Pedobiologia 51 (2007) 175–184

Available online at www.sciencedirect.com

ScienceDirect

Pedobiologia

www.elsevier.de/pedobi

SOIL ECOLOGICAL AND ECONOMIC EVALUATION OF GENETICALLY MODIFIED CROPS – ECOGEN

Agricultural studies of GM maize and the field experimental infrastructure of ECOGEN

Mathias Neumann Andersen[a,*], Christophe Sausse[b], Bernard Lacroix[c], Sandra Caul[d], Antoine Messéan[b,1]

[a]*University of Aarhus, Faculty of Agricultural Sciences, Research Centre Foulum, P.O. Box 50, 8830 Tjele, Denmark*
[b]*Centre Technique Interprofessionnel des Oléagineux Métropolitains, Centre de Grignon BP 4, 78850 Thiverval Grignon, France*
[c]*ARVALIS – Institut du vegetal, station Inter-instituts, 6, chemin de la côte vieille, 31450 Baziège, France*
[d]*Environmental Plant Interactions Programme, Scottish Crop Research Institute, Invergowrie, Dundee DD2 5DA, UK*

Received 15 March 2007; accepted 21 March 2007

KEYWORDS
Herbicide-tolerant maize;
Bt-maize;
Cry1Ab;
Genetically modified maize;
Ploughing;
Reduced tillage

Summary

Within the ECOGEN project, long-term field experiments with genetically modified maize, *Zea mays* L. were conducted to study agro-ecological effects on the soil fauna and agro-economic implications of the technology. Here, we describe the study-sites, experimental layout and results of agro-economic relevance. Experiments were conducted during 2002–2005 in Denmark (Foulum), northern France (Varois) and the Midi-Pyrenees region of southern France (Narbons). MON810 *Bacillus thuringiensis* (*Bt*)-varieties expressing the Cry1Ab protein, and a T25 glufosinate-ammonium (Basta) tolerant variety expressing the pat-gene encoding phosphinotrinacetyl-transferase were compared with near-isogenic non-*Bt* varieties, and conventional maize varieties. At Foulum, the maize was harvested for silage. There were no significant differences in yield between *Bt*-maize and a near-isogenic non-*Bt* variety, while a small difference in N-concentration of dry matter was detected in 1 year in a range of a measured quality parameters. Similar yield and quality were found in ploughed and reduced tillage treatments in all varieties. At Varois, the maize was harvested at ripeness and no significant differences in grain yield between *Bt*-maize and near-isogenic non-*Bt* varieties were found. These results were expected, as only Narbons harbours significant corn-borer populations. At Narbons, the number of *Sesamia* and *Ostrinia* corn-borer larvae were significantly lower in the *Bt*-maize than in a near-isogenic non-*Bt* variety and for *Sesamia* even less than in conventional varieties sprayed with pesticides to control corn-borer infestation. Here, *Bt*-maize produced a higher grain yield and grain size than a near-isogenic

*Corresponding author.
E-mail address: MathiasN.Andersen@agrsci.dk (M.N. Andersen).
[1]Present address: Institut National de la Recherche Agronomique, Eco-Innov, 78850 Thiverval-Grignon, France.

0031-4056/$ - see front matter
doi:10.1016/j.pedobi.2007.03.005

non-*Bt* variety or allowed a significant reduction in pesticide use. Concentrations of Cry1Ab in the *Bt*-varieties were sufficient to effectively control corn-borer larvae. In soil, Cry1Ab was close to the limit of detection and the protein did not accumulate in the soil year on year.

Introduction

The global commercial area of genetically modified (GM) plants reached 90 M ha in 2005 and that of GM maize expressing the *Bacillus thuringiensis* toxin (*Bt*) was 11.3 M ha (James, 2006). Even so, the environmental release of GM plants is controversial in some world areas due to the questions relating to the potential impacts on human health and the environment, including effects on non-target soil organisms (Wolfenbarger and Phifer, 2000). The *de facto* moratorium from 1999 to 2003 on the approval of GM crops in the European Union reflected this controversy and postponed extensive cultivation in the member states. Nevertheless, due to the economic advantages they may bring at farm level, GM crops are likely to be cultivated at larger scale in the EU, as is the case in Spain since 1997 and four other EU countries since 2005.

To date, the number of studies which address potential agronomic benefits in different agroecological settings in Europe is still relatively small, as are the studies on the potential for effects on non-target soil organisms (see for example, reviews by Groot and Dicke, 2002; Bruinsma et al., 2003). It was to address this information void that the EU-funded ECOGEN project (www.ecogen.dk) was initiated. This interdisciplinary study followed the advice of previous expert panels in adopting a tiered approach to risk assessment, i.e., laboratory, glasshouse and long-term field experiments (Angle, 1994), with an emphasis on soil communities and ecosystem functioning (Jepson et al., 1994; Trevors et al., 1994), as reported in other articles of this Pedobiologia special issue. In the present paper, we describe the three field sites within the ECOGEN project established in North, Mid and South European climatic zones where mainly *Bt* maize and a non-*Bt* near-isogenic control were grown for 3 or 4 years in permanently established plots. Due to the political sensitivity around the whole approval procedure, it proved difficult to obtain varieties with other GM traits for the experiments, but a single herbicide-tolerant (HT) variety was included at Foulum for the last 2 years. The field experiments comprised the "full-scale" experimental infrastructure of ECOGEN for soil fauna studies and allowed agricultural studies to be performed on cultivation practices – including tillage, pesticide and herbicide use – and the effects on yield and economic gross-margins.

Materials and methods

Field sites and experimental design

Foulum (North Jutland, Denmark) is the most northerly site at 56°30′N, 9°35′E. Varois is the intermediate location in Varois et Chaignot (Bourgogne, France), 47°34′N, 5°13′E. Narbons is the most southerly, located in Montesquieu Lauragais (Midi-Pyrenées, France) 43°26′N, 1°27′E. The climatic data obtained from the nearest meteorological stations were daily average values of air temperature, precipitation, global radiation, relative humidity, wind speed and wind direction. Figure 1 shows the summer temperatures at the three sites. While Foulum had a mean temperature over all four seasons 2002–2005 of 12.3 °C, the mean temperature in Narbons was higher at 18.4 °C, with Varois intermediate at 16.6 °C. Thus Foulum is at the northern border of maize cultivation with low temperatures in May to mid-July 2004 (and to a lesser extent also in 2005) that can significantly decrease the overall yield level. The summer of 2003 was unusually hot in France, with mean temperatures above 25 °C. It was also dry, the precipitation deficit reached almost 700 mm at Narbons, and while this was compensated by irrigation at this site, the maize suffered from drought at Varois.

The layouts of the long-term field experiments are shown in Fig. 2 for the last year of the project, 2005. At Foulum, different maize varieties were grown in a four-block design with four randomized plots surrounded with grassland in each block during 2002. The maize varieties were MEB307 (a MON810 *Bt*-variety), Monumental (the conventional variety near-isogenic to MEB307 but without the *Bt*-trait) and another conventional variety DK242. In 2002–2003, MEB307 was grown in two plots within each block. The experiment was repeated with the

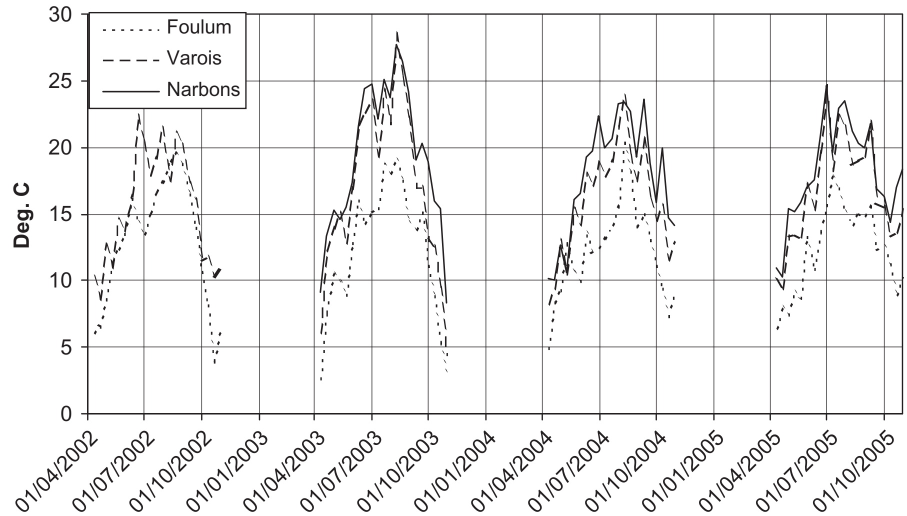

Figure 1. Average air temperature for 10-day periods during the four summer seasons (1 April–1 October) at the three experimental sites at Foulum (Denmark), Varois (Central France) and Narbons (Southern France).

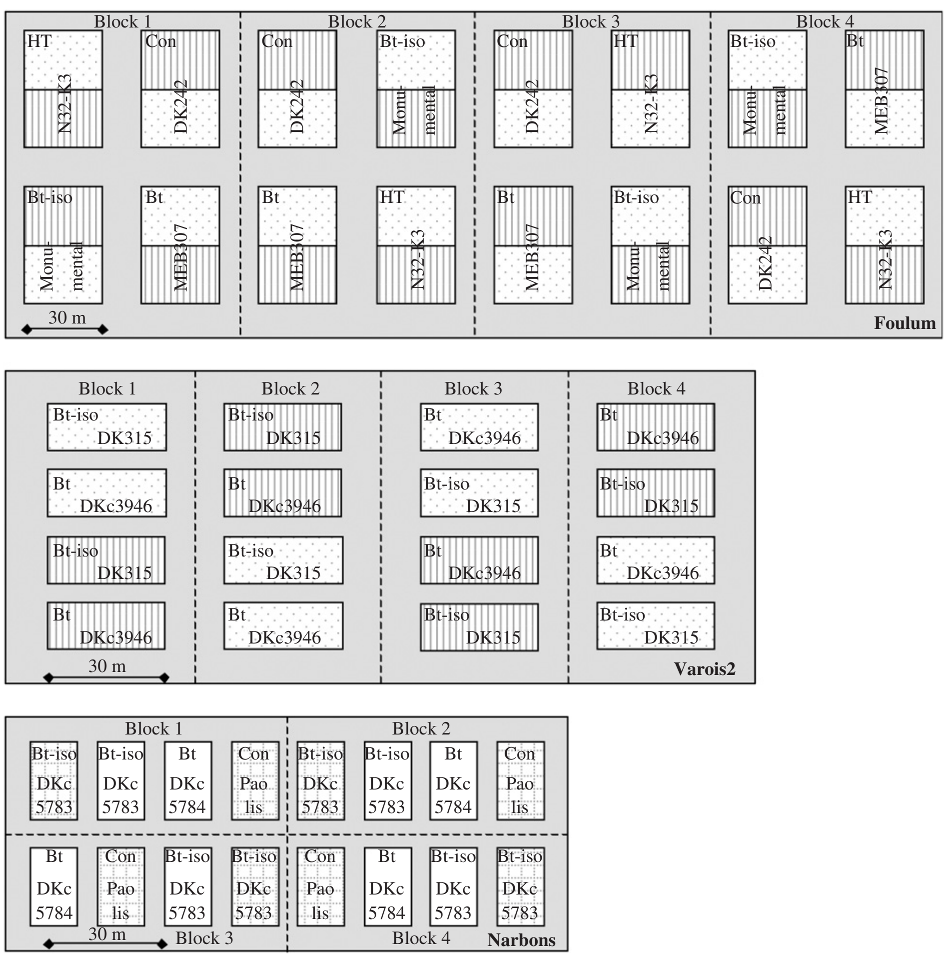

Figure 2. Layout in 2005 of the long-term field experiments with maize at the three ECOGEN sites in Europe: Foulum in Jutland, Denmark; Varois in Bourgogne, France; and Narbons in Midi-Pyrenées, France. The type of variety is indicated in the upper left corner of each plot and the variety name inside the plots. For Foulum and Varois2 plots patterned with lines ploughing and dots indicate reduced tillage. For Narbons plots with cross-hatching indicate pesticide application while white plots were without spraying.

GM and conventional varieties in the same plots during the following years 2003–2005. The field was ploughed in autumn of 2001 and prepared for sowing by application of $30\,\mathrm{t\,ha^{-1}}$ of cattle slurry. At seeding, May 2002, $150\,\mathrm{kg\,ha^{-1}}$ 17–9–0 NPK fertiliser was applied which was supplemented in June with $200\,\mathrm{kg\,ha^{-1}}$ calcium–ammonium–nitrate fertiliser containing ca. 27% N. The maize plants were harvested for silage production in October. During 2003–2005, each plot was divided in two halves: one part ploughed to a depth of 25 cm (as previous) and one part with reduced tillage from a rotary cultivator with vertical blades going to a depth of 8–10 cm. The experiment was thus amended to a split-plot design to assess the feasibility of fuel- and cost-saving tillage especially when HT maize varieties are introduced. However, due to non-availability in Europe of HT varieties, it was not before 2004–2005 the maize variety N32-K3, which is a T25 glufosinate-ammonium tolerant variety, was introduced in one main-plot of each block. In these, the broad-spectrum Basta herbicide with 20% glufosinate-ammonium as the active ingredient was substituted for the conventional herbicides used to control weeds in the other three varieties. Other cultivation practices were as in 2002.

At Varois, during 2002–2004 (Varois1, not shown in Fig. 2) there were two maize varieties MEB307 and Monumental, as at Foulum, in a four-block design with randomised plots. The field was ploughed, and seeded in May; after seeding $130\,\mathrm{kg\,ha^{-1}}$ ammonium nitrate fertiliser was applied. Pre- and post-emergence herbicides and insecticides were applied as standard practice to all plots. The maize was harvested when ripe in October and grain yield determined. During 2004–2005, a second experiment (Varois2, Fig. 2) was conducted at a nearby location with the *Bt*-variety DKc3946 and the conventional variety DK315. These were combined with conventional (ploughing, harrow and rotary harrow) versus reduced tillage (only harrow and rotary harrow) in a four-block split-plot design with 16 plots. Tillage was the main-plot factor and variety was the subplot-factor. Cultivation practices other than tillage were the same as in 2002–2003.

At Narbons, the experiment was conducted during 2003–2005 in a four-block design with completely randomized plots. There were three maize varieties in 2003: DK532Bt (MON810 *Bt*-variety), DK532 (a conventional variety near-isogenic to DK532Bt but without the *Bt*-trait) and DK312 (an unrelated conventional variety). In 2004–2005, three maize varieties were combined with either of two pesticide application strategies (Fig. 2). These were: (1) DKc5784 (Mon 810 *Bt*-variety), seed coated but without supplementary pesticide sprayings (PAS *Bt*); (2) DKc5783 (a conventional variety near-isogenic to DKc5784 but without the *Bt*-trait) treated as (1), i.e. PAS *Bt*; (3) DKc5783 seed coated and supplemented with two sprayings to control corn-borer infestation with Decis Protech $1.33\,\mathrm{l\,ha^{-1}}$ containing deltamethrine $15\,\mathrm{g\,l^{-1}}$(PAS Con.), and (4) Paolis (a registered conventional variety) treated as (3), i.e. PAS Con. The field was ploughed and seeded in May; after seeding $180\,\mathrm{kg\,ha^{-1}}$ ammonium nitrate fertiliser was applied. Pre- and post-emergence herbicides were applied as standard practice to all plots. The plots were irrigated regularly throughout the growing season and maize was harvested when ripe in October and grain yield determined.

At the start of the experiments, composite soil samples from all sites were collected from 0 to 30 cm depth in each plot with a standard soil-auger. Textural composition was determined after removal of C and $CaCO_3$ by dispersing a sample in sodium pyrophosphate solution and measuring clay and silt content with a hydrometer. Sand fractions were determined after wet sieving. Organic matter (OM) content was determined by combustion of a sample in O_2, followed by IR-measurement of the evolved CO_2, and using a factor of 1.7 for the conversion between C-content and OM-content. Content of calcium carbonate was determined by measuring the volume of CO_2 evolved when the sample was treated with HCl. pH was measured in an aqueous soil-water 1:2.5 (v/v) suspension with a pH-electrode. Cation exchange capacity (CEC) was determined by exchange with ammonium in an acetate solution at $\mathrm{pH} = 7.0$ and spectrophotometric determination of ammonium after exchange with sodium. Exchangeable sodium and potassium were measured on a flame spectrophotometer and calcium and magnesium by atomic absorption spectroscopy. Extractable phosphorus was determined after extraction with 0.5 M $NaHCO_3$ and spectrophotometric measurement.

For analysis of Cry1Ab protein, soil sampled from the upper 10 cm within maize rows but between plants (Griffiths et al., 2005) was sieved through a 4 mm diameter mesh and kept cold until frozen at $-80\,^{\circ}\mathrm{C}$ before determination using enzyme-linked immunosorbent assay (ELISA) kits (EnviroLogix QuantiPlate Kit for Cry1Ab/Cry1Ac, Portland, USA) as described earlier (Griffiths et al., 2006).

Based on their texture, the soils could be classified (Table 1) according to USDA (2003). They showed characteristic trends in composition of agricultural soils (Scheffer and Schachtschabel,

Table 1. Average topsoil (0–30 cm) composition determined by physical soil analyses at the three ECOGEN field sites

Site	Soil solids (%)							
	Clay (< 2 μm)	Silt (2–20 μm)	Coarse silt (20–50 μm)	Fine sand (50–200 μm)	Coarse sand (200–2000 μm)	Calcium carbonate	Organic matter	Soil type
Foulum	8.2	9.7	13.5	34.4	27.8	0.0	6.4	Sandy loam
SE	±0.2	±0.3	±0.4	±0.6	±0.3	±0.0	±0.12	
Varois1	30.7	10.9	16.9	9.6	3.1	18.8	4.8	Clay
Varois2	24.3	24.0	25.8	16.0	8.1	0.2	1.8	Silt loam
SE	±0.7	±0.2	±0.4	±0.5	±0.6	±0.1	±0.03	
Narbons	25.5	17.7	17.3	15.5	15.5	7.1	1.5	Clay loam
SE	±0.9	±0.7	±0.5	±0.4	±1.4	±1.4	±0.03	

Soil type classified according to USDA (2003) based on texture. For calculation of standard error of the mean (SE) number of observations (*n*) was 16 for Foulum, Varois2 and Narbons, and 1 for Varois1.

Table 2. Average chemical properties of topsoil (0–30 cm) at the three ECOGEN field sites

Site	pH (H_2O)	P (mg 100g^{-1})	K (mEq 100g^{-1})	Mg (mEq 100g^{-1})	Ca (mEq 100g^{-1})	CEC (mEq 100g^{-1})
Foulum	6.2	5.5	0.30	0.59	10.89	26.73
SE	±0.1	±0.2	±0.01	±0.02	±0.49	±0.58
Varois1	8.1	12.7	1.06	0.40	42.42	43.80
Varois2	7.0	7.3	0.85	0.84	16.34	–
SE	±0.1	±0.5	±0.06	±0.03	±0.50	
Narbons	8.2	15.8	0.16	0.09	7.59	15.83
SE	±0.1	±2.4	±0.01	±0.01	±0.37	±0.95

See Table 1 for information on statistics.

1979) related to temperature and other climatic variations between the sites. OM content decreased from North to South Europe, though the Foulum-soil was especially enriched with charcoal from repeated burning of heather vegetation. Calcium carbonate contents increased from North to South, although the site of Varois1 was an exception to this pattern. The pH of the soils (Table 2) reflected the contents of calcium carbonate and the degree of base-saturation of the total CEC. The soil at Varois is rich in potassium while the Narbons soil has a high content of extractable phosphorus.

Maize material harvested for silage at Foulum was analysed at the Central Laboratory of the Danish Institute of Agricultural Sciences using standard methods of analysis on one sample per plot. Ash content was determined after combustion of organic material at 550 °C (in accordance with Directive 71/250/EEC); Total N by complete combustion of sample in oxygen and measurement in thermal conductivity cell after removal of water, carbon oxides and sulphur oxides according to Hansen (1989); Total P and K by combustion of sample at 450 °C, extraction in HCl/HNO_3, followed by spectrophotometric quantification of a coloured complex between phosphate and vanadium molybdate for P (Directive 71/393/EEC; Stuffins, 1967) and flame photometry for K; Crude fibre was determined after extraction of sample with 0.13 M H_2SO_4, acetone and 0.23 M KOH (according to Directive 92/89/EEC). In vitro Enzyme-Digestible Organic Matter by successive extraction in pepsin-hydrochloric acid, cellulase–hemicellulases–amyloglucosidase, and acetone with subsequent determination of the residual non-digestible matter according to Weisbjerg and Hvelplund (1993). The feeding value for cattle was calculated in Scandinavian Feed Units (SFU) using the formula of Weisbjerg and Hvelplund (1993), which assigns weights to the different constituents. One SFU is approximately equal to 7.89 MJ of Net-energy (NE) (Sehestedt et al., 2004).

For analysis of Cry1Ab protein, *Bt*- and non-*Bt* maize plants (a mixture of stems and leaves), were freeze-dried and milled (Retsch mill, 0.2 mm sieve) before determination using enzyme-linked immunosorbent assay (ELISA) kits (QuantiPlate kit for *Bt*-Cry1Ab/1Ac protein, EnviroLogix, Portland, USA) see Griffiths et al. (2006).

Statistical procedures contained in the SAS software package (SAS Institute Inc., 1999) were employed to analyse data. Single year results were analysed using conventional analysis of variance (PROC ANOVA). Data including more years were analysed with the PROC MIXED procedure to account for correlations between measurements in the same plots.

Results

Maize yield and quality

The yield of silage maize in Foulum showed a considerable year-to-year variation (Table 3) that may be related to summer mean temperatures. April–October 2004 had the lowest temperature of 12.1 °C while 2002 had the highest mean temperature of the four seasons with 12.7 °C. No significant differences in yield were found between the

Table 3. Yield of dry matter (DM) and Scandinavian Feed Units (SFU) and quality variables of silage maize at Foulum for each of the years 2002–2005 giving mean values for each variety ($n = 4$–8) and mean values for each tillage system ($n = 16$), however, in 2005 only one composite sample per variety was analyzed for chemical composition

Year	Factor	Subfactor	Yield (t DM ha^{-1})	N (%)	P (%)	K (%)	Crude fibre (%)	Enzyme digestable OM (%)	Yield (SFU ha^{-1})
2002	Variety	MEB307	12.331	1.58a	0.180	1.48	23.6a	66.9b	9399b
		Monumental	11.939	1.54b	0.171	1.42	23.8a	67.1b	9116b
		DK242	12.894	1.52b	0.169	1.51	22.0b	68.0a	10003a
	P, H_0		0.07	0.014	0.10	0.28	0.0001	0.023	0.04
	$LSD_{.95}$		0.810	0.036	0.011	0.10	0.49	0.84	689
2003	Variety	MEB307	11.685	1.25	0.168	1.13	25.0	64.4	8481
		Monumental	11.722	1.23	0.163	1.07	25.4	64.8	8541
	P, H_0		0.85	0.40	0.37	0.09	0.19	0.48	0.79
	$LSD_{.95}$		0.584	0.064	0.014	0.07	0.85	1.41	660
	Tillage	Ploughed	11.726	1.24	0.166	1.14	25.2	64.5	8518
		Reduced	11.682	1.24	0.166	1.06	25.2	64.6	8505
	P, H_0		0.81	0.62	1.00	0.09	0.93	0.90	0.93
	$LSD_{.95}$		0.367	0.037	0.004	0.10	0.78	1.36	323
2004	Variety	MEB307	7.117a	1.42b	0.202b	1.43b	25.6b	63.2a	5023a
		Monumental	7.026a	1.45b	0.213b	1.40b	25.0bc	62.8a	4974a
		DK242	7.476a	1.40b	0.202b	1.26b	24.8c	63.5a	5311a
		N32-K3	1.975b	2.27a	0.271a	2.17a	27.3a	59.7b	1319b
	P, H_0		0.0001	0.0001	0.0001	0.0100	0.0001	0.0001	0.0001
	$LSD_{.95}$		0.539	0.07	0.013	0.51	0.55	0.90	360
	Tillage	Ploughed	5.823	1.65a	0.222	1.58	25.6	62.3	4125
		Reduced	5.974	1.62b	0.222	1.55	25.7	62.2	4189
	P, H_0		0.07	0.03	0.83	0.38	0.95	0.76	0.23
	$LSD_{.95}$		0.165	0.03	0.007	0.076	0.37	0.72	114
2005	Variety	MEB307	9.765b	1.32	0.189	1.13	26.3	62.2	6817
		Monumental	9.689b	1.26	0.174	0.98	26.1	63.2	6889
		DK242	10.314a	1.22	0.177	0.97	24.6	63.8	7438
		N32-K3	8.952b	1.28	0.183	1.00	25.5	64.0	6446
	P, H_0		0.009	–	–	–	–	–	–
	$LSD_{.95}$		0.660	–	–	–	–	–	–
	Tillage	Ploughed	9.673	1.26	0.183	1.04	25.8	63.2	6871
		Reduced	9.687	1.28	0.179	1.00	25.5	63.3	6924
	P, H_0		0.95	–	–	–	–	–	–
	$LSD_{.95}$		0.427	–	–	–	–	–	–

Percentages are w/w. Hypothesis H_0 is no difference between varieties or tillage treatments and is tested with a *F*-test. If $P<0.05$ for H_0, differences are further discriminated with a *t*-test indicated by lettering: values, which do not share a common letter, are significantly different at the 0.05 level. There was only one significant interaction between varieties and tillage treatments on yield of DM in 2004.

Table 4. Yield, grain weight and corn-borer populations at the Narbons site as affected by variety-pesticide regime

Variety	Pesticide application strategy	Grain yield (t DM ha^{-1})	Grain weight (mg $kernel^{-1}$)	Sesamia (number $plant^{-1}$)	Ostrinia (number $plant^{-1}$)
DKc5784	PAS *Bt*	10.1a	301a	0.1c	0.0b
DKc5783	PAS *Bt*	8.4b	277b	1.9a	0.5a
DKc5783	PAS Con.	9.6a	284b	0.5b	0.1b
Paolis	PAS Con.	9.2ab	279b	0.8b	0.1b
$LSD_{.95}$		1.00	15	0.34	0.10
P for H_0		0.029	0.022	$<$ 0.0001	$<$ 0.0001

DKc5784 is the *Bt*-variety and DKc5783 is its near-isogenic counterpart, while Paolis is an unrelated conventional variety. The crop management practices were either a conventional pesticide application strategy to control corn-borer larvae (PAS Con.) or a no application suitable for *Bt*-varieties (PAS *Bt*). Results for 2004 and 2005 were aggregated in the analysis, and the number of observations is 8 for each mean value. Hypothesis H_0 is no difference between variety-pesticide regimes and is tested with a *F*-test. If $P<0.05$ for H_0, differences are further discriminated with a *t*-test indicated by lettering: values, which do not share a common letter, are significantly different at the 0.05 level. There were no effects of year or interaction between year and treatment.

Bt-maize MEB307 and the near-isogenic line Monumental in any of the 4 years and the *Bt*-trait had no effect on yield at this site. The conventional DK242 variety seemed to be slightly better adapted to the temperature conditions in Foulum and gave a significantly higher yield of SFU in 2002 and dry matter in 2005. The much lower yield of the Basta-tolerant N32-K3 variety in 2004 was due to a 1.5-month delay in seeding. The tillage system had little effect on yield, which was very similar for ploughed and reduced tillage with the exception that in 2004 the Basta-tolerant N32-K3 variety gave a higher yield of dry matter in reduced tillage plots, creating a significant interaction between variety and tillage system. Beside this effect of late seeding, there was no interaction between tillage system and the choice of variety-herbicide combination. Although reduced tillage is believed often to result in increased weed abundance, both conventional maize herbicides and Basta were able to control weeds adequately. For the individual chemical analyses (concentration of N, P, K, crude fibre and enzyme digestible OM) that together with DM-yield determined the yield of SFU, there were few significant differences between varieties. Out of these, there was a difference between *Bt*-maize MEB307 and the near-isogenic Monumental only in one case, the former having a higher N-content at harvest 2002: 1.58% versus 1.54%, which is a small difference, when compared to year-to-year variations. In general, the $LSD_{0.95}$ values for the measured yield and quality variables were smaller than year-to-year variations (Table 3), showing that the experimental design with four replicates would have detected major differences between *Bt*-maize and the near-isogenic variety if such had existed.

In Varois, there were no significant differences in grain yield between *Bt*-maize and the near-isogenic variety. As in Foulum, the absence of a significant infestation level of the European corn-borer did not provide good test conditions for showing a yield benefit of *Bt*-maize. There were also no significant differences in yield between the tillage systems (data not shown).

Narbons provided a better location for testing of the possible agricultural benefits of the *Bt*-trait in maize, since significant populations of the Mediterranean corn-borer (*Sesamia nonagrioides*), as well as the European corn-borer (*Ostrinia nubilalis*), are present in most seasons. For 2004 and 2005, where the experimental designs were identical, with the same varieties in the same plots, an aggregate analysis (Table 4) showed that there were significant benefits of the *Bt*-maize DKc5784 with regard to grain yield and grain weight, when compared to its near-isogenic variety DKc5783 without pesticide sprayings. The *Bt*-maize also tended to perform better than conventional varieties treated with pesticides to control corn-borer infestation, although this result was statistically significant only for the grain weight. Counting of corn-borer larvae supported these results on maize yield and grain weight, since the populations of both species were significantly lower in the *Bt*-maize than in its near-isogenic variety without pesticide sprayings (Table 4). The population of *Sesamia* in the *Bt*-maize was also lower than in conventional varieties sprayed with pesticides.

Cry1Ab content in plant and soil

Analyses of plant leaf material (Table 5) showed the content of Cry1Ab protein to mostly be in the range of 5–15 mg kg^{-1} dry matter in the *Bt*-varieties. Conventional varieties had contents of

Table 5. Cry1Ab content in soil and plant material at three sites and 2 years

Site	Variety	Treatment	2004			2005				
			May	Oct.		May	Aug.		Oct.	
			Soil	Soil	Plant	Soil	Soil	Plant	Soil	Plant
Foulum	MEB307	Red. Till	0.70	1.86	10.61	1.05	1.82		1.05	13.10
	Monumental	Red. Till	0.66	1.57	0.01	0.93	1.75		0.88	0.00
	N32-K5	Red. Till	0.72	1.55	0.00	0.95	1.75		0.91	0.00
	MEB307	Ploughing	0.68	2.20	12.03	0.97	1.74	23.69	1.14	14.04
	Monumental	Ploughing	0.66	1.56	0.01	0.93	1.66	0.00	0.90	0.00
	N32-K5	Ploughing	0.67	1.51	0.01	0.94	1.69	0.00	0.90	0.00
	$LSD_{.95}$	Variety	0.018	0.19	0.588	0.05	0.055	n/a	0.06	1.168
	$LSD_{.95}$	Tillage	0.014	n.s.	0.464	n.s.	0.045	n/a	n.s.	n.s.
	$LSD_{.95}$	V × T	0.025	n.s.	0.831	n.s.	n.s.	n/a	n.s.	n.s.
Varois	DKc3946	Red. Till	0.60	1.62	3.38	1.10			1.67	7.83
	DK315	Red. Till	0.60	1.40	0.00	0.88			1.55	0.00
	DKc3946	Ploughing	0.60	1.57	4.08	0.89			1.60	8.61
	DK315	Ploughing	0.60	1.40	0.00	0.88			1.58	0.00
	$LSD_{.95}$	Variety	n.s.	0.060	0.668	n.s.			0.047	n.s.
	$LSD_{.95}$	Tillage	n.s.	n.s.	n.s.	n.s.			n.s.	n.s.
	$LSD_{.95}$	V × T	n.s.	n.s.	n.s.	n.s.			n.s.	n.s.
Narbons	DKc5784	PAS *Bt*	0.63	1.50	5.09	1.00	1.77	4.70	1.71	4.50
	DKc5783	PAS Con.	0.63	1.36	0.00	0.97	1.68	0.00	1.67	0.00
	DKc5783	PAS *Bt*	0.63	1.38	0.02	0.95	1.71	0.00	1.69	0.00
	Paolis	PAS Con.	0.63	1.40	0.06	1.02	1.71	0.00	1.69	0.00
	$LSD_{.95}$	Variety	n.s.	0.060	0.474	n.s.	n.s.	1.542	0.03	1.617

Units are $\mu g\,kg^{-1}$ dry soil, or $mg\,kg^{-1}$ plant dry matter. The plant material analysed consisted of a mixture of stems and leaves from Foulum and leaf material only from Varois and Narbons. The pesticide application strategies in Narbons were either conventional pesticide application to control corn-borer larvae (PAS Con.) or no application suitable for *Bt*-varieties (PAS *Bt*).

the protein not significantly different from zero. Cry1Ab protein concentrations found in the soil were increased in the plots with *Bt*-varieties but they did not seem to increase from year to year (Table 5).

Discussion

The absence of a significant infestation level of the European corn-borer did not provide test conditions for showing a yield benefit of *Bt*-maize at the two northernmost locations in Foulum and Varois. The results reported here demonstrated that there were minimal differences between the *Bt*-varieties and their near-isogenic counterparts without the *Bt* trait with respect to yield and main quality characteristics related to the feeding value of silage maize as investigated in Foulum. The summer mean temperatures in Foulum seemed to influence the yield level, which was highest in 2002 and quite low the other years. An effect that was probably exacerbated by the selection of varieties that was grown in the experiment. Both the GM-varieties MEB307 and N32-K3 are bred for more southerly, higher temperature conditions. In Narbons, the infestation level of corn-borer was higher. Under these conditions, the number of two species of corn-borer larvae on the near-isogenic non-*Bt*-variety DKc5783 was significantly higher than on the *Bt*-variety DKc5784 (Table 4). For the *Sesamia* corn-borer this was the case even when the non-*Bt*-varieties were sprayed with pesticides to control the corn-borer. A negative correlation between corn-borer infestation and maize yield has previously been demonstrated, and was estimated to be in the range of 2–3% yield loss per larve/plant (Dillehay et al., 2004, and references therein). Our results from Narbons generally correspond with this, since yield and grain weight of the *Bt*-variety was significantly higher than in the near-isogenic non-*Bt*-variety. It appeared that repeated pesticide application did not provide as good a protection level as the Cry1Ab content of the *Bt*-maize, since grain size was still significantly reduced in the near-isogenic non-*Bt*-variety and yield also tended to be reduced. Although the latter tendency was not significant, it is in line with the above correlation. It is well known that the larvae of the corn-borer are difficult to kill by pesticide application, since they reside inside stems and cobs, and thus are protected from direct contact with surface-applied chemicals. The MON810 Cry1Ab protein, on the other hand, is constitutively expressed in all parts of the maize plant (Mendelsohn et al., 2003) and thus is ingested by the larvae when they feed on the plant. In summary, *Bt*-maize produced a higher grain yield and better grain size than an unsprayed near-isogenic non-*Bt*-variety, or allowed a significant reduction in pesticide use by saving two times spraying each year while producing the same yield.

The concentrations of Cry1Ab measured in the *Bt*-varieties were in the range reported by others (Mendelsohn et al., 2003) to effectively control corn-borer larvae and were consistent with previous findings. Over the first 2 years of the trial at Foulum (2002 and 2003) the concentration of *Bt* protein in the leaves of MEB307 at harvest was ca. 10 mg kg^{-1} (Griffiths et al., 2005), while the same variety grown under glasshouse conditions gave 9 mg kg^{-1} in Foulum soil and 14 mg kg^{-1} at the Varois site (Griffiths et al., 2006). There were differences between the concentrations of Cry1Ab in the *Bt*-maize varieties grown at the different sites (Table 5), but this could be a site effect as there were no *Bt*-varieties common to the three sites.

Experiments at Foulum and Varois showed that reduced tillage could be applied without losses in yield and quality when compared to conventional ploughing. Both conventional herbicide application and use of Basta in the glufosinate-ammonium tolerant variety at Foulum were able to keep the weed populations at acceptable levels. Some of the conventional herbicides used in maize cultivation are considered a threat to groundwater resources because of risk of leaching (Kjaer et al., 2004). In so far as GM herbicide tolerant varieties can be applied or developed that are tolerant to more environmentally friendly herbicides, this may be of benefit to the environment. However, the concentration and persistence of proteins specifically formed by the GM varieties and introduced in the soil ecological system would also need to be considered. We investigated the concentrations of Cry1Ab-*Bt*-protein in the soil at the field sites. The measured values were close to the limit of detection and the protein did not accumulate in the soil year on year. The concentrations in the field were less than determined for the same soil/variety combinations in a glasshouse experiment where up to 43 μg kg^{-1} was detected (Griffiths et al., 2006). Mendelsohn et al. (2003) reported a half-life in soil of 1.6 days for degrading plant tissue and 8.3 days for purified protein in soil. Baumgarte and Tebbe (2005) were able to detect Cry1Ab-protein after the winter period in field soil grown with MON810-maize the previous year, but the content was very low. The values determined in our field plots were of the same order as the 4 μg kg^{-1} detected under *Bt*-maize in a Canadian field study, leading to the conclusion that much of the *Bt*-protein entering the soil decomposes quickly but that a small fraction is protected in relatively recalcitrant residues (Hopkins and Gregorich, 2003). The quantification of *Bt*-protein in soil was confounded by the low concentrations in soil and interference from soil factors, as suggested by the seasonal variation in the amounts of *Bt*-protein apparently detected even under non-*Bt* maize (Table 5). The use of new extraction techniques may enable a more accurate determination of the amounts and dynamics of *Bt*-protein in soil (Shan et al., 2005).

Acknowledgements

This research was supported by the EU RTD Quality of Life Programme; contract ECOGEN (http://www.ecogen.dk), no. QLRT-2001-01666. SCRI receives grant-in-aid from the Scottish Executive Environment and Rural Affairs Department. We thank Monsanto for supplying seed.

References

Angle, J.S., 1994. Release of transgenic plants: biodiversity and population-level considerations. Mol. Ecol. 3, 45–50.

Baumgarte, S., Tebbe, C.C., 2005. Field studies on the environmental fate of the Cry1Ab *Bt*-toxin produced by transgenic maize (MON810) and its effect on bacterial communities in the maize rhizosphere. Mol. Ecol. 14, 2539–2551.

Bruinsma, M., Kowalchuk, G.A., van Veen, J.A., 2003. Effects of genetically modified plants on microbial communities and processes in soil. Biol. Fertil. Soils 37, 329–337.

Dillehay, B.L., Roth, G.W., Calvin, D.D., Kratochvil, R.J., Kuldau, G.A., Hydeet, J.A., 2004. Performance of Bt corn hybrids, their near isolines, and leading corn hybrids in Pennsylvania and Maryland. Agron. J. 96, 818–824.

Griffiths, B.S., Caul, S., Thompson, J., Birch, A.N.E., Scrimgeour, C., Andersen, M.N., Cortet, J., Messean, A., Sausse, C., Lacroix, B., Krogh, P.H., 2005. A comparison of soil microbial community structure, protozoa and nematodes in field plots of conventional and genetically modified maize expressing the *Bacillus thuringiensis* CryIAb toxin. Plant Soil 275, 135–146.

Griffiths, B.S., Caul, S., Thompson, J., Birch, A.N.E., Scrimeour, C., Cortet, J., Foggo, A., Hackett, C.A., Krogh, P.H., 2006. Soil microbial and faunal community responses to *Bt*-maize and insecticide in two soils. J. Environ. Qual. 35, 734–741.

Groot, A.T., Dicke, M., 2002. Insect-resistant transgenic plants in a multi-trophic context. Plant J. 31, 387–406.

Hansen, B., 1989. Determination of nitrogen as elementary N, an alternative to Kjeldahl. Acta Agric. Scand. 39, 113–118.

Hopkins, D.W., Gregorich, E.G., 2003. Detection and decay of the *Bt* endotoxin in soil from a field trial with genetically modified maize. Eur. J. Soil Sci. 54, 793–800.

James, C., 2006. Preview: global status of commercialized transgenic crops: 2005. ISAAA Briefs No. 34. International Service for the Acquisition of Agribiotech Applications, Ithaca, New York.

Jepson, P.C., Croft, B.A., Pratt, G.E., 1994. Test systems to determine the ecological risks posed by toxin release from *Bacillus thuringiensis* genes in crop plants. Mol. Ecol. 3, 81–89.

Kjaer, J., Olsen, P., Barlebo, H.C., Juhler, R.K., Plauborg, F., Grant, R., Gudmundsson, L., Brüsch, W., 2004. The Danish Pesticide Leaching Assessment Programme. Monitoring results May 1999–June 2003. Geological Survey of Denmark and Greenland, Copenhagen. 110pp. (Report available at: ⟨http://pesticidvarsling.dk/xpdf/vap-results-99-03.pdf⟩).

Mendelsohn, M., Kough, J., Vaituzis, Z., Matthews, K., 2003. Are *Bt* crops safe? Nat. Biotech. 21, 1003–1009.

SAS Institute Inc., 1999. SAS OnlineDoc®, Version 8. SAS Institute Inc., Cary.

Scheffer, F., Schachtschabel, P., 1979. Lehrbuch der Bodenkunde, 10. Aufl. Ferdinand Enke Verlag, Stuttgart.

Sehestedt, J., Söegaard, K., Danielsen, V., Roepstorff, A., Monrad, J., 2004. Grazing with heifers and sows alone or mixed: herbage quality, sward structure and animal weight gain. Livestock Prod. Sci. 88, 223–238.

Shan, G., Embrey, S.K., Herman, R.A., Wolt, J.D., Weston, D., Mayer, L.M., 2005. Biomimetic extraction of *Bacillus thuringiensis* insecticidal crystal proteins from soil based on invertebrate gut fluid chemistry. J. Agric. Food Chem. 53, 6630–6634.

Stuffins, C.B., 1967. The determination of phosphate and calcium in feeding stuffs. Analyst 92, 107–111.

Trevors, J.T., Kuikman, P., Watson, B., 1994. Transgenic plants and biogeochemical cycles. Mol. Ecol. 3, 57–64.

USDA, 2003. Keys to Soil Taxonomy, 9th edition. US Department of Agriculture, Natural Resources Conservation Service, Government Printing Office, Washington, DC (Available at: ⟨http://www.soils.usda.gov/technical/classification/tax_keys/keysweb.pdf⟩).

Weisbjerg, M.R., Hvelplund, T., 1993. Determination of net-energy content (FEK) in raw materials and cattle feed mixtures. Research Report No. 3, Danish Institute of Animal Science, Foulum, Denmark, 39pp (in Danish).

Wolfenbarger, L.L., Phifer, P.R., 2000. The ecological risks and benefits of genetically engineered plants. Science 290, 2088–2093.