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Spatial distribution of *Aglais urticae* (L.) and its host plant *Urtica dioica* (L.) in an agricultural landscape: implications for *Bt* maize risk assessment and post-market monitoring

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Over the past decades, genes of *Bacillus thuringiensis* var. *kurstaki* (Berliner) (*Bt*) coding for protein toxins have been engineered into maize for protection against the European Corn Borer (*Ostrinia nubilalis* (Hbn.)). However, these transgenic plants may have an impact on non-target organisms. In particular, a potential hazard was identified for non-target lepidopteran larvae, if they consume *Bt* maize pollen on their host plants. Risk can be defined as a function of the effect of an event (hazard) and the likelihood of this event occurring. Although data on toxicity (hazard) are available from many lab and field studies, knowledge about the environmental exposure of European lepidopteran larvae is incomplete at the population level. Therefore we studied the distribution of small tortoiseshell caterpillars (*Aglais urticae* (L.)) and its host plant in an agricultural landscape in Germany, to estimate the potential population exposure to maize pollen. The results showed that larvae of the small tortoiseshell developed primarily on freshly sprouted nettle stands (*Urtica dioica* (L.)) in field margins, rather than adjacent to hedges and groves. However, the main distribution was at margins of cereal (non-maize) fields, where 70% of all larvae were found. This may be due the fact that cereals covered 54% of the survey area, while maize only covered 6.1%. On the other hand, maize fields seem so show higher food plant densities than cereal crops. The results must be interpreted carefully, as the data basis of the present study is very small, and the situation can vary between years due to crop rotation or other changes in agricultural practices. Therefore it is still questionable whether the small tortoiseshell is significantly exposed to maize pollen. For a conclusive risk assessment, more replications and surveys of larger areas in different intensively managed agricultural landscapes over several years are needed.

Keywords: Lepidoptera / GMO / risk assessment / post market monitoring / GIS

INTRODUCTION

Over the last decade, genes of *Bacillus thuringiensis* var. *kurstaki* (Berliner) (*Bt*) that encode lepidopteran-specific toxins (Cry1A(b), Cry1A(c), Cry9) were engineered into maize for protection against the European Corn Borer (*Ostrinia nubilalis* (Hbn.)). However, questions have been raised on the environmental impact of these transgenic plants on non-target organisms (Dale et al., 2002; Jepson et al., 1994; Poppy, 2000). In particular, lepidopteran species might be affected due to the specific activity of the toxin (Felke et al., 2002). During anthesis, pollen-covered leaves of host plants are consumed by lepidopteran larvae. This occurs for host plants that grow as weeds within maize fields, but also for host plants growing in maize field margins. As a consequence of intensification of agricultural practices and the loss of (semi-) natural habitat types, field margins are becoming increasingly important for species conservation (*e.g.* Boatman, 1994; Robinson and Sutherland, 2002).

Risk is a function of hazard and exposure (den Nijs and Bartsch, 2004). In the case of lepidopteran species,

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the potential hazard is the toxicity of pollen containing *Bt* toxin, and the likelihood of the event is the environmental exposure of lepidopteran larvae to the pollen (Sears et al., 2001). Presently, toxicity data of *Bt* pollen of different *Bt* events on lepidopteran species are available from laboratory and field studies (Dively et al., 2004; Felke et al., 2002; Gathmann et al., 2006; Hellmich et al., 2001; Jesse and Obrycki, 2002; Lang, 2004; Losey et al., 1999; Stanley-Horn et al., 2001; Wraigth et al., 2000; Zangerl et al., 2001). In contrast, information on their exposure is incomplete for Europe. Different studies estimated pollen on host plants of different Lepidopteran species (Dively et al., 2004; Gathmann et al., 2006; Lang et al., 2004; Pleasants et al., 2001; Shirau and Takahashi, 2005). In the US, comprehensive field studies were carried out for the monarch butterfly, based on host plant and larval distribution, in order to estimate the potential impact of *Bt* maize cultivation (Sears et al., 2001).

In Germany, potentially exposed species were identified by a mainly theoretical approach by Schmitz et al. (2003). They showed that approximately 7% of the German Macrolepidoptera species mainly occur in farmland areas near maize fields during maize pollen shed. Data on the distribution and hence the exposure of European lepidopteran species in agricultural landscapes on a population level are still lacking, but are essential to complete the risk assessment. Many studies investigated the distribution of adult butterflies instead of larvae (*e.g.* Lang, 2004), an approach that is questionable for a comprehensive risk assessment, because adults of some species are visiting field margins only for feeding without reproducing at these sites (Firbanks et al., 2003). Thus the coincidence of adult abundance and larval exposure to maize pollen to larvae is not always certain (Schneider et al., 2003).

The aim of this study was to acquire data on the distribution of the lepidopteran species, the small tortoiseshell, *Aglais urticae* (L.), and their host plant in a fine-structured agricultural landscape during the time of maize pollen shed. By this example, we wanted to show how lacking data on the potential exposure of Lepidoptera population to *Bt* pollen for an environmental risk assessment could be gathered.

We chose this species because (i) *A. urticae* is susceptible to *Bt* toxin (LD50: 32 consumed Bt176 pollen grains per larvae, Felke and Langenbruch, 2005). (ii) The small tortoiseshell is one of the most widespread butterflies in Europe. This common species usually occurs in great numbers in all kinds of habitats except woodlands (Ebert and Rennwald, 1991). (iii) The second generation develops during July and August, coincidently with maize pollen shed (Schmitz et al., 2003). (iv) The larvae are monophagous on patchily distributed nettles (*Urtica urens* L., *Urtica dioica* L.), live gregariously in conspicuous webs, and do not disperse until their last molting, which made mapping of potential food plants and the distribution of larvae easy, (v) nettles frequently occur in field edges and (vi) larvae are easily determined in the field. This project was a pilot study to test the suitability of the presented method as part of risk assessment or post-market environmental monitoring in Europe.

RESULTS

In the survey area, we mapped 13 different habitat types. The landscape was dominated by cereals, sugar beets, maize, and pastures. Minor crops were oilseed rape, alfalfa, clover, and meadows. In addition to agricultural areas, there were hedges, groves, set aside fields, and rural areas. Urban settlement covered only 0.7% of the area (Tab. 1).

The occurrence of nettle patches was investigated on a total length of 32 769 m field edges and margins (Tab. 1). In total, an area of 7389 m^2 was covered with nettles. Fifty-three percent of all nettles were located near hedges or groves, whereas all others were situated in completely open landscape without bushes or trees. About two thirds of all nettles belonged to tall nettle patches (5272 m^2) and one third to short nettle patches (2117 m^2). Distribution of height classes was significantly associated with habitat types. Sixty-four percent of tall nettles grew near hedges or groves. In contrast, 72% of short nettles were located in open fields. In addition, the habitat type tended to have an influence on the number of nettles growing in field edges, but these results should be interpreted carefully, because the data were gathered only one year.

Altogether, we found 40 batches of larvae on nettle stands in the whole area during maize pollen shed. No larvae were found in stands beside maize fields. The mean density was 0.54 batches of larvae per 100 m^2 area grown with nettles. Clearly, the small tortoiseshell preferred patches of short nettles for egg laying. Eighty percent of all batches of larvae were found there ($\chi^2 = 14.4$, $n = 40$, $P < 0.05$). Overall we found 1.5 larval batches per 100 m^2 on short nettles compared to 0.15 larval batches on tall nettles. Accordingly, most larvae of the population developed near intensively used field edges ($\chi^2 = 28.9$, $n = 40$, $P < 0.05$). Only 3 larval batches were found near hedges, two on set aside fields, pastures and meadows, but 31 on edges of intensively used fields. By far, the most larval batches (28) were found near cereal fields. Associating this result with the number of nettles growing

Table 1. Distribution of habitat types in the research area. Presented are habitat type, total size of habitat types, length of investigated field edges, area of field edges covered with nettles.

Habitat	Number of fields	Size (ha)	Percent of total area	Length of field edges (m)	Nettle area in field edges (m^2)[1]	Area of field edge covered with nettles (m^2/100m)	Batches of larvae
Crops							
Cereals	17	140.0	56.6	18013	2210.2	12	28
Sugar beet	6	22.8	9.2	1824	71	4	1
Maize	6	15.8	6.4	1711	336	20	0
Oilseed rape[2]	0	0	0	517	633.6	122	2
Set aside fields	3	2.5	1.0	583	375.4	64	2
Set aside meadows	5	0.4	0.2	232.6	70.2	30.2	0
Pasture	13	55.7	22.5	7129	2144.2	30	2
Clover meadows	1	0.9	0.4	96	0	0	0
Alfalfa	1	0.5	0.2	105	0	0	0
Meadow	2	3.5	1.4	1212	272	22	2
Total	**54**	**242.1**	**97.9**	**31422.6**	**6112.6**	**19.5**	**37**
Non productive habitats							
Hedges and groves[3]	11	3.5	1.4	6102[4]	3945.5[4]	64.6[4]	3
directly adjacent to field edges	n.d.	n.d.	n.d.	4756	2669.5	56.1	0
without direct contact to crop fields	n.d.	n.d.	n.d.	1346	1276	94,8	3
Urban settlement[5]	2	1.8	0.7	n.d.	n.d.	n.d.	0
Total (without double counting)	**67**	**247.4**	**100**	**32768.6**	**7388.6**	**22.6**	**40**

[1] Size of a nettle stand were calculates with the equation: length (m) × 1 m × density factor, where the density factor was: thin 0.33 (thin); 0.66 (medium) or 1 (dense).
[2] Edges of the oil seed field was inside the study area, field was outside.
[3] Main focus for the comparison was crop versus non productive areas. Therefore we did not differentiate between hedges, groves and small forests.
[4] Some hedges and groves were near to crop fields and were counted twice.
[5] Includes farms, gardens and roads.

in field edges, edges of cereal fields seemed to be the favored habitat for developing larvae (Tab. 1). However, detailed statistical analyses of the impact of crop type on the distribution of larvae were not feasible, due to the small size of the surveyed area and the small number of different crop fields.

DISCUSSION

It is well documented that a number of lepidopteran species may be affected by *Bt* toxins, and that some may be present in maize fields (Schmitz et al., 2003; for a review see Evans, 2002). However, exposure of any population of Lepidoptera to the toxin is restricted to those consuming the *Bt* plants or its products. In the vicinity of the *Bt* maize fields, larvae may be exposed to the toxin when *Bt* maize pollen is deposited on plants on which they are feeding (EFSA 2005a; b). One possibly affected species is *A. urticae* on its host plant *U. dioica*. In our survey, only a few nettle stands and no larvae were found in field margins of six maize fields (6.1% of the area). Thus it is questionable whether in our specific survey area the small tortoiseshell populations were significantly exposed to maize pollen. The occurrence of stinging nettle in northern Europe is probably restricted to open woodland on peaty soils, but the plant has reached widespread distribution in central Europe due to accumulation of organic matter provided by human habitation and agriculture (Zabel and Tscharntke, 1998). As expected, we found nettle regularly in the survey area. *A. urticae* females prefer laying eggs on nettle patches in open landscapes. Most of the larval batches were found on nettle patches in edges of intensively used fields. These nettle batches were characterized by a shorter plant height, because field margins were regularly mown during summer. Females prefer to lay their eggs on freshly sprouted, shorter nettles because of their better nutritional quality compared to older nettles. At the start of flowering, the content of water, nitrogen and soluble proteins decreases dramatically (Pullin, 1987). Additionally, sunny sites are the preferred egg laying habitats. Development time and mortality of small tortoiseshell larvae is dependant on microclimate (Bryant et al., 1997). Most larvae – 28 egg batches out of 40 – were observed in field edges of cereals. It seems that this habitat type is of relatively high quality for larvae, even though nettle density was very low, with 12 m^2 per 100 m field edge. Field edges of other crops were barely used as larval habitat. Nevertheless, it is worth a discussion whether maize field edges are rarely used as larval habitats for *A. urticae* in general, because the data presented here are based on observations of only six maize fields in one year. The phenology of lepidopteran species could be shifted due to different climate conditions (Woiwod, 1997). Gathmann et al. (2006) showed that larval development and maize flowering for the species *Plutella xylostella* L. and *Pieris rapae* L. barely overlapped in some years, but were coincident in other years. Also, crop rotation could alter the area of maize cultivation and the association of field edges with high densities of nettles and maize fields. Additionally, changing landscape management *e.g.* mowing of field edges could affect results. At the end of July 2004, field edges were mown in some cases, so that only very small patches of host plants could be found there. This makes clear that management options such as mowing, pesticide use, habitat fragmentation or pollution may have more adverse effect on lepidopteran species than growing *Bt* plants (Davis et al., 1991; Longely and Sotherton, 1997; Mulder et al., 2005; Pimentel and Raven, 1999; Ries and Debinski, 2001; Warren, 1997). Further investigation may comprise more replications over several years, larger areas, and different intensively used landscapes with a higher proportion of maize growing. To what extent different management options, such as crop rotation, herbicide or insecticide use, affected the occurrence of larvae could not be estimated in this study due to a lack of information about agricultural management practices in the survey area. In general, this information will be of interest, because in future assessments such influencing factors could be identified and compared to a potential harm caused by *Bt* pollen. In contrast to field edges, on nettle batches near hedges and groves, only 3 larval batches were found. There, mostly old nettle patches were found, because these areas were not mown. However most nettle patches were found near hedges and groves, but are of less importance as larval habitats for *A. urticae*.

At first appearance, this lepidopteran species fulfils substantial pre-conditions as indicator species for a risk assessment or post-market environmental monitoring. The small tortoiseshell is sensitive to the *Bt* toxin, a monophagous species with a wide range of distribution, and easy to determine. Additionally, the patchy distribution made it easy to find larval habitats. This will make monitoring very cost-effective. For the field work and data analysis of an area of 250 ha, we needed about 50 hours man-power.

But in 2004 and 2005 nearly no caterpillars of the second generation of the small tortoiseshell were found during maize flowering. This corresponds to the fact that in 2004 all over Central Europe the species was seemingly more rare than in 2003, which may be the result of the dry and hot summer in 2003 (Hensle, 2004). The diapause disposition is not only regulated by the circannual periodicity of the duration of daylight, but also by congenital factors (Niehaus, 1982). Therefore this phenomenon may be due to a partial extinction of the two-generation individuals as well as to some kind of migratory events. If this fact could be confirmed on different region within the next years, *A. urticae* should be considered an indicator species is questionable suitability. An additional disadvantage of *A. urticae* as an indicator is that it is a highly mobile species, which influences its association to agro-ecosystems and can lead to large differences from year to year. Furthermore, the second generation of at least partly polyvoltine lepidopteran species often tend to show a greater distribution in time than the single generation of a strictly univoltine species – a fact that can influence the degree of coincidence with maize flowering. Therefore other species should also be taken into account as potential indicator species.

In a theoretical approach based on a data-base query, Schmitz et al. (2003) identified 96 species of Macrolepidoptera typically occur in the German agricultural landscape that may be in contact with *Bt* maize pollen due to their phenology and habitat preference. The number of potential indicator species is reduced, if the authors take into consideration other factors, such as regional scale and the frequency of host plants. In a field survey within the same study, the authors found 17 lepidopteran species on five different host plant species on 18 maize field edges near Bonn and Aachen, but seven species were rare. In a field survey in the same region within three years, Gathmann et al. (2006) found on two host plant species larvae of nine lepidopteran species. Only two species were abundant enough for statistical analysis. In other studies, the diversity and abundance of adult butterflies were mapped on maize field edges on a regional scale in the Northern part of Bavaria. Altogether, 77 species could be identified as potentially endangered by *Bt* maize pollen. According to the authors, four of these species are possibly highly endangered, whereas for 33 species the potential risk could not be determined, due lack of ecological data (Felke and Langenbruch, 2005). On 20 maize field edges Lang (2004) found 33 lepidopteran species on five locations in Bavaria, South Germany. For eight species, egg-laying behavior was observed, and larvae or pupae were observed in the maize field edges.

In additional observations in the area of this study between 2003 and 2005 (unpublished data), we identified 27 alternative lepidopteran species. If factors such as phenology, host plant distribution, cost effectiveness are taken into account, none of these species is ideal, and three species are partly suitable indicator species, respectively (Tab. 2). All studies showed that the number of possible indicator species is limited, and differs in different regions or at different spatial scales.

The spatial and temporal distribution patterns of populations in the landscape are of interest in ecology (Caldow and Racey, 2000; Jeanneret et al., 2003; Kraus et al., 2003). In particular, in conservation ecology and risk assessment of anthropogenic effects on biodiversity, the need of these data is evident. Extended studies of harm to the monarch butterfly by *Bt* pollen showed that for a comprehensive risk assessment, spatial distribution of host plants and larvae is essential. High mortality rates from lab studies (Felke et al., 2002; Losey et al., 1999) become relative, if exposure to the larvae is assessed as exposure in the field at the population level (Dively et al., 2004; Sears et al., 2001). However, for most European lepidopteran species these data are still incomplete. The presented pilot study indicates how these data can be generated, and what labor investment is needed.

Recently, the EFSA GMO Panel considered whether the abundance of non-target Lepidoptera in or close to maize fields of the events Bt11 (EFSA, 2005a) and 1507 (EFSA, 2005b) should be monitored according to the requirements of European legislation. Although there was no requirement for case-specific monitoring of the abundance of non-target Lepidoptera in *Bt* maize, it is still important to consider future *Bt* maize plants with higher *Bt* pollen toxicity, as well as cost-effectiveness.

If exposure data are needed for risk assessment or post-market environmental monitoring, in future studies larger areas and additional information *e.g.* cultivation management should be taken into account. Additionally, a comparison of different intensively used landscapes is necessary to get a comprehensive data set. This could be a basis for modeling the potential hazard of *Bt* plant cultivation for lepidopteran species, but could also be used to assess side effects of pesticides or changes of agricultural landscapes.

MATERIALS AND METHODS

The study area was situated to the west of Aachen at 209 to 251 m a.s.l., and covered around 259 ha in 2003. We have chosen this area because it is a quite representative part of the fine-structured agricultural landscape in this region, containing agricultural crop land, grassland, hedges, woodlands, granges and many different kinds of field paths (unimproved or tarred) accompanied by typical field margins. First we mapped the biotope-types and field crops, to calculate the proportion of the area that each occupied within the study area. The plant mapping of nettles – the small tortoiseshell's food plant – was carried out in the study area in order to estimate the size, quality and distribution of the potential butterfly larval habitats just before mapping of Lepidopteran larvae. We assumed that the common nettle and the small nettle were the exclusive food used by the larvae. Further on, we focused on field margins, hedge margins and other linear landscape elements, mainly because it is quite easy to map such structures. The few nettle stands growing within grasslands were not recorded. During mapping, the parameters (i) length (in m) of each nettle stand, (ii) stand density (in three classes: thin = factor 0.33 in calculating the area covered by the nettles; medium = factor 0.66; dense = factor 1), (iii) height of each stand (two categories: short (< 0.5 m), tall (> 0.5 m)), and (iv) adjacent habitat type were recorded. In order to simplify analysis, we preferred to define long but thin stands, instead of many short but dense stands. Therefore both the shortest stand and the shortest distance between stands was 1 m. The stand density was used to estimate the area covered by the nettles, whereas to simplify matters we assumed the width

Table 2. List of lepidopteran species found in field edges in the investigated area during 2003–2005 and criteria to assess suitability as indicator species. Presented are species, number of generations (NG), phenology (Ph), preferred habitats (H), mobility (M), mode of nutrition and host plants (HP), effort for determination (D), frequency of host plants in field edges (F), best methods and frequency of catches (Me), data on susceptibility against *Bt* toxin are available (S), and summarizing evaluation of the suitability of the species as indicator (B).

Species	NG[1]	Ph[2]	H[3]	M[4]	HP[5]	D[6]	F[7]	Me[8]	S	B[9]
Cucullia chamomillae (Denis and Schiffermüller)	1	5–7/8	1	n.a.	o (*Chamomilla, Matricaria*)	1	2	D, S, 3	no	3
Aglais urticae (L.)	2(–3)	5–6, 7–8	1	n.a. – M	m (*Urtica*)	1	3	vd, 3	yes	2
Scoliopteryx libatrix (L.)	2	5–7, 7–9	3	2–3	o (*Salix, Populus*)	2	1	D, 3	no	3
Hypena proboscidalis (L.	2	9–5, 6–8	2	3	M (*Urtica*)	1	3	D, 3	no	2
Perizoma alchemillata (L.)	1	7–9	1–2	2–(3)	o (Lamiaceae, *Galeopsis* spec.)	1	1	D, 3	no	3
Vanessa cardui (L.)	2	6–9	1	n.a. – M	p (frequently on thistles)	2	2	vd, 4	no	3
Pieris rapae (L.)	2–3	5–10	1	n.a. – M	o (Brassicaceae)	2	1	D, S, vd, 3	yes	3
Cucullia absinthii (L.)	1	7–10	1	n.a.	m (*Artemisia*)	2	1	D, 2	no	3
Aetheria dysodea (Denis and Schiffermüller)	1(–2)	6–9	1	n.a.	o (*Lactuca, Sonchus*)	2	1	D, vd, 3	no	3
Vanessa atalanta (L.)	2	5–8	2	n.a. – M	m (*Urtica dioica*)	2	3	vd, 4	no	2–3
Macroglossum stellatarum (L.)	1	6–8	1–2	4 – M	m (*Galium* spec.)	2	1	vd, 1	no	3
Spilosoma lutea (Hufnagel)	1(–2)	7–10	2–3	2–3	p	2	n.a.	vd, 1,	no	3
Melanchra persicariae (L.)	1(–2)	7–10	2	3–4	p	2	n.a.	D, S, 1	no	3
Ectropis crepuscularia (Denis and Schiffermüller)	2	4–7, 7–10	3	2–3	p	2	n.a.	D, 2	no	3
Spilosoma lubricipeda (L.)	1(–2)	6–11	2–3	2	p	2	n.a.	D, vn, 2	no	3
Caradrina morpheus (Hufnagel)	1	7–10/11	1	3	p	2	n.a.	D, 3	no	2
Phragmatobia fuliginosa (L.)	2	9–5, 6–8	1	3	p	2	n.a.	vn, S, 2,	no	3
Noctua pronuba (L.)	1	8–4/5	1	4 – M	p	2	n.a.	vn, 3	no	3
Abrostola triplasia (Hufnagel)	1(–2)	6–10	2	2–(3)	m (*Urtica*)	2	3	D, 2	no	2
Lacanobia oleracea (Linnaeus)	2	6–8, 8–10	1	3–(4)	p	2	n.a.	Dl, S, Vn, Vd, 2	no	3
Macdunnoughia confusa (Stephens)	2(–3)	6–10	1	4 – M	p	3	n.a.	D, S, 2	no	3
Autographa gamma (Linnaeus)	2–(3)	6–10	1	4 – M	p	3	n.a.	D, S, 3	no	3
Eupithecia centaureata (Denis and Schiffermüller)	2	6–7, 7–10	1–2	2–3	p	2	n.a.	D, S, Vd, 3	no	3
Eupithecia subfuscata (Haworth)	1	7–10	2	2–3	p	2	n.a.	D, 3	no	2
Eupithecia absinthiata (Clerck)	1	7–10	1	2	o (Asteraceae: *Achillea, Artemisia, Eupatorium, Solidago, Senecio Tanacetum*)	2	2	D, Vd, 3	no	3

Table 2. Continued.

Species	NG[1]	Ph[2]	H[3]	M[4]	HP[5]	D[6]	F[7]	Me[8]	S	B[9]
Eupithecia trisignaria Herrich-Schäffer	1	7–10	3	2	o (Apiaceae, *Angelica sylvestris, Heracleum sphondyllium*)	2	3	D, Vd, 3	no	3
Eupithecia tripunctaria Herrich-Schäffer	2	5–7, 7–9	1–2	2–(3)	o (Apiaceae, *Heracleum sphondyllium*)	2	3	D, Vd, 3	no	2

[1] In parenthesis the number of partial generations is given.
[2] Numbers indicate time period (month) of larval development, based on Wirooks and Theissen (1998; 1999).
[3] Habitat types: 1 = open agricultural landscapes, 2 = open agricultural landscapes and woodland areas, 3 = rare in open agricultural landscape, frequent in wood land areas.
[4] Flight distances (based on Hausmann, 1990): 1 < 100 m, 2 = several 100 m, 3 = up to 1 km, 4 = several km; M = migratory butterflies (based on Eitschberger et al., 1991).
[5] m = monophagous, o = oligophagous, p = polyphagous, based on Wirooks and Theissen (1998; 1999).
[6] 1: after introduction easy, 2: only through experts, 3: extremely difficult to determine.
[7] 1 = rare, 2 = occasionally, 3 = frequent, n.a. = not available, because larvae are extremely polyphagous.
[8] d = dislodging, s = sweep net, vn = visual assessment at night, vd = visual assessment during day, 1 = rare, 2 = occasional 3 = frequent, 4 = dependant on number of immigrated females.
[9] 1 = very suitable, ideal, 2 = partly suitable 3 = unsuitable. Criteria to meet class 1 (very suitable) are one generation coincident with maize pollen shed, frequent in open agricultural landscapes, low mobility, larvae are mono- or oligophagous, host plants are frequent in field edges, larvae are easily to determine and can be easily collected in high abundances.

of all nettle stands to be 1 m in the mainly linear biotopes. When it was significantly broader, we preferred to define two different stands in parallel. We could then estimate the size of a nettle stand with the equation: length (m) × 1 m × density factor. Nettle patches were categorized into two height classes (short, tall). The height of the stands was estimated as an indicator of the age of the nettles. Therefore we assumed that nettles shorter than 0.5 m mostly have been mown.

The gregarious, conspicuous caterpillars can easily be found in two generations, mainly during May to June and again from July to August (Wirooks and Theissen, 1998; 1999). Sometimes even a partial third generation can be observed (Ebert and Rennwald, 1991). In order to get data on caterpillars actually at risk of consuming pollen containing *Bt* toxin, we only mapped the second generation of caterpillars. The mapping took place at four days in July 2003 (21.7, 22.7, 25.7 and 31.7). We investigated all detected nettle stands in order to find the caterpillars of the small tortoiseshell. We wanted to count the caterpillar groups belonging to one egg batch, because it is the only way to compare the caterpillar density of such a gregariously living species between different sites. One egg batch comprises between 40 and 60 eggs (Ebert and Rennwald, 1991). Therefore we always carefully investigated the surroundings after every finding of caterpillars or pupae to find out from how many egg batches the detected specimens may have arisen. Places where caterpillars were found were associated with data from the nettle mapping in geographical information system (GIS, Arcview). GIS systems provide abilities to organize, analyze, synthesize and display information gathered in the field over both space and time (Niemi and McDonald, 2004). Therefore field size, area of nettle stands and places where larvae were found, were digitalized (Fig. 1). Additionally, nettle stands were characterized by the attributes height and density. On the basis of these data, we could calculate and compare the relative density of caterpillar groups with regard to various factors, such as adjacent crops or height of the nettle stand. Distribution patterns of larvae were analyzed with a χ^2-Test.

At the same time, it is important to know the human labor intensity necessary for carrying out monitoring on a landscape level. The data collected here are based on the capacity of two experienced biologist with expertise in Lepidoptera sampling and detection.

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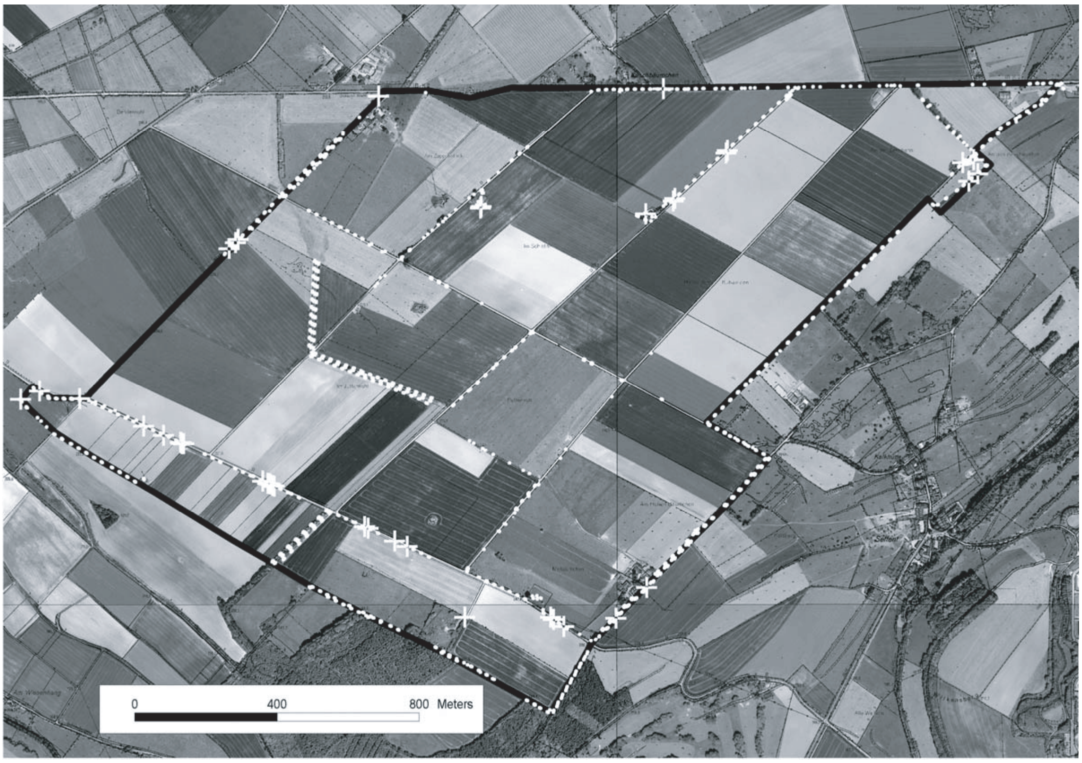

Figure 1. Digitalized airial scope of the investigated area. All edges of fields, hedges, woodlands and roads were assessed in the area marked in black. White crosses represent the larval batches found on nettle stands (white points).

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