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Monitoring the impact of *Bt* maize on butterflies in the field: estimation of required sample sizes

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The monitoring of genetically modified organisms (GMOs) after deliberate release is important in order to assess and evaluate possible environmental effects. Concerns have been raised that the transgenic crop, *Bt* maize, may affect butterflies occurring in field margins. Therefore, a monitoring of butterflies was suggested accompanying the commercial cultivation of *Bt* maize. In this study, baseline data on the butterfly species and their abundance in maize field margins is presented together with implications for butterfly monitoring. The study was conducted in Bavaria, South Germany, between 2000–2002. A total of 33 butterfly species was recorded in field margins. A small number of species dominated the community, and butterflies observed were mostly common species. Observation duration was the most important factor influencing the monitoring results. Field margin size affected the butterfly abundance, and habitat diversity had a tendency to influence species richness. Sample size and statistical power analyses indicated that a sample size in the range of 75 to 150 field margins for treatment (transgenic maize) and control (conventional maize) would detect (power of 80%) effects larger than 15% in species richness and the butterfly abundance pooled across species. However, a much higher number of field margins must be sampled in order to achieve a higher statistical power, to detect smaller effects, and to monitor single butterfly species.

Keywords: Lepidoptera / abundance / species richness / field margins / genetically modified organisms / transgenic crop / *Bacillus thuringiensis* / surveillance / monitoring / non-target effects / sample size calculation / statistical power analysis

INTRODUCTION

Over the last decade numerous transgenic crops have been engineered for specific traits (Nap et al., 2003). Prominent examples are crops modified with genes of the bacterium *Bacillus thuringiensis* (*Bt* crops). *Bt* crops produce proteins that are toxic to some pest insects that feed on these plants (Van Rie, 2000). Despite their potential economic value, there have been public and scientific concerns about potential adverse environmental effects of transgenic crops when cultivated commercially (Conner et al., 2003; Lövei, 2001; Villiger, 1999). In several countries, legislation has been enacted to ensure the identification and evaluation of potential environmental risks associated with genetically modified organisms (GMOs). The legislative framework of the European Community is based on the EU Directive 2001/18/EC, specifying a pre-release risk assessment of GMOs and post-release monitoring both for research purposes and their placing on the market (European Parliament and Council, 2001). The EU Directive further separates post-release monitoring into "case-specific monitoring" and "general surveillance". Case-specific monitoring should address specific hypothetical problems associated with particular characteristics of the transgenic organism (which may arise during pre-release risk assessment), while general surveillance should focus on unanticipated and possible long-term effects. Each applicant requesting either a release of GMOs into the environment or placing GMOs on the market is obliged by the EU Directive to submit a detailed post-release monitoring plan with the official application. What and how to monitor are currently under discussion, and several proposals about content and scope have been made (Jepson et al., 1994; Schmitz et al., 2003; Wilhelm et al., 2002; Züghart and Breckling, 2003).

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Of all transgenic insect-resistant crops, *Bt* maize is most advanced in the EU registration process and will probably be the first genetically modified crop to be cultivated commercially throughout Europe. Consequently, a monitoring plan must be developed according to EU guidelines. The two maize events that have been submitted for application in the European Community were both transformed with *B. thuringiensis* var. *kurstaki* genes to express an insecticidal lepidopteran-specific crystalline (Cry) protein (delta-endotoxin) against the European corn borer (Lepidoptera, Pyralidae: *Ostrinia nubilalis*), a pest feeding on maize (Van Rie, 2000). As the *Bt* toxin is specifically active on lepidopteran species, the impact on non-target organisms has been considered negligible. However, concerns have been raised that *Bt* maize may affect Lepidoptera other than pest species as well (Felke et al., 2002; Hansen Jesse and Obrycki, 2000; Losey et al., 1999). Most commercial *Bt* maize hybrids express the toxin also in the pollen, which may then be deposited on host plants of lepidopteran larvae occurring in field margins close to maize fields (Pleasants et al., 2001). So far, lethal and negative sub-lethal effects of *Bt* maize pollen consumption for larvae were demonstrated for a variety of butterfly species in the laboratory, the absolute effect depending on the particular maize event, the amount of pollen consumption, the lepidopteran species, the instar of the larvae and other factors (Felke and Langenbruch, 2001; Felke et al., 2002; Hansen Jesse and Obrycki, 2000; Hellmich et al., 2001; Losey et al., 1999). Field experiments published to date have confirmed possible adverse effects of the *Bt* maize event 176 on some butterfly larvae, while event 810 seems to be much less toxic (Stanley-Horn et al., 2001; Wraight et al., 2000; Zangerl et al., 2001). Considering the wind dispersal of maize pollen, the possible deposition of pollen on host plants of butterfly larvae near maize fields, and possible adverse effects of *Bt* maize pollen consumption on lepidopteran larvae, a case-specific monitoring plan for butterflies occurring in field margins appears to be essential for commercial cultivation of transgenic *Bt* maize. Such a monitoring of field edges seems especially necessary in Europe, with its often small structured agricultural landscapes containing many field margins. Butterflies have also been suggested for "general surveillance" monitoring (Züghart and Breckling, 2003), because they are a well-known group often used to indicate environmental changes, for signalling effects of management practices and for monitoring succession (Brunzel and Plachter, 1999; Feber et al., 1996; Firbank et al., 2003; Johnson et al., 1995; Kruess and Tscharntke, 2002; Miller, 1990; Steffan-Dewenter and Tscharntke, 1997; Wagner et al., 1996). Despite the vast amount of information about butterflies occurring on arable land, no comprehensive study has yet been published on butterfly species occurring in maize field margins. Moreover, no protocols for using butterfly monitoring in GMO environmental impact assessments exist.

This study considers aspects relevant for the design and implementation of butterfly monitoring programs along maize field margins. In order to design such a monitoring plan, we need to know what butterfly species occur in the habitats concerned, how much effort is needed per sample, how habitat characteristics influence sampling success, and how many samples are needed. This study does not aim to evaluate the impact of *Bt* maize on butterfly species or populations and resulting ecological implications, neither does it advocate bioindicator species, but it focused on the experimental design of a butterfly monitoring plan suited specifically to *Bt* maize cultivation. The objectives were (i) to complete a baseline inventory of the butterfly community occurring in maize field margins, (ii) to analyze the effects of sampling effort, field margin area and habitat diversity on monitoring results, and (iii) to calculate the required sample sizes necessary to detect potential effects of *Bt* maize based on the original field data of this study.

RESULTS

Abundance and species number of butterflies

Altogether, 809 individuals of 33 butterfly species were recorded in 20 maize field margins during 1910 min of monitoring (Tab. 1). Estimated species richness was between 38.5 species (Abundance-base Coverage Estimator) and 39.2 species (Incidence-base Coverage Estimator), which suggested that about 84%–86% of the occurring species had been recorded. On average, 9.25 species and 39.65 individuals were recorded per field margin (Tab. 2). The most abundant species were *Pieris rapae* (38.07% of the observations) and *P. napi* (9.27%), all other species representing less than 5% (Tab. 1). The most abundant species were also observed in more field margins (Tab. 1). Twenty-one percent of the observed species are listed in the federal and state Red Data Books, but generally in minor classifications (Tab. 1).

Parameters affecting abundance and species number of butterflies

Species richness was positively correlated with total monitoring time ($r = 0.64$, $P < 0.05$, Fig. 1A), number of

Table 1. Species list of adult butterflies monitored in margins of maize fields in Bavaria, South Germany, 2000–2002. Presented are the total number of observations, the relative abundance (= frequency), and the percentage of margins where the species were observed (= occupancy).

Species	Red data book		Observations	Frequency	Occupancy
	BY[2]	BRD[3]	(n)	(%)	(%)
Papilio machaon L.[1]		V[4]	16	1.98	40
Leptidea sinapis L. / *reali* Reiss.	D[5]	V[4]	2	0.25	10
Colias hyale L. / *alfacariensis* Ribbe			1	0.12	5
Gonepteryx rhamni L.			10	1.24	25
Pieris brassicae L.			26	3.21	20
Pieris rapae L.[1]			308	38.07	95
Pieris napi L.[1]			75	9.27	80
Anthocharis cardamines L.			10	1.24	25
Apatura iris L.	V[4]	V[4]	1	0.12	5
Apatura ilia Denis & Schifferm.	V[4]	3[6]	2	0.25	10
Limenitis camilla L.	V[4]	3[6]	1	0.12	5
Nymphalis io L.[1]			29	3.58	60
Nymphalis c-album L.			3	0.37	15
Nymphalis urticae L.[1]			34	4.20	75
Vanessa atalanta L.[1]			19	2.35	55
Vanessa cardui L.[1]			20	2.47	45
Araschnia levana L.[1]			12	1.48	15
Argynnis paphia L.			10	1.24	5
Boloria lathonia L.			20	2.47	40
Clossiana selene Denis & Schifferm.	3[6]	V[4]	2	0.25	5
Melanargia galathea L.			30	3.71	25
Maniola jurtina L.			38	4.70	45
Aphantopus hyperantus L.			37	4.57	30
Coenonympha pamphilus L.			37	4.57	70
Pararge aegeria L.			4	0.49	5
Lasiommata megera L.			2	0.25	10
Neozephyrus quercus L.			1	0.12	5
Lycaena phlaeas L.			4	0.49	10
Polyommatus icarus Rottemburg			20	2.47	35
Carterocephalus palaemon Pallas		V[4]	1	0.12	5
Thymelicus sylvestris Poda			12	1.48	15
Thymelicus lineola Ochsenheimer			19	2.35	30
Ochlodes sylvanus Esper			3	0.37	15

[1] Egg-laying behaviour was observed, or larvae or pupae were found; [2] after Bayer. LfU (2003), Red Data List Bavaria; [3] after Pretscher (1998), Red Data List Germany. [4] V = near Threatened; [5] D = data Deficient; [6] 3 = vulnerable.

Table 2. Variables recorded, their mean values, *sd*, and minimum-maximum values in the 20 field margins studied, Bavaria, 2000–2002.

Variable	Mean	1SD	Minimum	Maximum
Margin length (m)	643.35	475.53	149	2000
Margin width (m)	10.40	4.36	4	18
Margin area (m^2)	3969.53	3008.05	745	10100
Number of habitat types (n)	4.05	1.43	2	6
Monitoring time (min)	95.50	63.64	30	305
Site visits (n)	4.15	2.72	1	13
Study seasons (n)	1.35	0.67	1	3
Lepidoptera species (n)	9.25	4.13	3	17
Lepidoptera abundance (n)	39.65	27.66	6	103

habitats (r = 0.50, $P < 0.05$, Fig. 1C), number of study seasons (r = 0.50, $P < 0.05$), number of visits (r = 0.48, $P < 0.05$), and butterfly abundance (r = 0.86, $P < 0.001$). Likewise, abundance of butterflies was positively correlated with total monitoring time (r = 0.70, $P < 0.001$, Fig. 2A), number of habitats (r = 0.46, $P < 0.05$, Fig. 2C), number of study seasons (r = 0.45, $P < 0.05$), and number of visits (r = 0.49, $P < 0.05$). Of the independent variables, monitoring time was inter-correlated with number of study seasons (r = 0.72, $P < 0.001$) and number of visits (r = 0.85, $P < 0.05$). To identify the key variables determining species number and butterfly abundance, a multiple linear regression was performed with the following independent variables (Tab. 2): total monitoring time, number of visits, number of study seasons (log transformed), *Bt* status of the maize field (dummy variable), field margin area, number of habitat types and study site (independent variables that did not contribute significantly to the model were subsequently ignored). Variation in species richness per field margin (Tab. 2) was best explained by monitoring time according to the regression equation:

number of species = 0.004 * monitoring time + 5.31 ($R^2 = 0.40$, $P < 0.01$, Fig. 1A). There was a trend that "number of habitats" affected species richness positively (Fig. 1C): including habitats into the model increased R^2 to 0.50, but decreased the P-value to 0.08. Margin area did not influence the number of butterfly species (Fig. 1B).

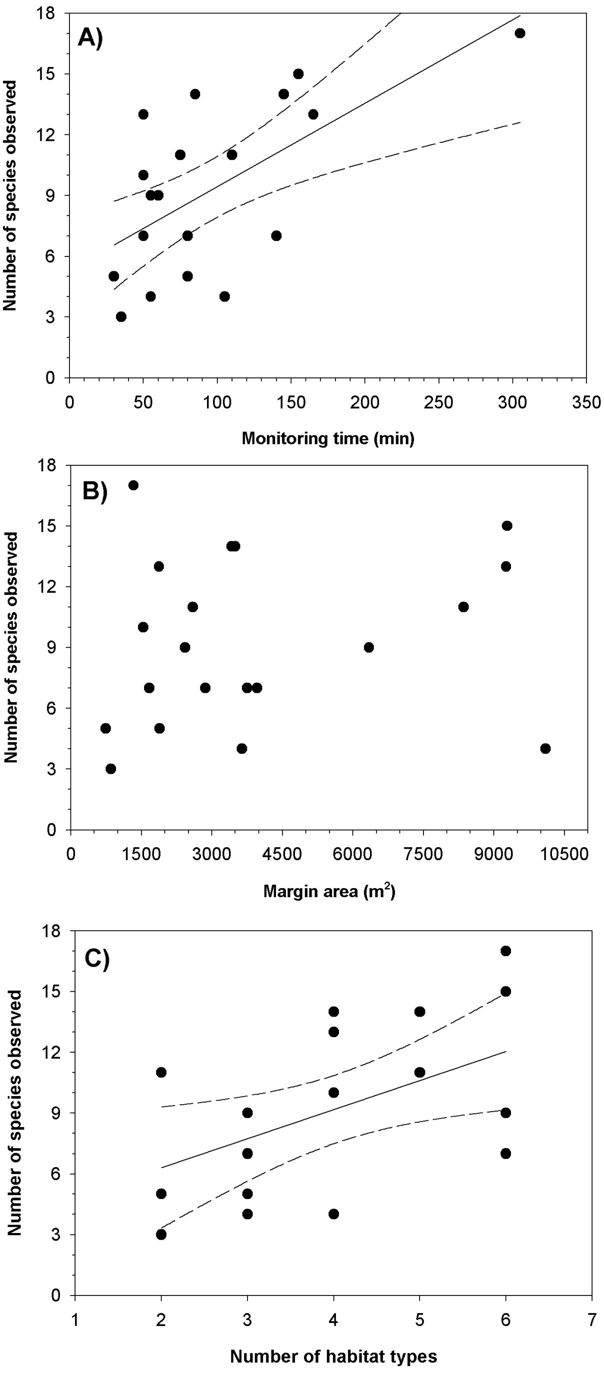


Figure 1. Influence of (**A**) monitoring time, (**B**) field margin area, and (**C**) number of habitat types on observed number of butterfly species occurring in field margins. In case of significant correlations ($P < 0.05$) the linear regression with 95% confidence interval is shown. Linear regression equation for (A): y = 5.31 + 0.04x, $R^2 = 0.40$, $P < 0.01$. (B) $R^2 = 0.03$, $P > 0.40$. (C): y = 3.45 + 1.43x, $R^2 = 0.25$, $P < 0.05$.

Butterfly abundance per field margin (Tab. 2) was best explained by monitoring time and margin area as modelled by the regression equation:

butterfly abundance = 0.305 * monitoring time + 0.004 * margin area – 6.234 ($R^2 = 0.69$, $P < 0.001$, Fig. 2A,B).

A model with monitoring time alone explained 49% of the total variance (Fig. 2A). The number of habitats was not identified as a significant factor by the multiple regression analysis, although it was positively correlated with butterfly abundance in a bivariate correlation (Fig. 2C).

Sample size and statistical power analysis

Table 3 displays the number of field margins that should be sampled to detect an effect of *Bt* maize on butterflies. For example, to detect a reduction of 5% in species richness with a probability of 80%, a total of 2156 field margins should be surveyed: 1078 margins along *Bt* maize fields and for comparison, another 1078 margins along conventional maize fields. Conversely, by monitoring 12 field margins (12 along *Bt* fields and 12 along conventional fields), only a species richness reduction of 50% or greater can be detected with 80% confidence. Depending on the coefficient of variation, single butterfly species varied greatly in the required sampling size (Tab. 3). The total abundance of butterflies, or the most abundant and frequently occurring species, *P. rapae*, *P. napi* and *Nymphalis io* require smaller samples. In contrast, the rarer species such as *Vanessa atalanta*, *Vanessa cardui*, *Boloria lathonia* and *Papilio machaon* would require a sampling effort several times greater than that suggested for species richness.

The number of sampled field margins also determines the probability of detecting a given difference between margins (Fig. 3). In both species number (Fig. 3A) and total abundance (Fig. 3B) monitoring about 150 field margins (species, Fig. 3A), or about 75 (abundance, Fig. 3B) would result in a probability of 80% to detect differences >15%, whereas smaller effects (<10%) would require larger sampler sizes for the same statistical power.

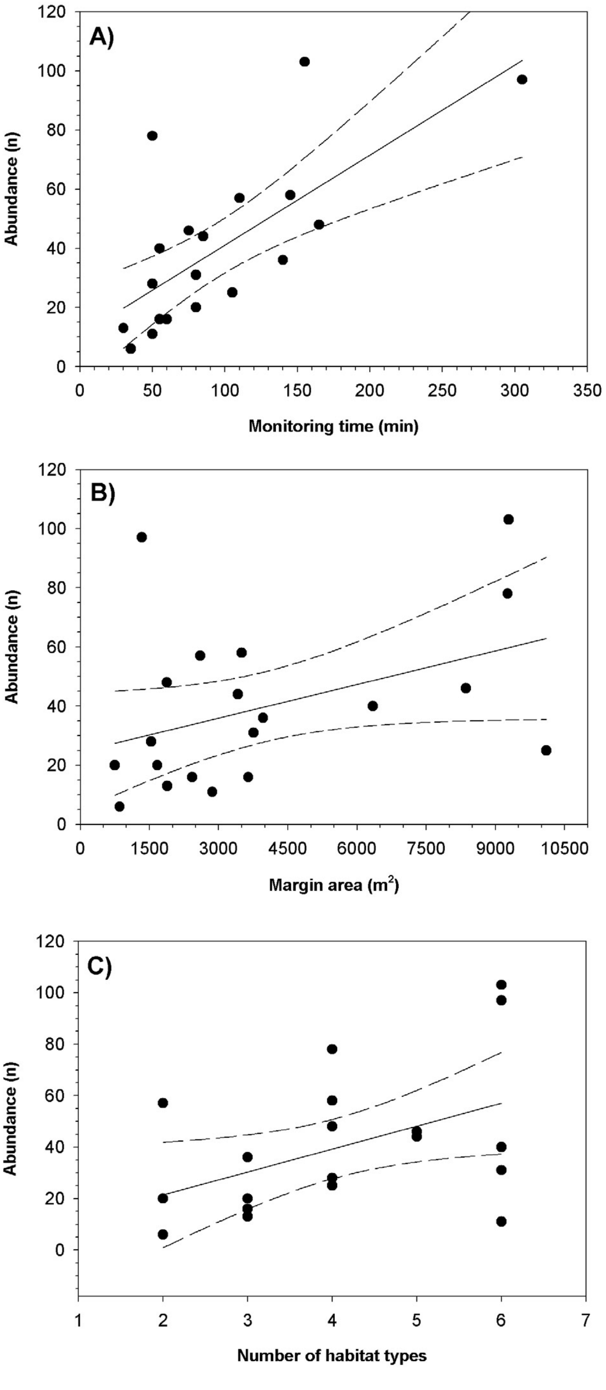


Figure 2. Influence of (**A**) monitoring time, (**B**) field margin area, and (**C**) number of habitat types on abundance of butterflies observed in field margins. In case of significant correlations the linear regression with 95% confidence interval is shown. Linear regression equation for (A): $y = 10.55 + 0.31x$, $R^2 = 0.49$, $P < 0.001$. (B) $y = 24.64 + 0.04x$, $R^2 = 0.17$, $0.05 < P < 0.10$. (C): $y = 3.53 + 8.92x$, $R^2 = 0.21$, $P < 0.05$.

DISCUSSION

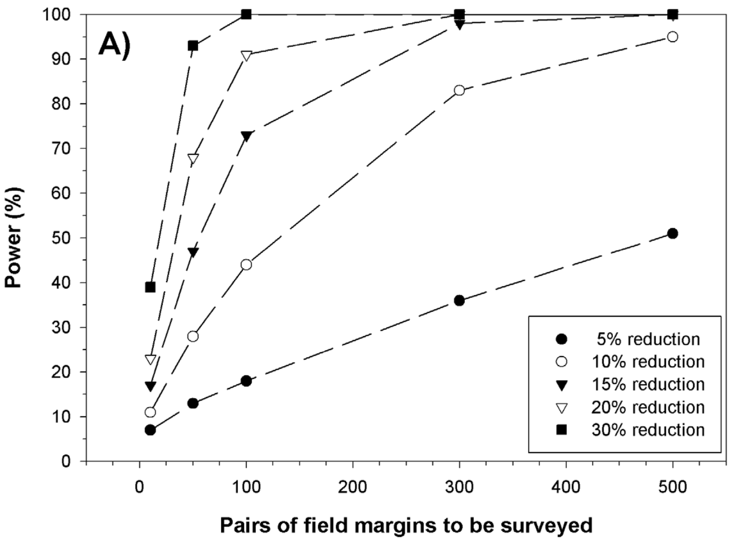
Species richness of butterflies tended to increase with the number of habitat types present within maize field margins. Diverse habitats support more butterfly species because these habitats contain more larval food plants (Niemelä and Baur, 1998; Steffan-Dewenter and Tscharntke, 1997; Thomas et al., 2001). Diverse biotopes

Table 3. Sample size calculation for monitoring the effect of *Bt* maize on species richness of butterflies, pooled abundance of all butterflies, and the abundance of 11 individual species. Given are the numbers of pairs of margins to be monitored in order to detect a difference of 5–50% between *Bt* and non-*Bt* field margins (*i.e.* doubling the numbers gives total sample size). Calculations are based on the average numbers (± 1SD) recorded in N field margins, and on the further assumptions that a two-sample t-test for independent data is used, the test is one-tailed, the statistical power is 80%, the significance level α is 0.05, and the sample size is equally distributed between the two samples.

Variable	N	Mean number per 60 min ± 1SD	Number of pairs of margins for detecting a reduction of							
			5%	10%	15%	20%	25%	30%	40%	50%
No. of species	15	5.95 ± 2.78	1078	271	121	69	44	31	18	12
All butterflies	15	23.29 ± 8.88	720	181	81	46	30	21	12	8
P. machaon	6	1.51 ± 1.26	3406	864	383	215	139	97	55	36
P. rapae	14	10.78 ± 4.70	940	236	106	60	39	27	16	11
P. napi	12	2.89 ± 1.31	1005	254	113	64	42	29	17	11
N. io	11	1.24 ± 0.59	1114	279	153	71	46	32	19	12
N. urticae	12	1.30 ± 0.95	2621	656	292	165	105	74	42	27
V. atalanta	9	1.30 ± 1.36	5451	1364	607	342	219	153	86	56
V. cardui	8	1.08 ± 1.11	5266	1317	586	330	212	147	83	54
B. lathonia	7	1.75 ± 1.51	3642	922	409	231	148	103	59	38
M. jurtina	7	1.79 ± 1.08	1814	449	201	114	73	51	29	19
C. pamphilus	10	1.61 ± 1.10	2288	580	257	145	94	65	37	24
P. icarus	6	1.34 ± 0.90	2216	555	250	141	90	63	36	23

may at the same time provide the nutritional resources for adult butterflies (Feber et al., 1996, 1999; Schneider et al., 2003; Steffan-Dewenter and Tscharntke, 2000), thus providing the necessary resources for both larval and adult stages in close proximity. While the size of the field margin had an influence on butterfly abundance, it did not affect species richness, in contrast to island biogeography theory predicting the increase of species diversity with habitat area (Connor and McCoy, 1979; MacArthur and Wilson, 1967). However, other studies on butterflies have shown that factors other than habitat size are important in determining butterfly species richness, including habitat qualities (Collinge et al., 2003; Munguira and Thomas, 1992; Schneider et al., 2003; Steffan-Dewenter and Tscharntke, 1997), disturbance (Feber et al., 1996), isolation of habitat patches (Dennis and Shreeve, 1997; Summerville and Crist, 2001; Thomas et al., 2001), landscape diversity (Hodgson, 1993; Weibull et al., 2000), and species metapopulation patterns (Gutiérrez et al., 1999). In general, narrow margins along intensively managed agricultural fields are poor habitats for many butterflies, and a limited set of "typical" species seem to occur on arable field margins, colonisation by other species being dependent on increases in habitat quality rather than margin size.

This study identified a butterfly assemblage occurring along maize field margins in southern Germany. The observed pattern was typical for invertebrate communities in agro-ecosystems comprised of a few dominants with remaining species occurring in relatively small proportions (Luff, 2002; Samu and Szinetár, 2002; Tischler, 1958). In general, the observed butterflies were common species typical of arable land, 33 species observed being within the expected range for richness (Ebert and Rennwald, 1991a; Feber et al., 1996; Kruess and Tscharntke, 2002; Munguira and Thomas, 1992; Steffan-Dewenter and Tscharntke, 1997). The proportion of threatened species listed in Red Data Books was relatively small, most species being categorized in minor classifications ("near threatened"). Although the species list presented in Table 1 is representative for maize field margins in Bavaria, it is by no means complete. This is demonstrated by the estimation of species richness, which suggested a total species number in the range of 38 to 39 species. The occurrence of species is expected to vary with region, habitat characteristics, management practices, and degree and scope of fragmentation (Collinge et al., 2003; Feber et al., 1996, 1997, 1999; Hodgson, 1993; Steffan-Dewenter and Tscharntke, 1997; Thomas et al., 2001; Weibull et al., 2000).



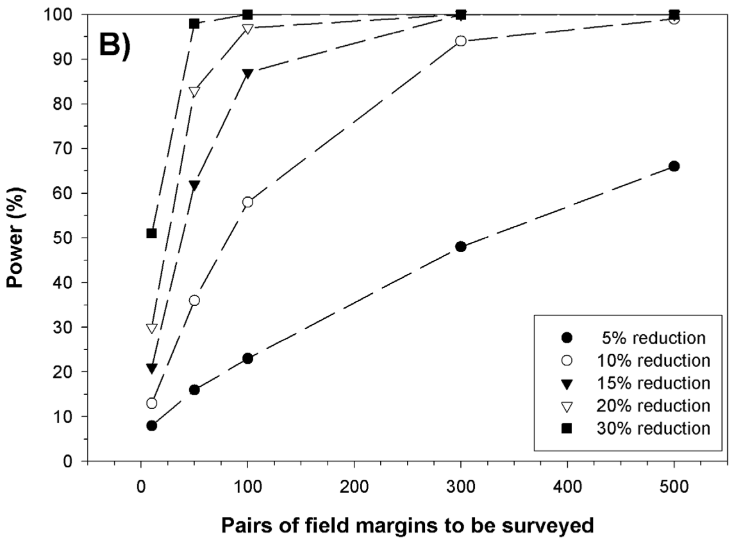


Figure 3. Power analysis for monitoring the effect of *Bt* maize on (**A**) species richness of butterflies, and (**B**) abundance of butterflies occurring in maize field margins. The probability of detecting a difference (= power) is shown for several different scenarios ranging from a 5% reduction to a 30% reduction of butterfly species and abundance. The sample size is given as the number of pairs of field margins to be surveyed, i.e. for total sample size the numbers must be doubled (*Bt* field margins plus non-*Bt* field margins). For the assumptions underlying the power analysis see Table 3.

Several of the species observed in field margins are unlikely to be affected by *Bt* maize, which potentially threatens larvae, because they reproduce at sites remote, while the adult butterflies only visit the flowers for feeding (Feber et al., 1996; Firbank et al., 2003; Schneider et al., 2003). Thus, it might be argued that monitoring adult butterflies is not the ideal stage to detect *Bt* maize effects. So far, published work is inconsistent, with some studies showing a positive correlation between number of larvae and adult butterfly abundance at a site, and others not (Munguira and Thomas, 1992; Schneider et al., 2003; Steffan-Dewenter and Tscharntke, 1997). However, for practical reasons the monitoring of adult stages is often adopted, because monitoring and identification of larval stages is often more difficult and time-consuming. Nevertheless, it should be noted that some single butterfly species can be monitored better in the larval stage than as adults (Hermann, 1999).

Not surprisingly, the employed sampling effort determined sampling success both in terms of species number and individual abundance. A monitoring time of 100 min per field margin appeared to be long enough to measure the average species richness and abundance. However, it is important to note that monitoring time *per se* does not necessarily secure relevant information provided by number of visits and number of study seasons. A higher number of visits and study seasons are important, because (i) different species occur at different times and in different abundance during one season (Ebert and Rennwald, 1991a,b), (ii) the occurrence of any one species may fluctuate from year to year both in space and time (Feber et al., 1996; Pollard, 1977, 1984; Thomas and Harrison, 1992), (iii) a higher number of visits and study seasons increases the chance to detect rare or cryptic, or less abundant species (Hermann, 1999), (iv) if various locations in different regions are sampled, a higher number of visits may compensate for differing sampling conditions among the study sites at the recording day, for example differences in weather, flower abundance, or management events (Schneider et al., 2003; Steffan-Dewenter and Tscharntke, 1997).

These results have important consequences for the design of a butterfly monitoring scheme. Assuming that the data presented here are representative, a large number of field margins should be monitored in field assessments of *Bt* maize cultivation, especially if small effects (<5%–10% reduction in species richness or total abundance) are expected. In particular, threatened butterfly species that are often the less abundant would require a greater sampling effort. The calculations presented are based on several assumptions that are not fully tested. Butterfly abundance may not be normally distributed, may differ with region or season, and the coefficient of variation of the count data may change with higher sample sizes. However, it is well known that the required number of field margins sampled depends on four major factors: the variance of the count data, the desired power of the test, the desired significance level, and the magnitude of effect to be detected. Reducing the variance of the data set would reduce necessary sample size, and such a reduction could be achieved by pooling abundance of different (indicator) species, by transforming the original data, or by specific spatial arrangements of the sampling plots (Perry et al., 2003). Moreover, it is important to standardize and record the variables responsible for the variance, or to take them into account in statistical analyses as covariates (monitoring time, habitat quality, margin size, flower abundance, landscape diversity, etc.).

The higher the power of the analysis, the greater the required sample size to prove a given effect. In the sample size calculation of this study, a power of 80% was selected, which is supposed to be adequate (Bourguet et al., 2002; Perry et al., 2003). The use of a less stringent significance level ($P = 0.10$) would decrease the required sample size, or increase the ability of the test to detect smaller differences, respectively (Diamond, 2003). Of course, there would be a concurrent increase in the probability of falsely concluding that there are differences when actually there are none. Sample size is also inversely proportional to the size of effect to be detected, *i.e.* increased sample sizes are required to detect smaller effects. Dutton et al. (2003) suggested a threshold value of 30% for entomophagous invertebrates, *i.e.* population effects of 30% and less (*e.g.*, in mortality) should be considered as safe from risk (see also Barrett et al., 1994). However, ecologically relevant differences such as long-term effects may occur at levels lower than 30%. If a monitoring program is designed to work as an early-warning system, it may be essential to be alerted at a lower threshold. Moreover, the desired threshold is not purely scientific, but is value-related, and will thus depend on public opinion and on political decisions about what environmental effects are acceptable. A possible monitoring protocol may be to employ a two-tiered approach. In the initial phase a P-value of 0.10 or higher may be used, which would reduce sample size as compared to a P-value of 0.05, and, at the same time, would make it more likely that negative effects will be detected, but risking type 1 error (*i.e.*, falsely concluding that there are differences when actually there are none). Since we are concerned with product safety, avoiding type 2 error is arguably more important here (*i.e.*, falsely concluding that there are no differences when actually there are). If significant results are obtained in the first phase, a second survey with reduced P and a larger sample effort could be made. Likewise, in a first tier sample, effort may be somewhat reduced in terms of species to be recorded and in terms of invested monitoring time, and increased in a second step, provided the first results indicate any effects.

MATERIALS AND METHODS

This study is part of a comprehensive research programme of the Bavarian State Ministry for Regional Development and Environmental Affairs concerning the development of methods for monitoring the potential environmental effects of transgenic plants (Lang et al., 2004). The present survey was carried out on five State Research Farms in Bavaria, Southern Germany, situated in different representative geographical areas (Tab. 4). Conventional maize and *Bt* maize fields of 1.5 ha–12 ha were established on each the farms, and adult butterflies were recorded during three years (2000–2002) in the uncropped margins adjacent to these fields. Maize cultivation and management of field margins followed

Table 4. Characteristics of the state research farms (precipitation and temperature values are ranges of long-term means recorded by weather stations located on research farms).

Site	Geographical position	Altitude above sea level	Annual average precipitation	Annual average temperature	Number of field margins surveyed
Grub	11°46'49" east Greenwich, 48°10'09" north the equator	514 m	800–1000 mm	7–8 °C	3 at conventional maize, 2 at *Bt* maize
Puch	11°13'00" east, 48°11'11" north	550 m	850–1000 mm	7–8 °C	1 at *Bt* maize
Baumannshof	11°32'16" east, 48°42'19" north	366 m	700–750 mm	7–8 °C	1 at conventional maize, 1 at *Bt* maize
Neuhof	10°47'10" east, 48°47'09" north	518 m	700–800 mm	7–8 °C	2 at conventional maize, 2 at *Bt* maize
Schwarzenau	10°12'40" east, 49°48'19" north	200 m	600–650 mm	8–9 °C	4 at conventional maize, 4 at *Bt* maize

standard agronomic practice. Butterflies were monitored by walking through the field margins once per sampling period, and by recording the number of all adult specimens observed within the boundaries of the margins. Standardized monitoring by walking given transects in a fixed time was not followed, because one aim of the study was to investigate the influence of observation time and margin area on the recorded data (see below). Recording was conducted from May to September, and during weather conditions suitable for butterfly activity (Pollard, 1977; Settele et al., 1999). Individual butterflies, which could not be identified in flight, were caught with a sweep net. The sibling species *Leptidea sinapis / L. reali*, and *Colias hyale / C. alfacariensis* were not distinguished. Species were identified using Tolman and Lewington (1998), and the nomenclature follows Settele et al. (1999). Larval stages were not searched for systematically, but any evidence of reproduction occasionally observed was registered (egg-laying behavior, eggs, larvae, or pupae). An overall number of twenty field margins were studied: five field margins in 2000, eleven field margins in 2001, and eleven field margins in 2002 (some margins were studied longer than one year, Tab. 2). The following variables were recorded per margin: study site, total species richness and abundance of individual adults of all butterfly species, monitoring time (per recording occasion, and overall), total number of visits (= number of sampling occasions), number of study seasons, *Bt* status of the adjacent maize field, margin length and width (giving margin area), and number of habitat types present within each margin (*cf.* Tab. 2 for descriptive data of the variables). The following habitat types were defined: grasslands, set-asides, grassy strips (width <1m), hedgerows, groves, damp ditches, forest edges, country lanes, and roads. Grasslands directly neighboring the study margins were not monitored but included in the tally of habitat types, because they harbored many reproducing satyrid butterflies with grass-feeding larvae that immigrated in adjacent study margins.

Species number of every field margin, *i.e.* of every sample, was entered into the biodiversity database *EstimateS*, version 5, in order to assess the completeness of the field margin inventory (Colwell, 1997). Sample order was randomized 50 times to obtain two nonparametric estimators of species richness, ACE (Abundance-base Coverage Estimator) and ICE (Incidence-base Coverage Estimators) (Chazdon et al., 1998; Colwell, 1997; Colwell and Coddington, 1994).

Pearson correlation analysis was used to test for associations of independent variables with butterfly species richness and abundance. In a subsequent analysis, a multiple linear regression analysis with stepwise selection was applied to the data to identify the key variables and joint effects. The probability-of-F-to-enter was 0.05, and for *P*-to-leave 0.10. In order to check whether the assumptions of regression were met, residuals were tested for normal distribution, constant variance, linearity, and independence. When necessary, data were log(x)-transformed to meet these assumptions. Additionally, the independent variables and resulting regression models, respectively, were tested for collinearity (which was not the case). All analyses were carried out using SPSS, version 11.

The program nQuery, release 4.0, was used to calculate sample sizes theoretically necessary to detect the impact of *Bt* maize on species richness and abundance of butterflies in field margins. Sample size was calculated for hypothetical effects ranging from 5% to 50% difference between field margins neighbouring *Bt* and neighboring conventional fields. The following assumptions were made: normally distributed data, equal sample size for both types of field margins, a t-test for two independent samples is applied to test for differences between the two types of margins, the test is one-sided with a significance level, α, of 0.05, and the power of the test is 80% (*i.e.* the probability of rejecting the null hypothesis at a given alpha). The basis of the sample size calculation was the simple sample variance of the butterfly data recorded in this study. Butterfly data were standardised by normalizing the observations to 60 min recording time (as observation time turned out to be the most influencing variable). Means and standard deviations were derived only from field margins sampled at least 50 min over three or more visits during spring (May), summer (June/July) and late summer (August) (Hermann, 1992, 1999; Mühlhofer, 1999; Steffan-Dewenter and Tscharntke, 2000). Based on the above criteria, I excluded data from 5 of the 20 field margins. Sample sizes were calculated for the following variables: species richness of butterflies, total butterfly abundance, and the abundance of 11 focal species. These 11 focal species were selected because they were observed at least in six of the 15 sufficiently sampled field margins (40% occupancy), the minimum required for representative means and variances. Only count data for margins where the respective species occurred were taken into account, *i.e.* zero values were excluded. Although excluding zero values reduces variance which subsequently affects sample size estimation, this approach was chosen because only field margins where the concerned species occur would be studied in a monitoring program in order to examine possible population declines.

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