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Effects of Bt maize on non-target lepidopteran pests

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Abstract: Genetically modified (GM) maize with the insecticidal capacity of *Bacillus thuringiensis*, (Bt maize, expressing Cry1Ab) was first authorized in Spain in 1998. Since then its cultivated area has increased year by year to reach 78'000 ha in 2008, representing 21% of the total maize-growing area in Spain. In the study area (Lleida, Catalonia, NE Iberian Peninsula) it represents almost 80% of the total. Bt maize provides an effective control of two key lepidopteran pests, *Sesamia nonagrioides* (Lefèbvre) and *Ostrinia nubilalis* (Hübner). However, in addition to the two corn borers, two other non-target Lepidoptera, *Mythimna unipuncta* (Haworth) and *Helicoverpa armigera* (Hübner), cause occasional but severe damage to maize. Effects of Bt maize on these two Lepidoptera were studied in laboratory and field trials. Some larvae of both species can survive and complete development when feeding on Bt maize. Field evaluations carried out from 2005 to 2008 showed no differences in the number of *H. armigera* larvae per plant between Bt and isogenic varieties in most of the trials. In the laboratory, *M. unipuncta* showed a larval survival of 15%, which is significantly lower than that recorded in isogenic varieties. Additionally, larval development in survivors was significantly longer when they were fed Bt maize. Adults resulting from larvae developed on transgenic maize laid 13% fewer eggs than those resulting from larvae developed on isogenic maize. When they had the choice, neonate *M. unipuncta* larvae preferred first *Sorghum bicolor*, then isogenic maize plants and finally Bt plants for feeding. Recorded differential mortality caused by Bt maize on non-target Lepidoptera in comparison with targeted corn borers may affect the composition and abundance of the Lepidoptera community in maize as a consequence of Bt maize deployment.

Key words: *Helicoverpa armigera*, *Mythimna unipuncta*, transgenic maize

Introduction

Bt maize has been sown in Spain since 1998. During the period 1998-2000, the area sown with Bt plants represented 5% of the total maize-growing area. This area has increased year by year and in 2007-2009 it represented 21% of the total (MARM, 2009). From 1998 to 2005 the only Bt maize sown was event Bt176. In 2005 this event was prohibited, and from 2003 to the present the varieties sown have been based on event MON810. Bt maize provides very effective control of the two key pests in our region: the Mediterranean corn borer, *Sesamia nonagrioides* (Lep., Noctuidae) and the European corn borer, *Ostrinia nubilalis* (Lep., Crambidae), but the effect on other lepidopteran pests that occur in maize fields is not so well known. In the study area maize is attacked by two other Lepidopteran species belonging to the Noctuidae family, *Mythimna unipuncta* and *Helicoverpa armigera*, but we have few data about the effectiveness of Bt maize against them. If these two species could survive on Bt maize they would benefit from the absence of corn borers. *M. unipuncta* feeds on leaves of several non-agricultural and cultivated gramineous plants such as maize, millet and weeds. When its populations are high it can consume all the leaves of a maize field. In our region for several years we have found maize fields totally devastated by *M. unipuncta* larvae close to other undamaged field. *H. armigera* is a species that feeds on crops belonging to very different botanical families. Little is known on the damage caused by this species on maize but it is always present in low number on the ear silks. It has also been observed in the study area on sunflower and, increasingly, on alfalfa.

The aims of this work were to determine whether Bt maize was as efficient against *M. unipuncta* and *H. armigera* as it is against corn borers, whether the larvae of the two species – which feed at least partially on Bt maize – could survive, and how feeding on Bt maize affects the development of *M. unipuncta*.

Material and methods

To achieve these objectives, the effect of Bt maize on *M. unipuncta* was studied by feeding the larvae with leaves of some Bt (P67 and DKC) and their corresponding isogenic varieties (P66 and TTR, respectively) and the effect on *H. armigera* was evaluated by recording in the field larval occurrence on some Bt and non-Bt varieties over several years.

To study the effect on *M. unipuncta* larvae, a laboratory rearing was established. For this, adults were caught with light traps installed in maize fields and then taken to the laboratory. The adults were caged with 4-5 leaf-maize plants and provided with a solution of 10% sucrose as the water and food source. Neonate larvae were individually placed in transparent cylindrical containers (3 cm ø and 5.5 cm height), in which they were fed with a piece of leaf of the non-transformed commercial varieties assayed. The pieces of leaf were renewed every day. The larvae developed in the same container until pupation and mortality and the duration of the development of each instar were recorded. Pupae resulting from these larvae were sexed and kept at 8 ºC until experimental use.

The viability of adults coming from these pupae whose larvae developed on Bt maize and non-Bt maize was studied by caging the adults (10 couples) in separate cages with non-Bt maize plants, counting the number of eggs laid by females and recording egg hatching.

To determine whether *M. unipuncta* neonates show any preference for feeding among different plants, 5 couples of adults were placed in cages with 6 different potted plants: 2 pots with two Bt maize (P67), 2 pots with two non-Bt maize (P66) and 2 pots with two *Sorghum bicolor* plants. This design was repeated 6 times. After the eggs hatched, the plants on which larvae started to feed were annotated every day for two weeks.

To determine whether *H. armi*gera larvae can survive on Bt maize plants, the number of larvae on several non-commercial varieties of Bt and non-Bt maize were counted (Table 1). Experimental units were plots of 27 x 38 m^2 in size. A complete randomized block design with 3 blocks (2005) and 4 blocks (2006-2008) was used. The plants observed were chosen at random on rows 10 to 14 and at a minimum of 7 m from the edge of each plot in order to minimize plot edge effects as much as possible. Fifteen plants per plot (3 points x 5 plants/point) were inspected carefully five times in the season and the number of *H. armigera* larvae was recorded. As a significant number of *H. armigera* larvae were usually found only on the 4th and 5th sampling dates, we used only these data for statistical analysis.

Statistical analysis

A one-way (Bt *vs.* non Bt) ANOVA was used to analyze the effects of Bt maize on the variables measured. Percentages were transformed by ASIN(SQRT(%/100)) to normalize data as much as possible.

Table 1. Number of Bt and non-Bt plots and data of the visual sampling performed to evaluate the effect of Bt maize on *H. armigera* larvae. Each year 2 fields with a complete randomized block design with 3 blocks (2005) and 4 blocks (2006-2008) was used.

	2005	2006	2007	2008
Number of Bt Plots	15	16	16	8
Number of Non-Bt Plots	6	8	8	8
Sampling 1	08/03/05	07/12/06	07/18/07	06/27/08
Sampling 2	08/22/05	07/27/06	08/07/07	07/17/08
Sampling 3	09/06/05	08/11/06	08/13/07	08/07/08
Sampling 4	09/26/05	09/01/06	08/30/07	08/11/08
Sampling 5				09/01/08

Results

Figure 1 compares the percentage of mortality of *M. unipuncta* larvae fed with Bt maize *vs.* the corresponding isogenic variety: when the larvae were fed with leaves of the two transgenic varieties (DKC and P67) the resulting mortality was significantly higher than in the larvae fed with leaves of the corresponding isogenic varieties (Tietar and P66). Although the mortality of the larvae fed with leaves of transgenic plants was high, a percentage of these larvae (14% of larvae fed on DKC and 17% on P67 varieties) survived to pupation.

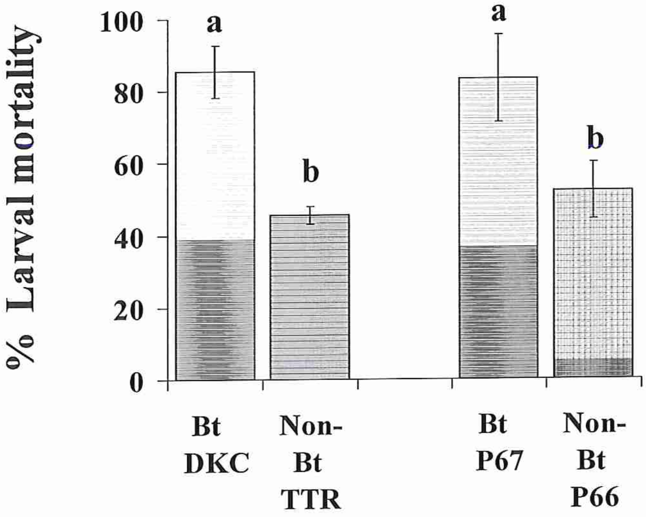

Figure 1. Percentage of *M. unipuncta* larval mortality when fed with two Bt maize varieties and the corresponding isogenic varieties. Within each treatment different letters above the columns indicate significantly different mortality.

Survival of larvae during development can be observed in Figure 2. The survival percentage of larvae fed with leaves of transgenic maize was similar in the two transgenic varieties (DKC and P67) and lower than in larvae fed with the two isogenic varieties (Tietar and P66). The highest mortality occurred in the first instar (L1) and in the last instar (the last one before pupation).

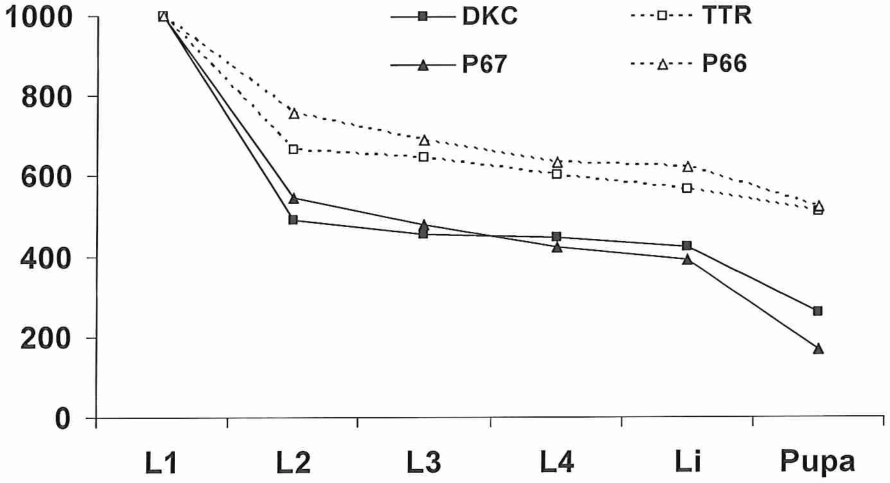

Figure 2. Survival of *M. unipuncta* larvae fed with two Bt maize varieties and the corresponding isogenic varieties during the different larval instars (Li indicates the last instar before pupation in all cases).

There were significant differences in the development duration of larvae fed with the two transgenic varieties in comparison with the larvae fed with the corresponding isogenic varieties ($p<0.05$): larvae on the two transgenic varieties showed longer development times than the isogenic ones (Figure 3).

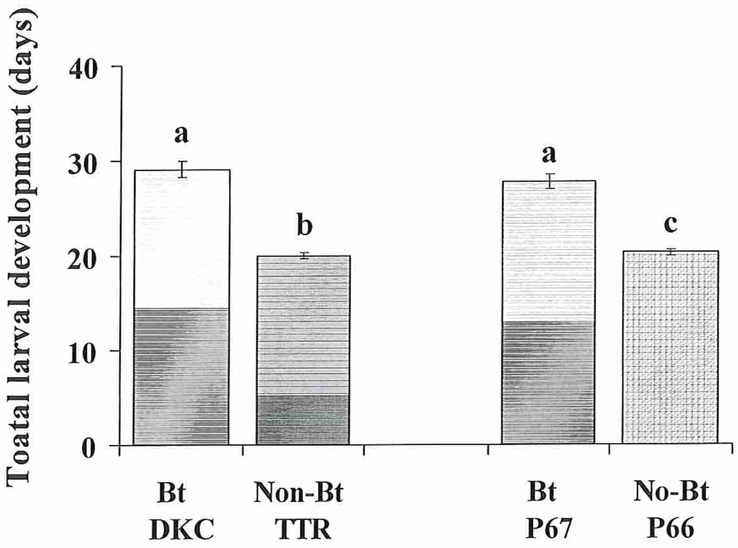

Figure 3. Duration in days of the larval development of *M. unipuncta* larvae fed with two Bt maize varieties and the corresponding isogenic varieties. Different letters above the columns indicate significantly different mortalities.

The majority of larvae fed with leaves of the two non-Bt varieties (P66 and Tietar) pupated after the 5th larval instar (Figure 4); none of them reached the 8th instar. None of the larvae fed with leaves of the Bt varieties (P67 and DKC) pupated after the 5th instar; the majority of them pupated after the 7th instar, and some after the 8th instar.

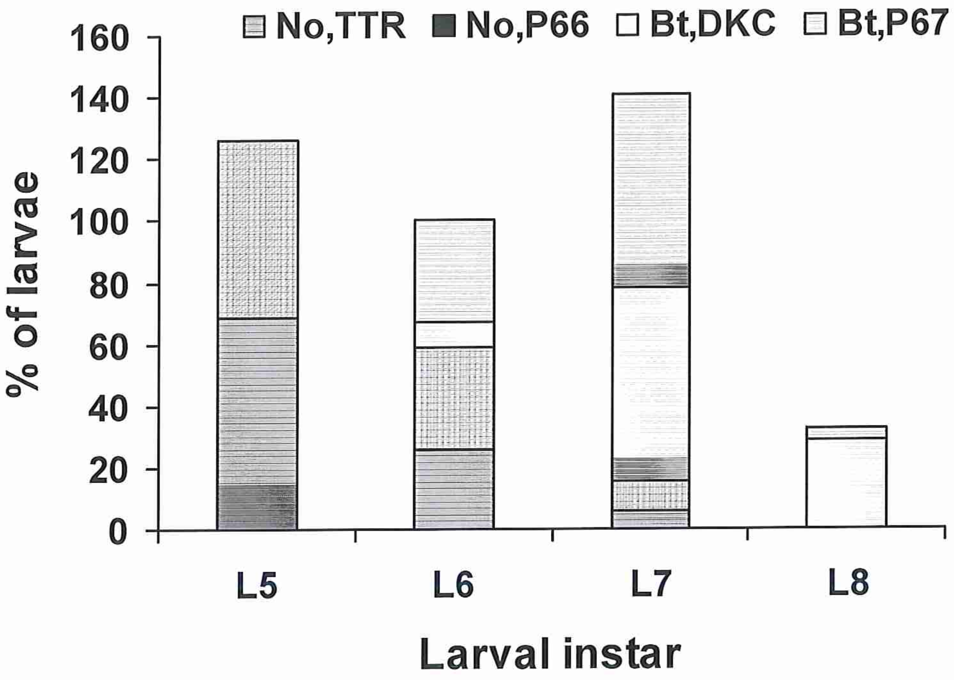

Figure 4. Percentage of *M. unipuncta* larvae that pupate after the 5th, 6th, 7th or 8th instar when fed on different Bt (Bt) or non-Bt (No) varieties.

The number of eggs per female coming from larvae fed with non-Bt maize *vs.* Bt maize can be seen in Figure 5. Females coming from larvae fed on Bt maize mated and laid viable eggs, indicating that larvae that survive on Bt maize can reproduce.

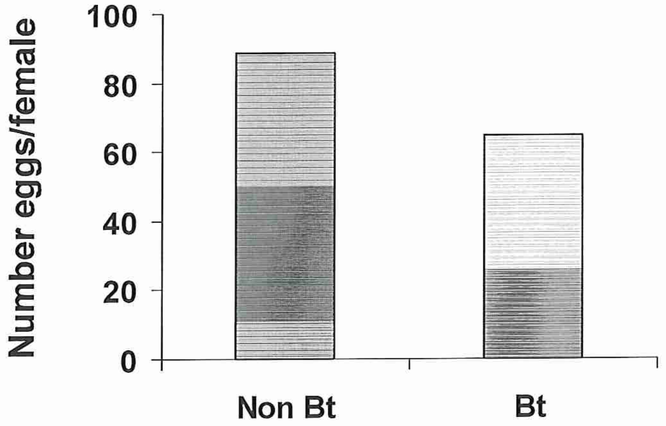

Figure 5. Number of eggs per female laid by adults of *M. unipuncta* that developed from larvae fed on the Bt and non-Bt maize varieties.

Host plant preference: Neonate larvae coming from *M. unipuncta* adults placed in cages with pots with *Sorghum bicolour* plants, Bt (P67) maize plants, and non-Bt (P66) maize plants started to feed on *S. bicolor* plants; when they finished with the leaves of this host, they moved to non-Bt maize and only when all leaves of this host plant were finished did they move to the Bt maize, whose leaves they also finished. These results are similar to the behavior of *M. unipuncta* larvae observed in the field. When one field is sown with a Bt (MON810) variety and an adjoining one with a non-Bt variety, the first suffers extremely high damage by *M. unipuncta*. When most of this field has been exhausted, the larvae start to feed on the leaves of the second field.

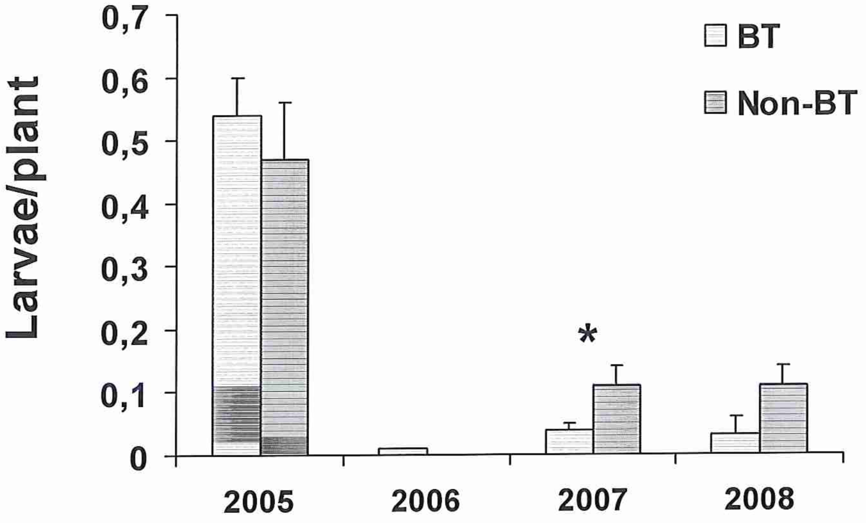

Figure 6. Number of larvae of *H. armigera* per plant in plots sown with Bt and non-Bt varieties that were monitored in 2005, 2006, 2007, and 2008.

Figure 6 shows the differences in the number of *H. armigera* larvae between Bt and non-Bt maize at the fourth sampling date in 2005, 2006, 2007, and 2008. The highest number of larvae per plant was recorded in 2005, when 50% of the plants had one larva, whereas in 2006 there were almost no plants with larvae. Only in one year, 2007, were significantly fewer larvae on non-Bt plants. These results – particularly those of 2005 – show that *H. armigera* larvae can feed and survive on some Bt maize varieties.

Discussion

The efficacy of Bt maize plants against corn borers has been widely studied, and among the most recent works are those of Huang *et al.* (2006) and Van den Berg and Van Wyk (2007). However, there are fewer works concerning the effect of Bt maize on other lepidopteran maize pests such as leaf and silk feeders. Pilcher *et al.* (1997) evaluated the efficacy of Bt maize with event 176 against *M. unipuncta* and against *H. zea* and obtained in the field similar results to those of this work. Although mortality was higher in *M. unpuncta* larvae fed on Bt maize than in those fed on the isogenic maize, 15% of the larvae reached pupation, but with longer developmental times. An absence of differences in the number or larvae per plant on Bt and isogenic varieties was also reported by Pilcher *et al.* (1997) for *H. zea*. More recently, Schaafsma *et al.* (2007) evaluated the effectiveness of three Bt corn events (176 and

Mon 810 with the Cry 1Ab toxin and TC1507 with the Cry 1F toxin) against *M. unipuncta*; they observed considerable plant damage on the three varieties assayed but did not record the larval survival rate. Our study shows that larval mortality occurred mainly just after the first and the last larval instars, being low in the intermediate instars. Larvae fed on the Bt maize took longer to complete development and needed more instars to pupate. These features are a common response to low quality, as stated by Esperk *et al.* (2007). To plan strategies for Bt-resistance prevention in insect pests, it is important to know the performance of the larvae surviving Bt maize. In the case of *M. unipuncta*, adults resulting from larvae fed on transgenic plants laid viable eggs though in lower numbers than those fed isogenic maize. Several authors (e.g. Van der Berg and Van Wyk, 2007) have studied females oviposition choice between Bt and non-Bt maize with *Sesamia calamistis*, but there are very few works that have studied the behavior of neonate larvae. In our work we demonstrate that when they have the choice of host plant, neonate *M. unpuncta* larvae choose first *Sorghum bicolor*, then isogenic maize plants, and finally Bt maize plants. It seems that neonate larvae can distinguish Bt plants and try to avoid them. Similarly, Yang *et al.* (2008) found indications that *H. armigera* larvae can distinguish parts of the plant with fewer Bt toxins to feed on and are more likely to survive on Bt cotton.

In summary, this work indicates that larvae of *M. unipuncta* and *H. armigera* can survive, at least partially, when feeding on Bt maize. As mortality of corn borers on Bt maize is very high, changes in the status of lepidopteran pests on maize must be considered. It may also be concluded from this work that when strategies to prevent Bt-resistance in corn borers are planned, potential consequences for *H. armigera* and *M. unipuncta* must be considered.

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References

Esperk, T., Tammaru, T. & Nylin, S. 2007: Intraspecific variability in number of larval instar in insects. J. Econ. Entomol. 100: 625-645.

Huang, F., Leonard, B.R. & Gable, R.H. 2006: Comparative susceptibility of European corn borer, southwestern corn borer and sugarcane borer (Lepidoptera: Crambidae) to Cry1Ab protein in a commercial *Bacillus thringiensis* corn hybrid. J. Econ. Entomol. 99: 194-202.

MARM 2009: http://www.mapa.es/agricultura/pags/semillas/estadistica/serie/maizgm98_06.pdf

Pilcher, C.D., Rice, M.E., Obrycki, J.J. & Lewis, L.C. 1997: Field and laboratory evaluations of transgenic *Bacillus thuringiensis* corn on a secondary Lepidopteran pests (Lepidoptera: Noctuidae). J. Econ. Entomol. 90: 669-678.

Schaafsma, A.W., Holmes, M.L., Whitstlecraft, J. & Dudley, S.A. 2007: Effectiveness of three corn events against feeding damage by the true armyworm (*Pseudaletia unipuncta* Haworth). Can. J. Plant Sci. 87: 599-603.

Van den Berg, J. & Van Wyk, A. 2007: The effect of Bt maize on *Sesamia calamistis* in South Africa. Entomol. Exp. Appl. 122: 45-51.

Yang, Y., Johnson, M.L. & Zalucki, M.P. 2008: Possible effect of genetically modified cotton on foraging habits of early instar *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae) larvae. Australian J. Entomol. 47: 137-141.