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Diversity and seasonal phenology of aboveground arthropods in conventional and transgenic maize crops in Central Spain

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Abstract

One of the major concerns regarding the release of Bt maize is its potential negative impact on non-target organisms present in this crop. In this paper, we compare the temporal phenology and community structure of the aboveground arthropods in commercial Bt maize fields in Central Spain with those of conventional maize crops, with or without an insecticide (imidacloprid) seed treatment, over a period of three years. Spiders, harvestmen, centipedes, ground beetles, rove beetles, carrion beetles, click beetles, earwigs and damsel bugs were captured in pitfall traps every year in sufficient number to provide meaningful phenological data. One predator spider and three omnivorous species of ground beetles have been consistently present in the maize fields: *Pardosa occidentalis*, *Poecilus cupreus*, *Pseudophonus rufipes* and *Pseudophonus griseus*, respectively. Rove beetles were caught to a lesser extent, with three dominant species: *Acrotona aterrima*, *Philonthus varians* and *Platystethus nitens.* The variability in activity–density patterns of the aboveground fauna was mainly influenced by the year, but no detrimental effects could be attributed to Bt maize. The only exception being the changes detected in rove beetles, although these differences were transitory and varied from year to year. No changes in species richness and diversity indices for spiders and ground beetles resulted from treatments. However, imidacloprid-treated maize caused a reduction in species richness of rove beetles, even though the abundance of the main species was not reduced. Our results suggest that Bt maize could be compatible with natural enemies that are common in maize fields in Spain.

Keywords: Bt maize; Non-target arthropods; Spiders; *Pardosa occidentalis*; Ground beetles; Rove beetles; *Platystethus nitens*

1. Introduction

The use of genetically engineered insect-resistant plants in agriculture has currently become a powerful tool providing an effective control of some key pests. The planting of transgenic maize expressing Cry1Ab toxin from *Bacillus thuringiensis* (Bt), an insecticidal toxin-specific to certain lepidopteran species, was approved in Spain in 1998 to control two major pests: the Mediterranean corn borer, *Sesamia nonagrioides* (Lefebvre) (Lepidoptera: Noctuidae), and the European corn borer, *Ostrinia nubilalis* (Hübner) (Lepidoptera: Crambidae). The first event commercialised, Bt 176 (var. Compa CB, Syngenta), was grown from 1998 to 2005. A mean surface of about 20,000–25,000 ha of Bt maize (5% of the total maize growing area) was cultivated between 1998 and 2002, increasing to 50,000–60,000 ha (around 12–15%) in the following years with the introduction of new hybrids derived from the event MON810 (http://www.mapa.es/es/agricultura/pags/semillas/estadisticas.htm). The high specificity of Bt maize and the reduction of insecticides used to control corn borers will likely result in a more favourable environment for natural enemies. However, laboratory and field studies have warned

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about adverse effects on non-target organisms exposed to the insecticidal toxin (Hilbeck et al., 1998; Losey et al., 1999; Harwood et al., 2005; Pilcher et al., 2005). Subsequently, a debate about the possible detrimental consequences of the deployment of Bt maize on non-target arthropods has intensified in recent years, and different approaches have been put forward to deal with the assessment of this environmental risk (Wolfenbarger and Phifer, 2000; Conner et al., 2003; Lövei and Arpaia, 2005; Romeis et al., 2006; Andow and Zwahlen, 2006).

Two of the most important components of the ground dwelling fauna in farming systems are ground beetles and spiders (Melnychuk et al., 2003; De la Poza et al., 2005). Both groups have proved to be beneficial for the control of agricultural pests (Riechert and Lockley, 1984; Kielty et al., 1999; Sunderland, 1999; Lang et al., 1999). Furthermore, they contribute to the faunal diversity of agriculture landscapes (Booij and Noorlander, 1992). As Bt plants express the insecticidal protein during the whole season, potential exposure of predators via prey feeding on Bt plants may be increased in comparison with Bt sprays. Different ways have been reported for non-target arthropods to come into contact with insecticidal toxins produced in transgenic plants: by feeding on plant parts themselves, through feeding on target or non-target herbivorous insects, or via the environment, i.e. the soil when toxins persist and do not lose their toxicity after plant parts or insects have died (Groot and Dicke, 2002; Zwahlen et al., 2003). Bt toxins are also released into the rhizosphere in root exudates from Bt maize (Saxena et al., 1999; Saxena and Stotzky, 2001), most likely producing greater concentrations in the soil than if they were only incorporated through normal plant dynamics.

The implication of Bt toxin exposure on the performance of non-target arthropods is still not clear in the case of long-term exposure occurring in the field, so field studies can be useful in identifying the overall effect on non-target arthropods. As part of a Spanish post-market environmental monitoring plan for the Bt maize hybrid Compa CB (Event Bt176), a three-year farm field study was initiated to assess the potential impact of Bt maize on predatory arthropods. The effect of Compa CB on the abundance of non-target arthropods was studied in two Spanish locations from 2000 to 2002 (De la Poza et al., 2005). Araneae (spiders), Carabidae (ground beetles) and Staphylinidae (rove beetles) were the most abundant groups collected by pitfall traps. Their abundance varied from year to year and between locations, but no clear tendencies relating to Bt maize were recorded when compared with the isogenic cultivar with or without imidacloprid seed treatment.

The present study enlarges on that of De la Poza et al. (2005) by focusing on the effects of Bt maize on species richness, diversity and seasonal phenology of ground dwelling arthropods present in maize crops in Central Spain.

2. Materials and methods

This study was conducted in a commercial maize field in the province of Madrid (Central Spain), over three consecutive years, from 2000 to 2002. The planting dates were 13th April 2000, 19th April 2001 and 9th April 2002. The three different treatments, contrasted by a randomized block design with three replicates each, were: Bt maize hybrid, Compa CB (Cry1Ab toxin, Event 176; Syngenta Seeds SA) (Bt+); the isogenic Dracma (Syngenta Seeds SA) (Bt−) and Dracma with imidacloprid insecticide seed-treatment (ImBt−). In this latter treatment, which is relatively common in this area for soil and other insect pest control, the seeds were dressed at 1400 cc of imidacloprid (Gaucho 35 FS®, Bayer) per 100 kg of seed (4.9 g a.i/kg). The surface of each block ranged between 0.5 and 0.6 ha, with corridors between them and an external border 2–5 m wide, depending on the availability of land each year. The fields were maintained according to conventional local farming practices, but without spraying insecticide. In all plots a postemergence herbicide mixture of 75% isoxaflutol + 47.5% atrazine (Spade + Atrazinax Flo, Rhône Poulenc, France) at 5–6 l/ha was sprayed soon after sowing.

Aboveground arthropods were sampled by using pitfall traps. Each trap consisted of a plastic cup 12.5 cm in diameter and 12 cm deep with a plastic funnel fitted inside its upper rim, and both flush with the ground surface. Under the funnel and inside the plastic cup, a plastic container of 150 cc (Resopal, Madrid), where the insects fell, was half filled with a 3:1 mixture of water and ethanol. The outer cup was punched to let the irrigation water drain off. Five traps per plot were placed diagonally across each plot, starting at least 6 m from the field boundary to minimize potential edge effect. Traps were operative for 3 days every 2 weeks and the sampling period lasted from mid-June to the end of September, giving a total of eight sampling dates per year. The number of arthropods captured in pitfall traps is a function not only of the density, but also of the activity of the sampled organisms and their behavior on encountering a trap. Hence, the term "activity–density" is used to refer to population estimates obtained using pitfall traps. The arthropods collected were categorized by order and family in all groups, and by genus and species in the predominant groups. Richness and α- and β-diversity indices were calculated for the predominant groups collected, namely, spiders, ground beetles and rove beetles. The Shannon–Wiener α-diversity index (H') was computed to detect changes in the community structure of the predominant groups among the three different treatments. This index is based on the proportional abundance of species and is thus affected by species richness, and was calculated using the following formula (Magurran, 1989):

$$H' = -\sum p_i \ln p_i$$

p_i being the proportion of individuals found in the ith species. Differences in species richness and diversity when comparing the three kinds of plots and years were tested by two-way ANOVA analyses. If overall differences among them were detected, pairwise comparisons were made employing the Newman–Keuls test.

β-Diversity measures the degree of difference (or similarity) of a series of habitats, taking into account the variation of species found in them. In this case, β-diversity was calculated to evaluate the degree of likeness of the three treatments. The easiest way to measure β-diversity between pairs of samples is by using similarity coefficients. Similarities between pairs of treatments was determined by means of the Sorenson quantitative coefficient, that makes an effort to weight shared species by their relative abundance, without being influenced by the abundance of the most frequent species (Magurran, 1989). The equation for calculating the Sorenson quantitative coefficient (C_N) is the following:

$$C_N = 2jN/(aN + bN)$$

aN being the total number of individuals in the first treatment, bN the total number of individuals in the second treatment, and jN the sum of the lower of the two abundances recorded for species found in both treatments. The value of the index is 1 in the case of complete similarity and 0 when the samples compared have no common species. This index was calculated each year for the entire period studied.

Once the dominant species had been determined, differences in their abundance from one treatment to another were tested by two-way ANOVA (year and treatment) with repeated-measures (date) analyses. Square root transformations were used to stabilize variances. Sphericity of the variance-covariance matrix was tested by Mauchly's W statistic. Since the assumption of sphericity of the data was not confirmed, treatment differences were tested using the F value generated by Pillai's trace. Treatment results were compared using the Tukey test when significant differences among variances were not found (Levene's test), and the Tamhane test when the contrary was found. The significance level of $P < 0.05$ was considered for all tests.

3. Results

3.1. Arthropod taxa in maize

The following nine groups of aboveground arthropods belonging to six different orders were consistently recorded every year and captured in sufficient number in Bt and non-Bt maize plots to provide meaningful phenological data: spiders (Araneae), harvestmen (Opiliones: Phalangiidae), centipedes (Chilopoda: Lithobiidae), ground beetles (Coleoptera: Caraboidea), rove beetles (Coleoptera: Staphylinidae), carrion beetles (Coleoptera: Silphidae), click beetles (Coleoptera: Elateridae), earwigs (Dermaptera: Labiduridae), and damsel bugs (Heteroptera: Nabidae) (Table 1). A total of 29,792 individuals comprised of the above groups were captured in the 45 pitfall traps during the 3 years of the study, covering a period of 24 days per year distributed from June to September in Bt and non-Bt maize. Among the above groups, 92% of them were generalist predators: spiders, ground beetles and rove beetles. The damsel bugs, all of them of the genus *Nabis*, and centipedes belonging to the family Lithobiidae are also mainly predators, although their feeding behaviour can be variable. Harvestmen and earwigs were primarily one species, *Phalangium opilio* (L.) and *Labidura riparia* (Pallas), respectively. Carrion beetles, which are mainly necrophagous or fungivorous, belonged to the species *Silpha tristis* Illiger, but most of the captures were larvae, which prey on insects and snails. Finally, click beetles feed on vegetable material or small prey.

Table 1
Total number of the surface-dwelling arthropod groups consistently captured in pitfall traps in Bt and non-Bt maize plots

Arthropod group[a]	2000	2001	2002	Total
Spiders (ARA)	3495	4996	4656	13,147
Ground beetles (COL)	4901	3535	3164	11,600
Rove beetles (COL)	787	538	951	2276
Centipedes (CHI)	245	327	272	844
Carrion beetles (COL)	99	64	536	699
Click beetles (COL)	217	77	314	608
Harvestmen (OPI)	59	79	195	333
Damsel bugs (HET)	100	32	54	186
Earwigs (DER)	31	36	32	99
Total	9934	9684	10,174	29,792

[a] Orders are in brackets: ARA, Araneae; COL, Coleoptera; CHI, Chilopoda; OPI, Opiliones; HET, Heteroptera and DER, Dermaptera.

3.2. Activity–density patterns

Temporal activity–density patterns of the arthropods oscillated from June to September, but differences in each taxonomic group were noticeable from year to year, but not for treatments (Fig. 1). Dynamics of spiders, ground beetles and rove beetles, the dominant groups, greatly depended on the year, showing different peaks that had no relationship with the treatment. However, in the case of rove beetles there was a significantly lower number of individuals ($F_{2,44} = 3.50$, $P = 0.04$) in Bt− plots with respect to Bt+ and ImBt− in the first sampling date of 2002. In the minority groups (<5 specimens collected per trap per sampling date) the temporal activity–density patterns were not clear since numbers of captures were low. In general, samples of harvestmen, centipedes and click beetles were rather similar irrespective of treatments but big differences from one year to another were found. Only centipedes in 2001 showed an isolated peak in August in Bt− fields compared with the other two treatments. Click beetles showed a conspicuous peak every year at the commencement of the summer that then sharply decreased until they became completely absent from mid July on

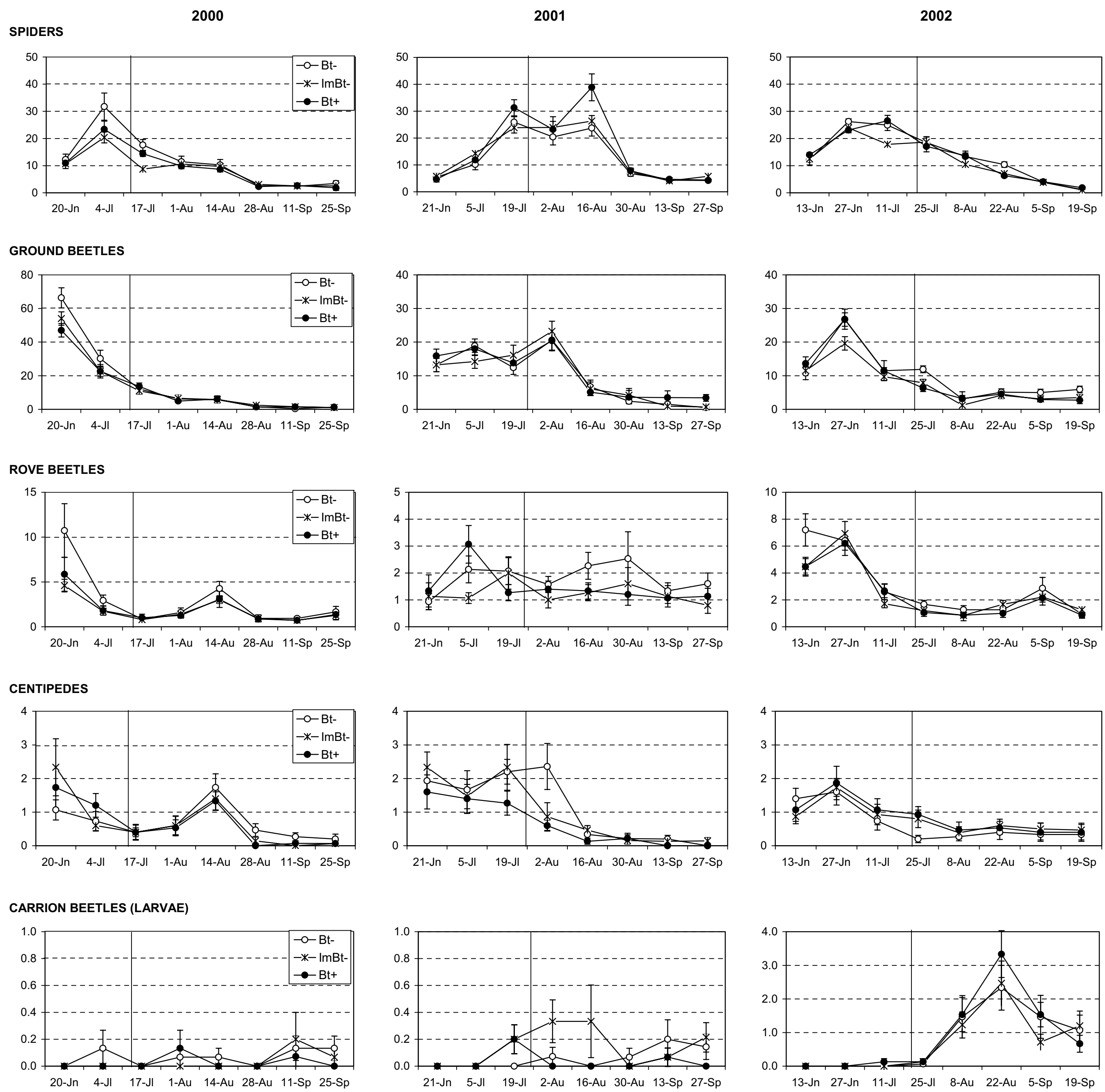

Fig. 1. Activity–density (mean number of individuals per trap ± SE) of the five most abundant ground-dwelling arthropod groups in Bt maize (Bt+), non Bt maize (Bt−) and maize treated with imidacloprid (ImBt−) in Central Spain in 2000–2002. The vertical lines in the graphics indicate the date of anthesis. Months on the *x*-axis are June (Jn), July (Jl), August (Au) and September (Sp).

(data not shown). The dynamics of carrion beetles, earwigs and damsel bugs were quite irregular throughout the 3 years (data not shown). Particularly noticeable were the differences from year to year in carrion beetle larvae (289 larvae collected in 2002, but only 15 and 28 in 2000 and 2001, respectively). Pollen shedding occurred mostly in mid-July, but no drastic changes in activity densities were observed in any of the non-target groups in Bt plots due to the presence of pollen, either during or after the anthesis (Fig. 1).

3.3. Indicators of community structure of spiders, ground beetles and rove beetles

A total of 37 species of spiders were found during the 3 years of sampling. Just six species accounted for 96% of all the spiders captured in the traps (Fig. 2): *Pardosa occidentalis* Simon (Lycosidae); *Zelotes* sp. (Gnaphosidae); the Lyniphiidae *Meioneta* sp., *Ostearius melanopygius* (O. P-Cambridge) and *Erigone dentipalpis* (Wider), and *Robertus* sp. (Theridiidae). *P. occidentalis* was the dominant species

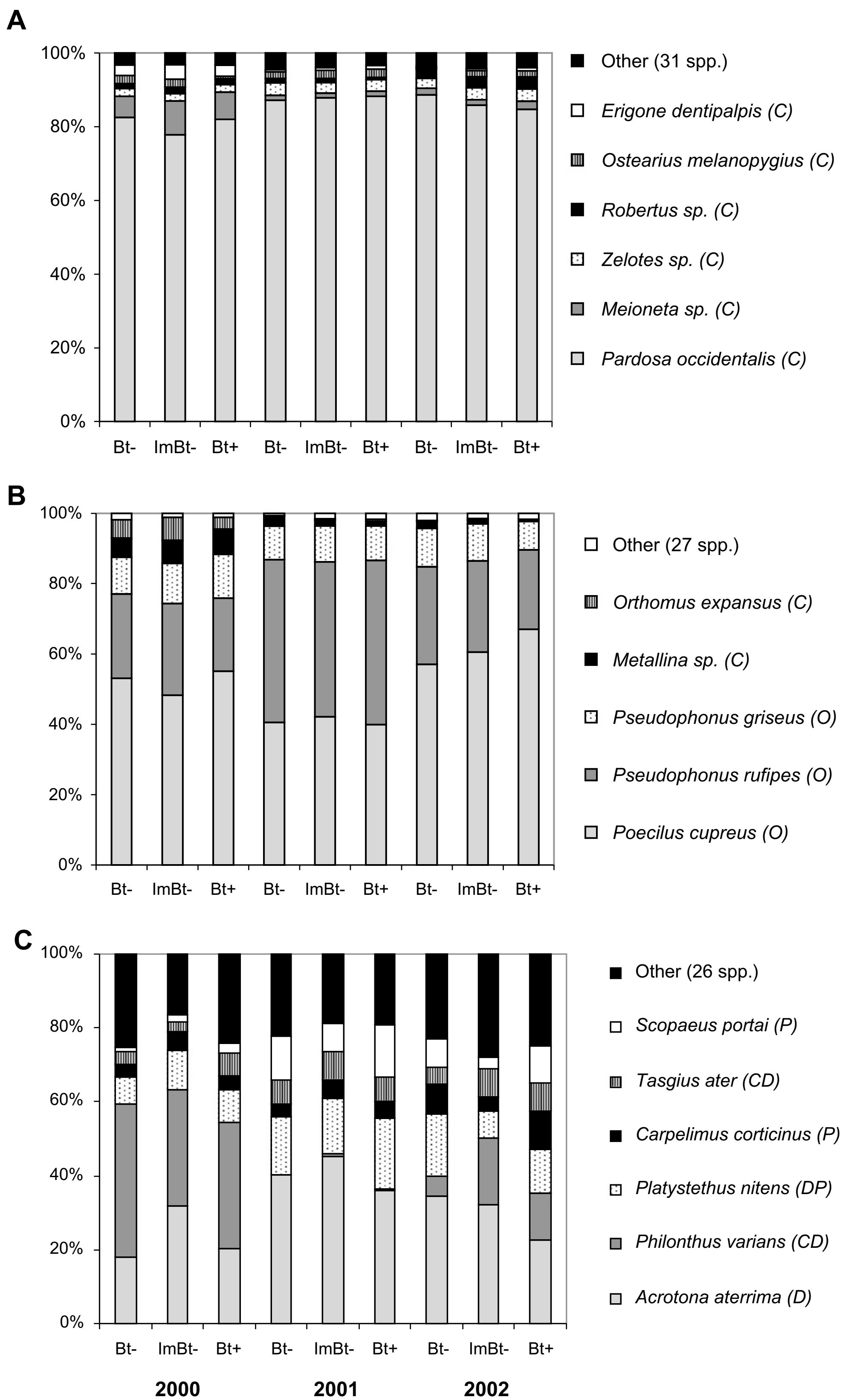

Fig. 2. Species composition of spiders (A), ground beetles (B) and rove beetles (C) in Bt maize (Bt+), non Bt maize (Bt−) and maize treated with imidacloprid (ImBt−) in Central Spain in 2000–2002. Letters after the species name indicate the trophic guild: C, carnivorous; P, phytophagous; O, omnivorous; D, detritivorous. The genus *Metallina* might include two species: *M. lampros* and *M. properans*.

(86% of the total) and its density was lower in 2000 than in 2001 and 2002 ($F_{2,112} = 55.01$, $P < 0.00$). However, no significant differences were found comparing treatments for this species ($F_{2,112} = 0.961$, $P = 0.386$), or for the other five most abundant species ($F_{2,112} = 0.55$, $P = 0.577$; $F_{2,112} = 0.484$, $P = 0.618$; $F_{2,112} = 0.168$, $P = 0.846$; $F_{2,112} = 0.546$, $P = 0.581$; $F_{2,112} = 0.011$, $P = 0.989$, in the same order as above). There were some changes in spider species composition depending on the year (Fig. 2), but no significant differences were found comparing the different treatments ($F_{2,110} = 0.516$, $P = 0.666$). Mean richness ranged from 11.0 species in ImBt− in 2000 to 17.0 species in Bt− plots in 2002, though significant differences among treatments were not found (Table 2). The diversity of species was higher in 2000 than in 2001 and 2002, with values between 0.81 and 0.92, but no significant differences from one treatment to another were observed in this case (Table 2).

Thirty-two species of ground beetles were collected in the pitfall traps in the 3-year study. Three species accounted for 93% of the total number of ground beetles captured: *Poecilus cupreus* L. (Pterostichidae), and the Harpalidae *Pseudophonus rufipes* De Geer and *Pseudophonus griseus* Panzer (Fig. 2). There were significant differences in the temporal density of the three common species depending on the year: for *P. cupreus* it was significantly lower in 2001 than in 2000 and 2002 ($F_{2,112} = 11.51$, $P < 0.00$), for *P. rufipes* it was higher in 2001 than in 2002 ($F_{2,112} = 8.67$, $P < 0.00$), and for *P. griseus* it was higher in 2000 than in 2001 and 2002 ($F_{2,112} = 10.77$, $P < 0.00$). Nevertheless, no differences in the density of these species in Bt+, Bt− and ImBt− plots were detected ($F_{2,112} = 1.92$, $P = 0.15$; $F_{2,112} = 1.11$, $P = 0.33$; and $F_{2,112} = 0.24$, $P = 0.79$ for *P. cupreus*, *P. rufipes* and *P. griseus*, respectively). Similar results were observed for spiders, namely differences in species composition due to the year, but not to the treatment (Fig. 2). Mean richness values for ground beetles were lower than in spiders, ranging from 6.3 species in 2001 to 12.7 species in 2000, although no significant differences in richness among treatments each year were detected. Similarly, the Shannon–Wiener diversity index calculated every year did not show any differences because of treatments (Table 2).

The third most abundant group was that of rove beetles with 32 species collected in the pitfall traps, and three of them comprised 59% of the total: *Acrotona aterrima* (Gravenhorst), *Philonthus varians* (Paykull) and *Platystethus nitens* (C.R. Sahlberg) (Fig. 2). The species composition of rove beetles was very variable every year. The clearest example was *P. varians* that was the dominant species in 2000 in the three plots, only two specimens were found in 2001 and became the third most abundant species in 2002 (Fig. 2). Nonetheless, no significant differences were observed for *P. varians* when comparing treatments ($F_{2,117} = 0.79$, $P = 0.46$), and the same was true for *A. aterrima* ($F_{2,117} = 1.44$, $P = 0.24$). Only *P. nitens* displayed significant differences due to the treatment ($F_{2,117} = 4.09$,

Table 2
Richness (average number of species ± SE) and Shannon diversity index (means ± SE) of spiders, ground beetles and rove beetles sampled in non Bt maize (Bt−), maize treated with imidacloprid (ImBt−) and Bt maize (Bt+), in 2000–2002

	Year	Treatment			Year (Y) *F*-ratio (*P*-value)	Treatment (T) *F*-ratio (*P*-value)	Interaction (Y × T) *F*-ratio (*P*-value)
		Bt−	ImBt−	Bt+			
Richness							
Spiders	2000	15.0 ± 1.2	11.0 ± 1.0	12.3 ± 0.7	7.19(0.01)*	3.08(0.07)	0.98(0.44)
	2001	14.0 ± 1.5	14.0 ± 0.0	13.7 ± 0.3			
	2002	17.0 ± 1.0	15.0 ± 1.0	15.7 ± 1.5			
Ground beetles	2000	12.7 ± 2.2	9.7 ± 0.7	9.7 ± 0.9	2.77(0.09)	0.19(0.83)	1.74(0.19)
	2001	6.3 ± 0.9	8.7 ± 2.3	9.7 ± 0.9			
	2002	9.7 ± 1.5	8.3 ± 0.7	8.3 ± 0.7			
Rove beetles	2000	12.7 ± 0.9	10.7 ± 0.3	12.0 ± 0.0	29.45(0.00)*	6.36(0.01)*	1.69(0.20)
	2001	12.0 ± 1.5	9.3 ± 1.2	9.0 ± 0.6			
	2002	15.3 ± 0.7	13.3 ± 0.3	16.0 ± 0.6			
Diversity							
Spiders	2000	0.82 ± 0.05	0.92 ± 0.09	0.81 ± 0.12	9.44(0.00)*	0.50(0.62)	1.22(0.34)
	2001	0.66 ± 0.02	0.63 ± 0.05	0.60 ± 0.02			
	2002	0.61 ± 0.03	0.70 ± 0.04	0.78 ± 0.07			
Ground beetles	2000	1.31 ± 0.18	1.35 ± 0.07	1.27 ± 0.07	9.49(0.00)*	0.62(0.55)	0.49(0.74)
	2001	1.09 ± 0.06	1.09 ± 0.06	1.11 ± 0.02			
	2002	1.11 ± 0.07	1.04 ± 0.06	0.91 ± 0.07			
Rove beetles	2000	1.85 ± 0.18	1.79 ± 0.06	1.99 ± 0.07	11.83(0.00)*	3.08(0.07)	0.62(0.65)
	2001	1.89 ± 0.01	1.66 ± 0.08	1.79 ± 0.12			
	2002	2.13 ± 0.14	2.05 ± 0.11	2.33 ± 0.01			

Means were compared by two-way ANOVA, and the *F* calculated for the two factors (year and treatment) and their interaction. Data are from 15 traps per treatment and eight sampling dates each year.

$P = 0.02$), since ImBt− plots had a lower number than Bt− ones, but there were no differences between Bt+ and Bt− plots. Likewise, there were also significant treatment-related differences in mean richness of rove beetles, but they were due to the lower number of species in ImBt− plots than in Bt− plots. In contrast, diversity was not significantly different in the three treatments (Table 2).

The Sorenson quantitative coefficient calculated for spiders and ground beetles to measure similarity between pairs of treatments showed high values, over 0.8, in every case (Table 3). In spiders the values ranged between 0.816 and 0.939, and in ground beetles values were slightly higher, between 0.847 and 0.964. Levels of similarity in rove beetles were, in general, lower than in spiders and in ground beetles, ranging between 0.726 and 0.880 in all the pairs of treatments compared, but the percentage of shared species between treatments was over 50% in all cases.

4. Discussion

Activity–density patterns were similar in Bt+, Bt− and ImBt− plots in all the groups studied. We found great temporal variation in arthropod dynamics for each year as well as over the entire period studied regardless of the treatment, indicating that various other factors rather than the use of transgenic plants could affect the populations. The differences in activity–density found in all groups of arthropods throughout the season are expected in agricultural landscapes, since they can respond rapidly to crop disturbances due to their high dispersive capacity (Booij and Noorlander, 1992). Therefore, it is important to perform this type of study in commercial fields wide enough to avoid underestimation that might occur in small plots (<0.05 ha), where abundance and diversity could be dominated by migration from surrounding habitat (Sisterson et al., 2004). The treatment with imidacloprid, a systemic insecticide used mostly to control sucking insect pests, was chosen to compare the effect of Bt maize with a control method used to reduce first generation *S. nonagriodes* (Pons and Albajes, 2002). This insecticide was not expected to interfere with the dynamics of the aboveground fauna of arthropods, though predators could be affected by feeding on contaminated prey or by the removal of prey due to the effect of the insecticide (Albajes et al., 2003). Significant differences in species richness and diversity due to the year, but not to the treatment, were found in the most abundant arthropod groups, spiders, ground beetles and rove beetles. Although these groups are dominated by large numbers of a few robust species, the analysis at the species level takes into account other scarce but potentially sensitive species that may otherwise be overlooked. Only in the case of rove beetles were differences in richness found due to the lower number of species in ImBt− compared to Bt+ and Bt− plots.

Table 3
Sorenson quantitative coefficient calculated for spiders, ground beetles and rove beetles, by means of paired comparisons of the three treatments: non Bt maize (Bt−), maize treated with imidacloprid (ImBt−) and Bt maize (Bt+) in 2000–2002

	Year	Bt− and Im/Bt−	Bt− and Bt+	Im/Bt− and Bt+
Spiders	2000	0.816 (13/26)	0.866 (12/26)	0.917 (12/21)
	2001	0.932 (16/23)	0.872 (17/22)	0.918 (14/24)
	2002	0.880 (19/25)	0.939 (20/25)	0.915 (18/23)
Ground beetles	2000	0.910 (13/23)	0.877 (11/24)	0.922 (9/20)
	2001	0.964 (10/13)	0.929 (8/17)	0.960 (10/17)
	2002	0.847 (11/18)	0.910 (11/19)	0.888 (8/20)
Rove beetles	2000	0.763 (14/21)	0.763 (15/20)	0.827 (12/18)
	2001	0.794 (12/22)	0.880 (11/21)	0.839 (11/17)
	2002	0.726 (17/23)	0.764 (18/26)	0.739 (16/24)

Numbers of shared species between treatments/numbers of total species of the two treatments compared are shown in brackets.

None of the six spiders studied at the species level were affected by the use of transgenic maize or seed treatment with imidacloprid. Lycosidae (wolf spiders) and Linyphiidae were the dominant families collected by pitfall traps. Wolf spiders are very important predators in agro-ecosystems for reducing pest populations such as Cicadellidae, Thysanoptera and Aphididae in maize crops (Lang et al., 1999). Different species belonging to this family were found, though the dominant species was *P. occidentalis,* a diurnal running spider that actively moves about searching for prey (Uetz et al., 1999) and, like the rest of the species of this genus, are very common in agricultural systems. Other field studies have also shown that spiders are not affected by different transgenic plants expressing Cry toxins of *B. thuringiensis* (Al-Deeb and Wilde, 2003; Duan et al., 2004; Candolfi et al., 2004). Likewise a laboratory assay revealed no adverse effects of Bt maize pollen consumption on the garden spider, *Araneus diadematus* Clerck (Araneae) (Ludy and Lang, 2006).

It has been shown that a small number of species dominate the ground beetle fauna in agro-ecosystems, and thus they can be considered an important component of the whole farming system (French et al., 2004). Just three species of carabids, *P. cupreus*, *P. rufipes*, and *P. griseus* accounted for 93% of the total captured. These species are omnivorous and, together with *Metallina* spp. are relatively common in the European agro-ecosystems (Andersen, 1999; Kielty et al., 1999; Lozzia, 1999; Dutton et al., 2003). Moreover, *P. rufipes* feeds on different prey in cereal fields, such as aphids (Edwards et al., 1979), snails and slugs (Ayre, 2001), and like many other ground beetle species, has also been described as an important consumer of vegetal material (Thiele, 1977). Thus, it could have entered into contact with the insecticidal toxin and the imidacloprid in many ways, though no effects were detected. In addition, some ground beetles have proved to be good indicators for testing the potential effects of Bt crops on non-target fauna (Duan et al., 2006; López et al., 2005). No detrimental effects of Bt maize on the total density of ground beetles were observed, which is in agreement with other field studies (Lozzia, 1999; Candolfi et al., 2004), and similar results were obtained with other Cry toxins (Al-Deeb and Wilde, 2003; Duan et al., 2004).

In a previous analysis, total abundance of rove beetles diminished in Bt+ plots compared with Bt− plots in central Spain (Madrid) in 2000 (De la Poza et al., 2005). A more detailed examination here, found no differences when the data were analyzed by sampling date in 2000, but showed a significant decrease on the first date of sampling for Bt+ and ImBt− plots in 2002. Furthermore, analysis of the abundance of the three dominant species found (*A. aterrima*, *P. varians* and *P. nitens*) showed that they were not significantly affected by Bt maize. Thus, the reduction of rove beetle abundance seems to be the result of a general decrease that could not be attributed to any of the predominant species. A decrease in the total number of rove beetles was also obtained by Jasinski et al. (2003), in one of six Bt maize plots using sticky traps, but they considered that it might be an artefact of an insecticide application of permethrin rather than because of some factor related to the transgenic nature of the hybrid. Further studies are needed to clarify the inconsistent results found on the abundance of rove beetles in Bt maize fields and to establish the key factors determining these differences.

Species richness of the two most abundant aboveground predators, spiders and ground beetles, did not significantly differ in Bt+, Bt− and ImBt− plots. However, we found that richness of rove beetles significantly decreased in ImBt− plots compared to Bt+ and Bt− plots. This result could be due to the fact that some species of rove beetles, such as *P. nitens*, are not strictly predators and might have fed on imidacloprid seed treated maize.

Diversity indices calculated for species of spiders, ground beetles and rove beetles in Bt+, Bt− and Im Bt− plots did not show any significant differences, and H' values for ground beetles were relatively high (>1 in most cases) as compared with other studies in maize crops (Ellsbury et al., 1998; Lozzia, 1999). The Shannon–Wiener index is affected by species richness, so the results obtained could be expected after checking that richness of spiders and ground beetles did not significantly change for the three treatments during the 3 years studied. Moreover, the possible lack of sensibility of H' to changes in the community could also be due to a lack of change in the dominance of the species. Results obtained with the Sorenson quantitative coefficient for measuring similarity between Bt− and Bt+ revealed not only very high, but also similar values each year, indicating a high degree of likeness. Similar results were found by Lozzia (1999) for ground beetle assemblages in Bt and non-Bt maize crops. It is worth mentioning that rove beetle similarity values are lower than in spiders and ground beetles, even if pairs of treatments have a higher percentage of common species. This is due to the fact that the Sorenson quantitative coefficient takes into account not only the number of common species, but also their relative abundance. So it seems that features of the crop system itself were mainly responsible for the composition of spider, ground beetle and rove beetle assemblages rather than the presence of transgenic plants in the plots.

Attention should also be paid to minority arthropod groups, since they could be responsible for biodiversity shifts in agro-ecosystems. Harvestmen, common in different crops in most temperate regions of the world, were mainly represented by only one species, *P. opilio*, a generalist predator that preys on small insects, earthworms, other harvestmen, spiders and other beneficial invertebrates, as well as dead insects and decaying material (Clark et al., 1994; Newton and Yeargan, 2002). Little is known about the possible effects of Cry1Ab expressed in Bt maize on this species, but this study reveals that their levels of activity were similar every year regardless of the treatment. Similarly, centipedes (Lithobiidae), facultative predators in many agricultural systems, were captured in the same quantities in transgenic and non-transgenic plots. Our results support those findings reported in the few studies that have been carried out showing the effect of Bt toxins on centipedes. Candolfi et al. (2004) did not find a decrease in the number of centipedes (Chilopoda) in Bt maize fields, and similarly Dively and Rose (2003) did not report any negative effect on the abundance of centipedes of the order Geophilomorpha due to the use of transgenic sweet corn. Activity–density patterns of click beetles and carrion beetles in the transgenic plots did not decrease compared with non-transgenic plots. Although carrion beetles of the subfamily Silphinae have attracted relatively little research interest, they provide a valuable ecological service (Hoback et al., 2001). Adults and larvae of *S. tristis* can be found on the carcasses of small animals and they also prey on snails (Prieto Piloña and Valcárcel, 2002). Damsel bugs are not strictly soil predators, since they usually live on the vegetation. However we have found a considerable number of them in pitfall traps, and they are frequent in Spanish maize fields (Asín and Pons, 1998). Till now, no transgenic crop has been reported to affect this predator (Reed et al., 2001; Ponsard et al., 2002; Jasinski et al., 2003; De la Poza et al., 2005), and any decrease in abundance found in some studies could be due to the reduction in the number of prey (Naranjo, 2002). Earwigs are also a common group found in maize crops in Spain (Asín and Pons, 1998). Adults of *L. riparia* are voracious, have high attack capacity, and might be an important component for the control of several insect pests (Shepard et al., 1973). There are no previous data about the possible effect of Bt toxins on this species, but we did not find any significant differences between transgenic and non-transgenic maize plots.

Given the importance of arthropods in agro-ecosystems, pest management with Bt crops should make an effort to maintain vigorous and healthy natural enemy communities, especially the generalist predators such as spiders and ground beetles, which are probably the most important components of the aboveground fauna in farming systems (Melnychuk et al., 2003). In this regard, no detrimental effects of farm-scale Bt maize have been observed on the activity–density patterns and diversity of the ground dwelling fauna. The only exception being the changes detected in rove beetles, although these differences were transitory and varied yearly. Our findings suggest that Bt maize could be compatible with the natural enemies that are common in maize fields in Europe.

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