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Short Communication

Effects of transgenic maize expressing the Cry1Ab protein (event MON810) on locally adapted earthworms in a sandy loam soil in the Central Eastern Cape, South Africa

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This field study investigated effects of growing *Bacillus thuringiensis* (Bt) maize (MON810) on local earthworms in the Central Eastern Cape, South Africa. Two Bt maize cultivars (DKC61-25B and PAN6Q-321B) and their near-isolines (DKC61-24 and PAN6777) were grown in the 2009/2010 and 2010/2011 summer seasons. Earthworms were sampled after six, nine and eighteen weeks in 2009/2010 and after six, twelve and twenty-one weeks in the 2010/2011 season. The four maize treatments had similar earthworm counts, irrespective of sampling time, in both seasons. Sampling time had no effect in the 2009/2010 season, whereas the earthworm counts at 21 weeks after planting (WAP) were lower than the other two sampling times during the 2010/2011 season. The findings suggested that, at least in the short-term, growing Bt maize does not have negative effects on the numbers of the earthworms in the Central Eastern Cape, South Africa.

Key words: *Bacillus thuringiensis* (Bt) maize, Cry1Ab protein, earthworm counts.

INTRODUCTION

Commercial production of genetically modified (GM) maize has rapidly increased in the past few years, which has led to concerns about the crop's effects on non-target organisms. Research findings on *Bacillus thuringiensis* (Bt) maize range from no effects on soil microorganisms, nematodes and earthworms (Saxena and Stotzky, 2001), minimum non-persistent, site specific effects, on nematodes, amoebas and protozoa (Griffiths et al., 2007), to significant growth reduction of earthworms after a prolonged exposure to Bt maize residues (Zwahlen et al., 2003).

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Earthworms are known to burrow the soil influencing soil processes, and aid in the fragmentation of plant residues and nutrient cycling, among other important functions (Feller et al., 2003). Few field studies have been conducted to address the effects of growing Bt maize on earthworms (Lang et al., 2006; Zeilinger et al., 2010). A study in the Northern Corn Belt of the USA showed minimal effects of Bt maize cultivars with Cry1Ab and Cry3Bb1 proteins on earthworm populations of *Aporrectodea caliginosa* species complex and *Lumbricus terrestris* species (Zeilinger et al., 2010). The authors recommended that more earthworm species, associated with maize, need to be studied particularly in other parts of the world, like the temperate parts of Australia, China and South Africa, which have abundant native species.

Earthworm species of Microchaetidae, Acanthodrilidae,

Table 1. Earthworm counts in soil grown with Bt maize cultivars and their near-isogenic lines.

Treatment	Count (worms m^{-2})
DKC61-25B*	28.5
DKC61-24	23.7
PAN6Q-321B*	25.7
PAN6777	19.7
LSD (p = 0.05)	9.39

*Bt maize cultivars.

and others native to South Africa face extinction due to human activities (Edwards and Bohlen, 1996). The general objective of this study was to determine the effects of the growing Bt maize crop on numbers of earthworms in the Central Eastern Cape, South Africa.

MATERIALS AND METHODS

Study site

The study was conducted at the University of Fort Hare Research Farm located at 32° 47' S; 26° 50' E" at an altitude of 508 m above sea level. The site is in a semi-arid area, with mean annual rainfall of 575 mm and a mean annual temperature of 18.1°C (Van Averbeke and Marais, 1991). The soil used in the study was a Haplic Cambisol (Eutric) (IUSS Working Group WRB, 2006), with pH 6.2 (1:2.5; soil: water), 0.84% organic carbon and 13.6% clay containing high mica and low kaolinite, hematite and quartz (Mandiringana et al., 2005).

Experimental set up and management

The study was established as a randomized complete block design (RCBD) with two Bt maize (MON810)(PAN6Q-321B and DKC61-25B), and their near-isolines (PAN6777 and DKC61-24), with three replications. The maize hybrids were planted on 18th December, 2009 (first season) and on 26th November, 2010 (second season) at 40 000 plants ha^{-1}. Basal fertilizer (2:3:2 (22); N:P:K) was applied at a rate of 25 kg N ha^{-1} at planting, and topdressing with Limestone ammonium nitrate (LAN; 28% N) was done at 50 kg N ha^{-1} at six weeks after planting. Weeds were controlled using Basagran® (bentazon) and Atrazine® at 2 L ha^{-1} applied post emergence. Bulldock 050EC was applied at a rate of 150 ml ha^{-1} in all treatments at 12 weeks after planting (WAP) to control stem borers in the near-isolines. Supplementary irrigation was applied when required using a sprinkler system.

Sampling

Sampling for earthworms was done after receiving a significant amount of rainfall when the worms were close to the surface of the moist soil. Sampling was done at six, nine and 18 WAP in the 2009/2010 season and at six, 12 and 21 WAP in the 2010/2011 season. A 1 m^2 quadrant was randomly placed in each plot and earthworms were sampled by excavating the soil to a depth of 50 cm, hand-sorting and counting (Edwards and Bohlen, 1996).

Data analysis

Data of earthworm counts were subjected to analysis of variance (ANOVA), after testing for normality, and the means were separated using the least significant difference (LSD) at P<0.05. The statistical analysis was done using the GenStat Release 7.22 DE statistical package (Lawes Agricultural Trust, 2008).

RESULTS AND DISCUSSION

There were no interaction effects of sampling time and maize cultivar on earthworm counts in both the 2009/2010 and 2010/2011 seasons. Earthworm counts were not different between the Bt maize and their non-Bt near-isogenic maize lines (Table 1). Earthworm biomass followed the same trends (data not shown), but its significance was limited because the data were only collected during the 2009/2010 season. Similarity in the number of earthworms among all the treatments was in agreement with Lang et al. (2006) and Zeilinger et al. (2010), who reported no significant effects of Bt maize cultivars on earthworm populations under field conditions, in South East Germany and northern US Corn belt, respectively. The *Proandricus* spp. was dominant at our site (Plisko, J.D., Natal Museum, personal communication), whereas Lang et al. (2006) studied the Lumbricidae, and the study by Zeilinger et al. (2010) was on *Lumbricus* and *Aporrectodea* species. Similarities in these findings suggest that counts of different earthworm species will not be negatively affected by growing Bt maize with the MON810 event. Lack of negative effects of Bt maize cultivars on earthworm counts has also been reported under laboratory conditions by other researchers including Saxena and Stotzky (2001). Clark and Coats (2006) even observed greater growth of an earthworm, *Eisenia fetida*, fed with leaf material of one Bt maize with the MON810 event, while the other did not have an effect when compared with the controls. This finding was explained by protein and sugar contents of the diet which were correlated with the growth of the worm. However, these nutritional parameters were not measured in our study.

Whereas, the earthworm counts were not affected by sampling time in the 2009/2010 season, the counts at 21 WAP were lower than those at 6 and 12 WAP in the 2010/2011 season (Table 2). This could be as a result of differences in moisture conditions experienced at the time of samplings. The findings of this study suggest that numbers of locally adapted earthworms in the Central Eastern Cape are not negatively affected by Bt maize (MON810), at least in the short-term.

Conclusion

Growing Bt maize (MON810) would not have negative effects on number density of locally adapted earthworms, at least in the short-term, in the Central Eastern Cape of

Table 2. Earthworm counts as affected by sampling time in the 2009/2010 and 2010/2011 seasons.

Sampling time (week)	Count (worms m^{-2})	
	(2009/2010 season)	(2010/2011 season)
6	26.7	32.5
9 - 12	21.8	29.1
18 - 21	26.9	19.4
LSD (P<0.05)	7.27	4.97

South Africa. Further studies should focus on populations of earthworms where Bt maize has been grown for medium to long-term.

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