid number. In fact the statistical analysis demonstrates that, over the two years, there was a difference in parasitism rate by *L. thompsoni* in Bt maize versus the near isogenic non-Bt maize. However four localities had higher parasitism percentages for Bt corn larvae. It was also shown that there was a significant variation in the parasitism rate in the fields and field locations. There were no significant differences in parasitism in the two years, and none of the interactions were significant. The biology of *L. thompsoni* parasitizing ECB larvae from isogenic and transgenic corn was investigated. The parasites emerging from borers reared on the two maize hybrids showed no significant difference in lifespan or adult longevity. However as corn borer populations decline in Bt corn and this study report a decline as well of the number of parasitoid, refuge areas, may moderate these indirect effects and they should considered also for conserving natural enemies.

Riassunto - *Effetti del mais transgenico su* Lydella thompsoni *Herting (Diptera Tachinidae) parassita di* Ostrinia nubilalis *Hb. (Lepidoptera Crambidae).*

Tramite studi di campo e biosaggi di laboratorio, sono stati valutati i possibili effetti del mais transgenico, che esprime la tossina CryIAb derivata dal *Bacillus thuringiensis* var. *kurstaki*, sul parassitoide di *Ostrinia nubilalis* Hb., *Lydella thompsoni* Herting. Negli anni 1998-1999 sono state raccolte in 9 differenti località larve di piralide da campi di mais transgenico ed isogenico e ne è stato valutato il tasso di parassitismo. Considerando il numero totale di larve di piralide raccolte, quelle prelevate nei campi di mais transgenico presentavano un minor numero di parassitoidi, sia in termini assoluti che in percentuale. Infatti le analisi statististiche hanno messo in evidenza una differenza significativa nel tasso di pa-

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rassitizazione di *L. thompsoni* rispetto alle piralidi rinvenute nei campi di mais transgenico ed isogenico. Tuttavia in 4 località la percentuale di parassitismo era maggiore per le larve provenienti dai campi di mais Bt. I risultati evidenziano anche una differenza statisticamente significativa nei livelli di parassitizzazione rispetto ai campi e alle località. Nessuna differenza è stata invece riscontrata in relazione agli anni. Inoltre è stata studiata la biologia di *L. thompsoni* che parassitizzava larve provenienti dai due tipi di campi di mais. Non sono state registrate differenze statisticamente significative, sia per quanto riguarda il tempo di sviluppo che la longevità degli adulti del tachinide emerso dalle piralidi che si erano nutrite di mais transgenico o isogenico. Tuttavia, poiché la popolazione di piralide è quantitativamente inferiore nei campi di mais transgenico e questo studio riporta un declino in termini numerici del parassitoide, sono effettuate alcune considerazioni sulle aree rifugio impiegate anche per la conservazione dei nemici naturali.

Key words: Bt corn, non target effects, parassitoid, CryIAb.

INTRODUCTION

The use of Bt transgenic plants raises some questions on the possible direct and indirect environmental effects, particularly on non-target organisms. Different experimental laboratory data have been published. Some researchers have studied the effects of direct feeding on Bt plants of non-target arthropods, e.g., Pilcher *et al.* (1997) studied, in the laboratory, the effects of Bt corn pollen containing the protein Cry1Ab on three species of predators *Coleomigilla maculata* DeGeer (Coleoptera: Coccinellidae), *Orius insidiosus* Say (Heteroptera: Anthocoridae) and *Crysoperla carnea* Stephens (Neuroptera: Chrysopidae). These insects feed on the eggs and young larvae of *Ostrinia nubilalis* Hb., and also on pollen, but there have been no reports of harmful effects on the pre-imaginal development, or the survival, of these insects fed on Bt pollen. Other studies have analyzed the possible tritrophic interactions on predators fed on prey, that had itself been fed on Bt plants. Dougan *et al.* (1996) tried to determine the effect on the biological cycle of *Hyppodamia convergens* Guerin-Meneville (Coleoptera: Coccinellidae), fed on the aphid *Myzus persicae* Sulzer, itself fed on leaves of transgenic potatoes that contain the toxin CryIIIA. The experiment did not highlight any differences that might demonstrate the potential harmfulness of the transgenic plant on this predator through its prey. On the other hand, Hilbeck *et al.* (1998) obtained results that indicate possible negative effects on *C. carnea* fed on larvae of *O. nubilalis* and of *Spodoptera littoralis* Boisduval. The mortality of the larvae of *C. carnea* was higher when fed on prey that had grown for 24-72 hours Bt corn leaves. These authors underline that it could have been the result of a combined effect between indirect exposure to the toxin and nutritional deficiency caused by a weak prey. Other studies have indicated possible effects of Bt plants on certain non target species (Losey *et al.,* 1999; Hilbeck *et al.*, 1999; Meier & Hilbeck, 2001). In fact, although the toxin CryIAb seems to be active only in particular taxonomical groups, little is still known about the effects of Bt corn on non-target organisms. The effects of transgenic corn on non target insects of corn can be direct, or else indirect by affecting the different trophic levels. It is particularly important to verify whether there are any negative effects of transgenic corn on the natural antagonists of the ECB. Such an influence can appear in the relationship of the prey-predator, prey-parasite, and even directly on the parasite or predator itself, lowering its fertility, ability to search, fitness, etc. The need for a study into this is even more evident if we think of the role natural enemies play in controlling the populations of harmful insects and in development of forms of resistance. *Lydella thompsoni* Herting (Diptera Tachinidae), a parasite of *O. nubilalis,* is quite widespread throughout eastern and southern Europe (Maini, 1973; Maini & Burgio, 1990; Manachini, 2000). In Europe the level of parasitism found in the ECB of *L. thompsoni* varies greatly with percentages ranging from 0.90% to 59% (Galichet *et al.*, 1985; Barbattini, 1989; Eizaguirre *et al.*, 1990; Grenier *et al.*, 1990; Cagan *et al.*, 1999; Manachini, 2000). This parasitoid was introduced into the United States for the biological control of the ECB as early as 1928, and for many years it was the major biological factor to contain phytophagous insects, with parasitism percentages reaching, in some areas, even 75% for the second generation of Crambidae (Jarvis & Guthrie, 1987). The fertilized, and ovoviviparous, female of *L. thompsoni* lays larvae in the holes made by *O. nubilalis* in corn stalks, or on the extruding excrement (Galichet *et al.*, 1985; Mahr, 1999). The larva of the ECB parasitised do not seem to suffer due to the *endophagous* larvae, which feed on haemolymph. Thus Bt corn can influence this tritrophic relationship at different levels. Even though *L. thompsoni* does not have a determining role in containing *O. nubilalis,* the factors influencing its parasitism level and the reasons for the decrease in its population in the United States and in some European areas (Jarvis & Guthrie, 1987; Burbutis *et al.*, 1981; Mason *et al.*, 1994; Jones *et al.*, 1995; Mahr, 1999) require further studies, especially in view of the possible use of genetically modified plants also in Europe. In fact, as there is such a close relationship between the parasite, the phytophagous larvae and the vegetation, any changes in one or more parameters linked to the host, the phytophagous or the parasite can influence this tritrophic relationship.

MATERIALS AND METHODS

Mature larvae of the fifth age of the *O. nubilalis* in isogenic and transgenic cornfields were collected directly from the corn plants (maize Bt 176), dissecting the stalk immediately before crop harvesting. The pupal cases of Diptera found in the field were identified following the indications of Grenier and Nardon (1983). Three hundred larvae from the isogenic corn crop and three hundred from the transgenic crop were collected at each locality. Each field was subdivided into 6 replicates and 50 larvae were collected randomly from each plot. In 1999 and 2000 the level of *L. thompsoni* parasitism was measured for ECB (European corn borer) sampled at 9 different localities in North Italy (Fig. 1).

After the over-wintering period the larvae of *O. nubilalis* were placed in climatic

Fig. 1 - Sampling localities in 1999 and 2000: 1. Santhià (TO); 2. Sillavengo (NO); 3. Cornegliano (LO) only for collection of 1999; 3'. Brignano (BG) only for collection of 2000; 4. Grumello (CR); 5. Cologna (RO); 6. Montebello (VI); 7. Minerbe (VR); 8. Vedelago (TV); 9. Morsano (PN).

cells (18:6 day:night, temperature 25°C for daylight hours and 18°C for night time hours, humidity 80% ± 5). Each day the eventual coming out of the pupae of the diptera was checked and recorded. The time that elapsed from the pupa to the emerging of the adult from the puparium was calculated as well as its adult life span . The diptera adult, kept in the above-mentioned conditions, was fed only a honey solution (10%).

Analysis of the data

The percentage of parasitism of *L. thompsoni* was calculated on the basis of the number of pupae emerging from the phytophagous larvae after the wintering period and from the few specimens found in the field itself. The data were analyzed as a contingency table, using a log-linear model using year, field and maize variety as structural variables and parasitism (parasitized or not) as the response. Data regarding the biology of *L. thompsoni* were analyzed using the two-way ANOVA test with the program SPSS 9.0 in relation to the corn hybrid.

RESULTS AND DISCUSSION

Natural parasitism

In all the considered fields *L. thompsoni* was the most widespread parasite, with the exception of a few specimens of *Eriborus terebrans* Gravenhorst (Hymenoptera Ichneumonidae) found in the province of Rovigo in 1999; it was the only entomoparasite non-oophagous of the ECB.

The experiment was carried out on mature phytophagous larvae, and some whole or empty pupae were found during the dissection of the stalks (tables 1 and 2). The results revealed no notable differences between the numbers of pupae found during the ECB sampling, in transgenic and isogenic corn fields, and after the overwintering period. In total, 278. *L. thompsoni* came out of overwintering *O. nubilalis*; 122 the first year and 156 the second year. On the whole, the ECB from the isogenic corn fields were more greatly parasitized than the ECB from the Bt hybrid, with 177 pupae and an average of 9.0 in the isogenic case and a total of 111 pupae and an average of 6.2 in the second the Bt hybrid.

Table 1 shows the data relative to the first year of experimenting. Note the clear differences in the abundance of *L. thompsoni* in the different areas, while there are no significant discrepancies in the number of parasites found in the fields of transgenic and isogenic corn in the same area. Furthermore the abundance of *L. thompsoni* in the fields of Bt and isogenic corn showed no statistically significant differences ($F=0.59$, $df=1,18$, $p=0.45$), though the number of *L. thompsoni* found in the fields of transgenic corn was a little over half that found in the isogenic corn fields.

Table 1 - Parasitism of O. nubilalis *collected in isogenic and transgenic corn crops during Autumn 1998, by* Lydella thompsoni.

Sampling site	Isogenic				Transgenic			
	Avarage ECB per stalk	Pupae Autumn 1998	Pupae Spring 1999	% Parasitism	Avarage ECB per stalk	Pupae Autumn 1998	Pupae Spring 1999	% Parasitism
1 Santhià (TO)	0.85	0	11	9.56	2.55	0	5	3.88
2 Sillavengo (NO)	0.97	2	20	13.92	3.01	1	8	7.34
3 Cornegliano (LO)	0.51	0	1	1.92	2.02	0	2	3.39
4 Grumello (CR)	0.72	1	5	4.41	2.00	0	7	6.19
5 Cologna (RO)	0.91	0	21	10.60	2.85	1	10	6.45
6 Minerbe (VR)	0.45	0	5	3.22	1.98	0	3	3.16
7 Montebello (VI)	1.01	1	3	5.88	3.72	0	2	3.85
8 Vedelago (TV)	0.88	0	4	3.41	2.55	0	2	3.28
9 Concordia (PN)	1.00	1	13	10.00	2.98	2	10	9.01
Total	0.81	5	83	7.72	2.63	4	49	5.54

Table 2 - Parasitism of O. nubilalis *collected in isogenic and transgenic corn crops during Autumn 1999, by* Lydella thompsoni.

Sampling site	Isogenic				Transgenic			
	Avarage ECB per stalk	**Pupae Autumn 1999**	**Pupae Spring 2000**	**% Parasitism**	**Avarage ECB per stalk**	**Pupae Autumn 1999**	**Pupae Spring 2000**	**% Parasitism**
1 Santhià (TO)	0.91	1	9	11.24	2,75	1	5	5.56
2 Sillavengo (NO)	0.96	2	21	13.53	3,55	2	9	10.97
3 Brignano (BG)	0.75	0	4	3.48	2,45	1	2	1.87
4 Grumello (CR)	0.72	0	5	3.76	2,10	0	8	6.96
5 Cologna (RO)	0.99	3	22	13.51	2,85	2	15	8.38
6 Montebello (VI)	0.41	0	2	2.04	2,01	0	6	5.88
7 Minerbe (VR)	1.15	0	6	4.00	3,68	0	2	1.34
8 Vedelago (TV)	0.98	1	7	6.67	2,57	0	2	1.63
9 Morsano (PN)	1.15	2	14	8.12	2,96	0	15	8.11
Total	0.89	10	94	6.87	2.77	7	62	4.88

This difference was mainly due to the lower number of Diptera in the transgenic cornfields in Novara and Rovigo than in the corresponding isogenic ones.

A similar situation was observed in the second research year. It can be seen (Table 2) that the number of *L. thompsoni* found in the fields of transgenic corn was lower than that for isogenic corn, however in some areas a greater number of *L. thompsoni* was found in the fields with the genetically modified hybrid (Grumello and Montebello).

Employing a log-linear model, for the two considered years, using year, field and maize variety as structural variables and parasitism (parasitized or not) as the response, there was a difference in parasitism rate by *L. thompsoni* in Bt maize versus the near isogenic non-Bt maize (Table 3). Also demonstrated was a significant variation in parasitism rate among fields or field locations; while no significant differences in parasitism in the two years, and none of the interactions were significant (Table 3).

The percentages of parasitism varied from 1.34% (Minerbe, transgenic, seen in 2000) to 13.92 (Novara, isogenic, seen in 1999), confirming the spatial variability of the parasitizing of *O. nubilalis* by *L. thompsoni* as already highlighted in previous studies (Maini, 1976; Platia & Maini, 1976; Barbattini, 1989; Manachini, 2000). Its presence in the Bologna area was confirmed by Maini (1975) who found it again with a variable degree of parasitizing from 9.96% on the plain to 16.27% in the hills and mountains. In other experiments (Platia & Maini, 1975) *L. thompsoni* was the most frequent parasite of larvae in the Forlì area with parasite percentages of 11.67% on the plains and 20.91 in hilly and mountain areas.

In the first year, the highest parasite levels were recorded in Novara and Porde-

Table 3 - Maximum likelihood analysis of deviance table for log-linear analysis of the effect of field, year and maize variety (Bt or non-Bt) on parasitism rate by Lydella thompsoni.

Source	**df**	g^2	**p**
Variety	1	4.24	0.0395 *
Field	8	50.69	<.0001**
Year	1	0.08	0.7737
Year*Field	8	3.36	0.9098
Field*Variety	8	11.83	0.1590
Year*Variety	1	0.13	0.7189
Year*Field*Variety	8	4.11	0.8471

none, followed by Torino and Rovigo. Whereas Novara had the highest percentage in the isogenic field of corn, Pordenone, Lodi and Cremona reported the opposite. All the other places recorded higher parasite percentages in the isogenic cornfields, with quite a large gap in the north west provinces. On average, the percentage of parasitism found for *O. nubilalis* was 6.9% in isogenic corn and 5.5% in the transgenic. Also in the second year the highest percentages of parasitism were recorded in Novara, Torino and Rovigo (Table 2). Pordenone had a slight decrease with respect to the first year, the situation in the transgenic and isogenic cornfields being similar. While in Piacenza and Vicenza the level of parasitism was greater in the fields of transgenic corn, in the other places studied the greatest number of pupae came from the ECB larvae collected in the traditional fields and, as in the first year, the difference was more noticeable in Torino, Rovigo and Treviso (Table 2).

Figure 2 shows the average of the percentages of *L. thompsoni* coming out of the larva in 1999 and in 2000. It interesting to note that, also considering both years, the isogenic fields showed a slightly higher percentage of parasitism than the transgenic, however 4 of the 9 localities had a higher parasitism level in the Bt corn.

However, the average percentage of parasitism in the isogenic cornfields was higher, 10.68% against 8.36% in the fields for *L. thompsoni* parasiting ECBs from Bt corn-fields.

The mortality rate for the period after the overwintering period is interesting: the ECB that had survived mortality on genetically modified corn was higher (Manachini, 2002) than on isogenic corn. However, smaller numbers of *L. thompsoni* emerging from the pupae from the first group does not necessarily mean a lower rate of parasitism in the field. Indeed, as the percentages were mainly calculated on pupae found after wintering, it could be possible that the ECB parasites were weakened by the intake of toxin protein and were not able to complete the period of diapause, resulting in a higher death rate. It would therefore be advantageous to check the presence of *L. thompsoni* directly in the ECB larvae collected in the field, however this is extremely

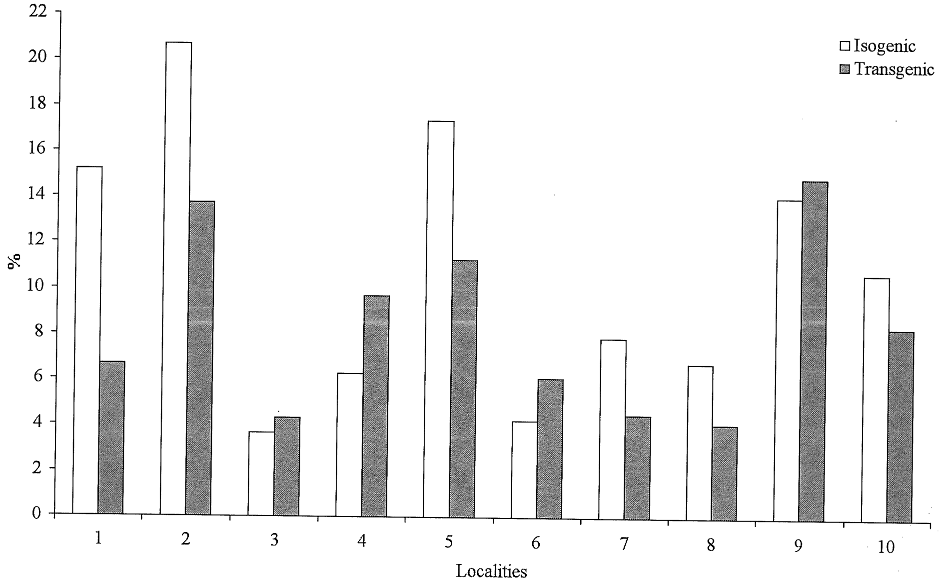

Fig. 2 - Average of parasitsm percentage, by *Lydella thompsoni,* parasitoid of *Ostrinia nubilalis,* collected in isogenic and transgenic cornfields during the year 1999-2000. 10) Total average of percentages.

difficult as it is almost impossible to recognize the parasitized larvae until the tachinid is almost a pupa.

The spatial variability in the parasitism rate of *O. nubilalis* by *L. thompsoni* (Table 3) has many different causes, one of the most important surely being asynchrony in the host and parasite cycle. However, it seems that the parasite can complete the first generation on various alternative hosts. Galichet (1986) captured *L. thompsoni* in the delta of the Rhone from April to October, with a peak in September. The larvae of the spring generation of the parasite develop on the larvae of *Archanara geminipuncta* Haworth, *A. dissoluta* Treitscke, *A. spargani* Esper and *Nonagria typhae* Thunberg, while the second part of the cycle takes place on *O. nubilalis* and *Sesamia nonagroides* Lefebre. In this region *L. thompsoni* parasitizes at least four host species in spring as well as another two in summer. In this way the cycle of the tachinid adapts to the area through multiple host and parasite interactions. A similar phenomenon has been noted in other areas (Galichet & Radisson, 1977; Figueiredo & Araùjo, 1996; Cagan *et al.*, 1999), and this could partly explain the diverse abundance of *L. thompsoni* in North Italy where the density of these lepidopters varies according to specific habitat availability. This means that any change in the agroecosystem conditions can affect the relationship between the tachinid and the Crambidae. In this context transgenic corn does not seem to have any significant effect, though a decrease in host numbers could lead to a reduction in the number of parasites, even if survival of the latter is not linked only to the existence of ECBs. Thus the introduction of genetically modified plants could lead to this hoped for decrease in ECB but it would, unfortunately, also decrease, the number of its natural enemies.

The introduction of refuge areas can be seen not only as a means of dissolving phytophagous resistance but also as a means of preserving this parasite.

Effects of CryIAb on the development of L. thompsoni

The average time lapse between the end of diapause of the ECB larvae and the emerging from the pupa was slightly longer for *L. thompsoni,* the parasite of *O. nubilalis* fed on Bt corn in the first year of the experiment (Tables 4 and 5) and the second (Tables 6 and 7). In fact *L. thompsoni* needed approximately one extra day to develop from the Bt corn ECB survivors (13.72 days vs 15.39 for those from *O. nubilalis* fed isogenic corn). However, the analysis of the variance did not highlight any statistically significant differences, neither on considering the years separately (1^{st} year $F= 0.10$, $df=1,17$, $p= 0.75$; 2^{nd} year $F= 2.47$, $df=1,17$, $p= 0.13$) nor on analyzing the results all together (year $F= 0.54$, $df=1,34$, $p= 0.46$).

Some pupae appeared immediately after the overwintering period, while there were cases where becoming a pupa took longer than one month. This variability can probably be linked to the degree of development of the parasite when it was collected and to individual variability.

It is interesting to note that normally, after the appearance of the pupa, development carried on to the end; in fact the number of pupae from which no adult

Table 4 - Development of Lydella thompsoni *emerging from the larvae of* Ostrinia nubilalis *collected in isogenic of corn fields during 1999.*

	N°pupae	Mxpa	Apa	Mnpa	NSF	Mxae	Aae	Mnae	Longevity*
1 Santhià (TO)	11	19	12.12	7	0	11	8.87	5	12.3
2 Sillavengo (NO)	20	31	5.05	1	3	14	11.94	9	18.3
3 Cornegliano (LO)	1	26	26	26	0	11	11	11	14.0
4 Grumello (CR)	5	15	14.08	14	0	11	6.02	3	11.2
5 Cologna (RO)	21	32	11.5	2	3	13	6.72	3	15.1
6 Montebello (VI)	3	39	33.33	25	0	11	9.33	7	10.5
7 Minerbe (VR)	5	16	10.41	3	0	10	8.6	7	11.4
8 Vedelago (TV)	4	20	11.5	6	0	10	8.75	7	13.2
9 Concordia (PN)	13	38	16.07	8	1	11	8.91	6	14.5
Average		26.22	15.56	10.22	0.77	11.33	8.90	6.44	13.38

Table 5 - Development of Lydella thompsoni *emerging from the larvae of* Ostrinia nubilalis *collected in transgenic corn fields during 1999.*

	N°pupae	Mxpa	Apa	Mnpa	NSF	Mxae	Aae	Mnae	Longevity*
1 Santhià (TO)	5	17	12.60	0	0	11	7.40	6	11.9
2 Sillavengo (NO)	8	14	5.15	3	1	14	11.85	6	18.3
3 Cornegliano (LO)	2	43	22.00	1	1	11	11	11	12.0
4 Grumello (CR)	7	35	10.57	1	0	21	10.83	4	12.1
5 Cologna (RO)	10	36	15.5	3	1	10	4.55	3	14.9
6 Minerbe (VR)	3	25	17.70	11	1	11	10.50	10	10.0
7 Montebello (VI)	2	42	39.00	36	0	11	10.50	10	10.5
8 Vedelago (TV)	2	14	12.00	10	0	7	6.50	6	13.2
9 Concordia (PN)	10	25	17.70	11	0	11	8.88	6	14.7
Average		27.89	16.91	11.89	0.44	11.89	9.11	6.89	13.07

Table 6 - Developmennt of Lydella thompsoni *emerging from the larvae of* Ostrinia nubilalis *collected in isogenic corn fields during 2000.*

	N°pupae	Mxpa	Apa	Mnpa	NSF	Mxae	Aae	Mnae	Longevity*
1 Santhià (TO)	9	13	10.51	6	0	11	8.87	6	14.3
2 Sillavengo (NO)	21	29	8.90	2	1	15	12.02	8	18.0
3 Brignano (BG)	4	17	13.50	8	0	11	9.50	8	13.2
4 Grumello (CR)	5	15	13.89	13	0	12	8.45	5	15.2
5 Cologna (RO)	22	28	11.48	5	3	12	7.21	5	16.7
6 Minerbe (VR)	6	17	10.56	3	0	11	8.67	7	16.3
7 Montebello (VI)	2	15	9	3	0	10	8.65	6	11.6
8 Vedelago (TV)	7	19	11.01	6	0	11	8.91	7	15.7
9 Morsano (PN)	14	29	17.05	7	3	14	9.06	4	13.7
Average		19.27	12.38	7.45	0.63	12.09	9.40	7.09	14.59

emerged was always very small. Furthermore the number of pupae was usually lower in the cases of parasites developed on ECBs fed on Bt corn.

The time of development from the appearance of the pupa to the adult emerging from the puparium was approximately 9 days for parasites emerging from ECBs collected from the isogenic and transgenic cornfields. However, there were cases where 21 days were necessary (Grumello, Table 5) and others where 3 days sufficed (Tables 4 and 5).

Table 7 - Development of Lydella thompsoni *emerging from the larvae of* Ostrinia nubilalis *collected in transgenic corn fields during 2000.*

	N°pupae	Mxpa	Apa	Mnpa	NSF	Mxae	Aae	Mnae	Longevity*
1 Santhià (TO)	9	16	15.50	15	0	12	10.00	8	11.5
2 Sillavengo (NO)	21	17	15.50	14	0	14	11.00	8	15.5
3 Brignano (BG)	4	18	14.00	10	0	10	8.50	7	13.0
4 Grumello (CR)	5	31	16.13	9	1	11	8.53	5	15.5
5 Cologna (RO)	22	16	11.50	7	0	11	9.00	7	16.5
6 Minerbe (VR)	6	21	16.80	8	0	13	8.60	4	13.7
7 Montebello (VI)	2	16	9	3	1	11	8.50	6	10.6
8 Vedelago (TV)	7	18	16.00	14	0	11	8.50	6	12.5
9 Morsano (PN)	14	14	9.00	5	0	12	8.00	5	14.6
Average		18.27	13.93	10.00	0.18	11.99	9.15	6.45	13.91

Mxpa = maximum number of days for the appearance of the pupa from overwintering ECB larvae after the diapause, **Apa** = average of days from the end of the diapause to the emergence of the pupa, **Mnpa** = minimum number of days for the emergence of the pupa from overwintering ECB larvae, **NSF** = number of pupa from which no adult emerged, **Mxae** = maximum number of days from the appearance of the pupa to adult emergence of the adult, **Aae**= average number of days from the appearance of the pupa to the emergence of the adult, **Mnae** = minimum number of days from the appearance of the pupa to the emergence of the adult, *= average longevity of the adult calculated in days.

No significant differences were noted in the longevity of adults emerging from the pupae coming from the ECB fed the two different corn hybrids. The adults of *L. thompsoni* coming from fields of isogenic corn lived slightly longer. In the first year the adults emerging from ECBs fed on isogenic corn lived, on average, 13.38 days while the adults from ECBs fed on Bt corn lived 13.07 days. In the second year of the experiment the former lived, on average, 14.59 days, the latter 13.91. The highest longevity was recorded for adults coming from cornfields sown, in the first year, with both the hybrid Bt176 and the normal (18.3 days). This figure was also confirmed the second year, although the adults emerging from ECBs from the genetically nodified hybrid lived, on average, 3 days less than other diptera in the same area. The average life span of the adults was about 13 days for the first year of the experiment and about 14 for the second. This increase can probably be correlated to improved breeding techniques and the dexterity gained during the experiment. Some differences do appear in the length of the cycle, though these are not statistically significant. Thus, from the results obtained, it would seem that the development of *L. thompsoni* is in no way affected by the fact that it parasitizes *O. nubilalis* fed on either conventional or transgenic corn.

CONCLUSIONS

The present research indicates that, over the two years, there was a difference in parasitism rate by *L. thompsoni* in Bt maize versus the nearby isogenic non-Bt maize. In fact the parasitism rate was higher in the isogenic corn filed. However because it was also demonstrates that there is significant variation in parasitism rate among fields or field locations, and in some localities the parasitism was higher in Bt corn fields, it should not concluded that transgenic corn does not have any noxious effects, neither direct nor indirect, on the activity of parasitism of *L. thompsoni.* Moreover not deleteriuos effect where recorded on the biology of *L. thompsoni.* The results could, in part, resolve the problems Emden (1999) foresaw about the possible repercussions of genetically modified plants on tritrophic relationships. This author reported how alterations to plants through genetic engineering has changed the capacity of such plants to release terpene and sesquiterpene. These substances are often indicated as useful traces for parasites to single out the habitat of their host or, in some cases, they can even be a real call for help on the part of the plant. However, no reference is made to Bt corn while there is a statement that this phenomenon has shown itself for the selection of the character regarding the DIMBOA effect.

Furthermore, it must not be forgotten that Hsiao *et al.* (1966) studied, in the laboratory, factors influencing the search for a host and the egg laying propensity of the adult females of this fly. They also experimented, with artificial parasitism, the development of *L. thompsoni* in *Galleria mellonella* L. and in other secondary hosts. Their results showed that the gnawed holes and fecal waste from these holes, due to *O. nubilalis* in the corn, is extremely attractive; however also other material from maize or different monocotyledons eroded by other lepidoptera like *Archanara unipuncta* Haworth and *A. oblonga* Grt. and *Papaipema* sp provokes the stimulus of egg laying in tachinid females. As far as artificial parasiting is concerned *L. thompsoni* has always been able to develop in the larvae of *G. mellonella,* however there has never been any positive outcome in other hosts like *Pieris rapae* L., *A. unipuncta* or *Tenebrio molitor* L.

Chafaux *et al.* (2002) found that the percentage of parasitism was statistically lower in the case of ECB from transgenic maize. They supposed that the differences were not imputable to asynchrony of the cycle of the Diptera comparing to the host, as studied previously by Plantevin and Grenier (1990). Many studies have shown that Bt Cry proteins are highly selective in killing the larvae of moths, but many others report that transgenic corn does not, in any way, affect beneficial insects including honey bees, lady beetles, green lacewing larvae, spiders, pirate bugs or parasitic wasps (Johnson *et al.,* 1997; Lozzia *et al.,* 1998; Manachini *et al.*, 1999). However there could be indirect effects on the natural enemies of the European corn borer, its predators, parasites and pathogens could decline as corn borer populations decline. In fact Sandlan *et al.* (1983) found an evident density-dependent response of the parasite, although there was no difference among the 4 maize genotypes with regard to parasitism rate.

The results of the present study show that *O. nubilalis* fed on Bt corn was not a secondary food for the tachinid, in that the tachinid identified its host in the Bt corn cornfield just as it did in isogenic cornfields; if this were not so then all the localities would show higher parasitism in the isogenic corn fields. However it should consider that even if statistical analysis did not show differences in the lifespan of the parasitoid, it is difficult to be sure that the 2 days of delay does not mean anything for the biology of the tachinid. Anyway considering all different variables that can affect the tachinid, it coul be considered that the tritrophic relationship between *O. nubilalis* and *L. thompsoni* does not seem to be influenced by the presence of Bt corn. However there still remains the problem that the presence of the parasite is, in any case, linked to the host and this host should not be completely eradicated from the area. Agreement with this is found in what has been set down by Integrated Pest Management programs and Bt Resistance Management. For example Andow (1996) reported a study focused on three natural enemies of maize phytophagous insect in Minnesota; the published interpretation of the response of is consistent with the theoretical prediction of how vegetational diversity influences and eventually increases parasite populations.

It could be that refuge areas will play a role in moderating the indirect effects due to smaller host populations, but very little data is available on this subject. It is to be noted that Bt corn fits into, and complements, an integrated pest management approach to farming that includes the conservation of biological control agents.

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Dr. Barbara Manachini - Istituto di Entomologia agraria, Università degli Studi di Milano, Via Celoria 2, I-20133 Milano. E-mail: barbara.manachini@unimi.it

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