Insect Science

Insect Science (2021) 28, 1159–1168, DOI 10.1111/1744-7917.12849

ORIGINAL ARTICLE

Can the growing of transgenic maize threaten protected Lepidoptera in Europe?

Gábor L. Lövei[1,2], Andreas Lang[3,4], Marco Ferrante[1,5] and Victor Bacle[1,6]

[1]*Department of Agroecology, Aarhus University, Slagelse, Denmark;* [2]*Institute of Applied Ecology, Fujian Agricultural and Forestry University, Fuzhou, China;* [3]*Büro Lang, Zell im Wiesental, Germany;* [4]*Department of Environmental Geosciences, University of Basel, Basel, Switzerland;* [5]*Centre for Ecology, Evolution and Environmental Changes, Azorean Biodiversity Group, Faculty of Agricultural and Environmental Sciences, University of the Azores, Angra do Heroísmo, Portugal and* [6]*La Painerie, Preaux du Perche, Perche en Noce, France*

Abstract We evaluated whether protected European butterflies can potentially be at risk if transgenic maize is extensively grown in Central Europe. We explored potential consequences of both insect resistant (IR) and herbicide resistant (HR) transgenic maize. IR maize can produce pollen that is toxic to lepidopteran larvae, and this puts butterfly species at possible risk if the presence of young larvae coincides with maize flowering, during which large quantities of maize pollen can be deposited on vegetation. By considering the timing of maize flowering in Europe and the phenology of the protected Lepidoptera species, we found that 31 species had at least one generation where 50% of the larval stage overlapped with maize flowering, and 69 species for which first instar larvae were present during maize pollen shedding. HR maize allows high concentration herbicide treatments on fields without seasonal limitation, which can drastically reduce weed densities. In cases where such weed species are host plants for protected butterflies, reduced host plant/food availability can result, causing population decreases. By using published information, we first identified the important weed species in major maize-growing European countries. Subsequently, we checked whether the host plants of protected Lepidoptera included species that are common maize weeds. We identified 140 protected species having food plants that are common weeds in one or more of the major European maize-growing countries. If HR maize is grown in Europe, there is a potential hazard that their food plants will seriously decline, causing a subsequent decline of these protected species.

Key words butterflies; conservation; environmental risk; food plants; GM crop; pollen toxicity

Introduction

Maize (*Zea mays*) is a tropical plant, and was domesticated in Central America from teosinte (Beadle, 1939). It was introduced to Europe by Cristopher Columbus, who brought it back already from his first voyage, the discovery of the Americas (Anghiera, 1907; Janick & Caneva, 2005). Since then it has become a widely grown crop plant on the European continent. In 2013, the total European area was 15 million ha, 60% of which was grown for seed and 40% for silage; the share of sweetcorn is negligible (FAO, 2018). The eight largest producers (in decreasing order: Romania, France, Hungary, Italy, Poland, Germany, Bulgaria, and Spain) represent >87% of the total European area devoted to maize (FAO, 2018).

Correspondence: Gábor L. Lövei, Department of Agroecology, Aarhus University, Flakkebjerg Research Centre, Forsoegsvej 1, DK- 4200 Slagelse, Denmark. Tel: +45 87158224; fax: +45 871 56 082; email: gabor.lovei@agro.au.dk.

Maize is also among of the first of the genetically modified (GM) crop plants. Today, both insect- (IR) and herbicide- (HR) resistant lines are available and, at least in the Americas, widely cultivated (Parisi *et al*., 2016). The EU register of authorized GMOs lists 27 different GM maize constructs for food and feed use, although most of them does not extend to field growing (http://ec.europa.eu/food/dyna/gm_register/index_en_new.cfm). In Europe, Spain grows most of the GM maize (ca.111 000 ha) amounting to 28% of the total area devoted to this crop in the country. Smaller quantities of GM maize have been grown in the Czech Republic, Slovakia, and Romania (Rostoks *et al*., 2019).

GM maize has also been subject to controversies about its environmental impacts. IR maize-fed caterpillars can harm the green lacewing, *Chrysoperla carnea* (Hilbeck *et al*., 1998), which was first confirmed (Dutton *et al*., 2002) then challenged (Obrist *et al*., 2006). The *Bt* toxin present in the GM maize lines is also expressed in the pollen, sometimes in concentrations high enough so that lepidopteran caterpillars ingesting such pollen may suffer sublethal effects or die (Losey *et al*., 1999; Lang & Otto, 2010). This hazard exists because maize is a wind-pollinated crop, producing large amounts of pollen, which carried by the wind can be deposited on various surfaces, including leaves of plants within and around maize fields. Insects feeding on these plants can inadvertently ingest this maize pollen that is toxic to them. The possibility, importance, and impact of this chain of events has been the subject of intense debate (Stanley-Horn *et al*., 2001; Dively *et al*., 2004). The larvae of the monarch butterfly, *Danaus plexippus*, one of the conservation icons in North America, live and develop on milkweed, *Asclepias syriaca*, which is an important weed in maize. Monarch larvae consuming maize pollen-dusted milkweed leaves are exposed to the *Bt*- toxin and can suffer high mortality (Hansen Jesse & Obrycki, 2000).

A similar scenario is also possible in Europe: the seasonally abundant maize pollen can be deposited on various plant surfaces, and herbivores feeding on those pollen-coated plants may be exposed to the *Bt*-toxin present in the pollen. This, for example, poses a potential risk to the peacock butterfly, *Inachis io* (Felke *et al*., 2010, Holst *et al*., 2013), the larvae of which feed on nettle, *Urtica dioica*, a common agricultural weed in Europe or other species whose host plant can be dusted with maize pollen (Shirai & Takahashi, 2005; Lauber, 2011; Lang *et al*., 2015). Although the European Food Safety Authority expert panel concluded that risk by IR maize pollen to protected butterflies in Europe can be avoided (EFSA, 2015), their standpoint is not based on any detailed analysis of seasonality, sensitivity, and biology of the protected Lepidoptera species in Europe, partly because much of this knowledge is still lacking. The EFSA opinion has also been challenged on its assumptions of pollen deposition (Hoffmann *et al*., 2014; Kruse-Plass *et al*., 2017), and thus the matter rests inconclusive. This makes it important to further scrutinize the potential impact of IR maize pollen on protected European butterflies.

Another mechanism through which GM maize can impact the flora and fauna is through changing the cultivation system, and thus modifying the environmental conditions in a landscape where such GM maize is grown. Such indirect impacts can be important and substantial, which is one of the main conclusions of the large-scale field trials done in the United Kingdom in the early 2000s (Firbank *et al*., 2003). Most of the GM maize grown in the Americas is HR (or both IR and HR). A major consequence of the widespread planting of HR maize in the United States was an increased use of broad-spectrum herbicides (Benbrook, 2016), with the consequence of substantially reducing the density of milkweed over vast areas. Thus, the host plant of *D. plexippus* was reduced on a landscape scale, which is a major cause of a serious decline of the species (Thogmartin *et al*., 2017, Stenoien *et al*., 2018). A similar chain of events can also occur in Europe, but so far, the potential consequences for protected butterflies of large-scale growing of HR maize have not been analyzed. Elimination of host plants can trigger extinction cascades, and this has also been documented in European butterflies (Pearse & Altermatt, 2013).

Our aim in this article is to provide an overall evaluation of potential exposure of protected European butterflies to IR maize pollen, and to identify protected species that feed on weed species common in maize, which therefore would possibly be exposed to the hazard of reduced host plant densities as a consequence of increased herbicide sprays under the scenario of widespread planting of HR maize in Europe.

Materials and methods

Protected Lepidoptera

For the analysis of exposure to IR maize pollen, we focused on protected species. We analyzed the Central European day-active Lepidoptera (Papilionoidea) within the political borders of Germany, excluding all moths except for the species listed in the Annexes of the Natura 2000 directive (European Council, 1992). The reasons for the geographical focus were that (i) Germany occupies a large part of the European continent, extending from the North Sea in the north to the Alps in the south; (ii) detailed information is largely available and comprehensive for this area, including the phenology and ecology of lepidopteran species as well as of flowering periods of maize. We chose the butterfly species from the following officially published listings of protected species: Annexes of the Natura 2000 directive (European Council, 1992), the European Red List for butterflies (Van Swaay *et al*., 2010), the national Red List for Germany (Binot-Hafke *et al*., 2011), and the regional Red List for the Federal State Baden-Württemberg (Ebert *et al*., 2008). Species that are extinct in Germany were not considered; we also excluded endophytically feeding species which are not expected to be exposed to pollen, and species that occur in mountainous and alpine regions above 700 m asl, where little or no maize is grown in Central Europe. The *Maculinea* (*Phengaris*) spp. (Lyacaenidae) were also excluded as their larvae start their lives within the inflorescences of legumes, and later continue development within ant nests (Hayes, 2015), thus an exposure to maize pollen appears unlikely. Of the remainder, we selected all species that were listed as "critically endangered" (CR), "endangered" (EN), "vulnerable" (VU), or "near threatened" (NT). Species names follow Gaedike *et al*. (2017).

Exposure of protected Lepidoptera species

We analyzed the temporal exposure of the larvae of the selected species to *Bt* maize pollen. First, the number of generations per season and the period of occurrence of larvae were identified for each species using information in Ebert (1991), Ebert and Rennwald (1991), SBN (1994), Settele *et al*. (2009), Bräu *et al*. (2013), and by consulting the following websites: Arbeitsgruppe Schmetterlinge Deutschlands (2017), Rennwald and Rodeland (2004), Wagner (2018), and Ziegler (2018). In Central Europe, butterfly larvae are usually active from March to October (Settele *et al*., 2009), and this period was checked for larval presence concurrent with the pollen shedding period of maize. Periods when larvae are inactive were ignored. Potentially, maize in Europe can shed pollen from mid-June until the end of August, depending on sowing date, cultivar, and climatic conditions (Emberlin *et al*., 1999; Treu & Emberlin, 2000; Feil & Schmid, 2001; Lang *et al*., 2004). We used two criteria to identify species that may be vulnerable. First, if the initiation of larval activity overlapped with maize pollen shedding, first instar larvae would probably be present, and they are the most sensitive to the *Bt* toxin (Dammak *et al*., 2016). We also identified species whose larvae were present throughout the period of pollen shedding, or half or more of one larval generation overlapped with maize pollen shedding.

The different generations of bi- and multivoltine species will differ in their overlap with the period of maize pollen shedding. For species with continuous, overlapping generations, we calculated the overlap of the total larval period with maize pollen anthesis. For bi- and multivoltine species with distinct generations, we counted the overlap for the respective generation with the largest exposure to maize pollen shed within a season. For the latter, this value is the relevant one, because an adverse effect on one generation will impact the following ones through the decreased reproductive output of the affected generation (Andow & Zwahlen, 2006).

With regard to spatial exposure, we assumed that any habitat type that Lepidoptera use can principally be exposed to maize pollen dispersal, albeit with different likelihood and intensity, except for mountainous and alpine regions. These would also include forest edges as well as nature reserves (Lang *et al*., 2015).

Food plants of protected Lepidoptera

We considered the major maize weeds in four principal maize-growing European countries: Spain, Germany, Hungary, and Romania (Dewar, 2009; Jensen *et al*., 2011), and cross-checked the list of host plants of Lepidoptera protected in the respective country. The list of protected Lepidoptera in the four target countries were obtained by consulting the above sources, as well as van Sway & Warren (1999) and van Sway *et al*. (2010). Host plants records of Lepidoptera were obtained from Jonko (2018) and Mazzei *et al*. (2017). We included a species potentially at risk if at least one species of weeds was included among the indicated host plants, or if the larva was a polyphagous feeder on Gramineae.

Results

Species potentially affected by IR maize pollen

At the European level, 37 butterfly species are considered as threatened, with 0.7% of them being "Critically Endangered," 2.8% "Endangered," and 5% "Vulnerable." A further 44 species are classified as "Near Threatened." Annex II of the Habitat's Directive includes 38 species, and Annex IV lists 41 species of

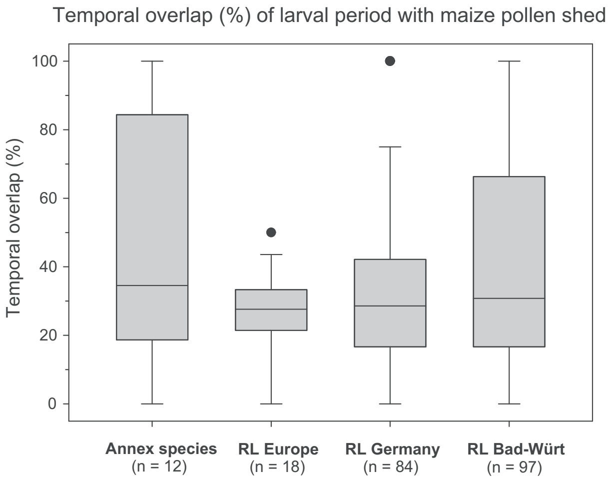

Fig. 1 Temporal overlap (%) of the larval period of protected Lepidoptera with maize pollen shedding period in mid-Europe. Annex species = butterfly and moth species listed on the Annexes of the Habitats' Directive, RL Europe = species listed on the European Red List of butterflies, RL Germany = butterfly species listed on the Red List of Germany, RL Bad-Würt = butterfly species listed on the Red List of Baden-Württemberg. Boxes show the 25% and 75% quartiles, the horizontal line within the box is the median, while 10% and 90% percentiles are indicated by the whiskers and outliers by dots.

Lepidoptera that merit special conservation considerations. Out of all those species, 32 of them (*Lycaena helle*, *L. dispar*, *Proserpinus proserpina*) had a larval generation with >50% overlap with pollen shedding in Central Europe (Germany), 3 of them being listed on one of the Annexes of the Habitats Directive (Table S1). The Red Lists of Europe, Germany and Baden-Württemberg contain 18 species, 84 species, and 97 species exposed, respectively, including the "Near Threatened" category (Table S1). This demonstrates that on a more regional level, several additional, protected species could be potentially affected. On average, the larvae of the protected Lepidoptera may be exposed to pollen shed for 25.7%–42.9% of their larval development period, with a median overlap of around 30% (Fig. 1). Species with two to three different generations within one season showed a higher mean overlap of at least one larval generation of 77.8% (sd = 19.85%, n = 25). Sixty-nine species had an overlap between the onset of a generation (when first instar larvae are present) and pollen shedding, and 8 of those were listed on the Annexes of the Habitats Directive (Table S1). Seventeen species fitted both conditions: the presence of first instar larvae and >50% of a generation overlapped with pollen shedding (Table S1).

Species potentially affected by lack of food plants

Five species were recorded as common weeds in maize fields in all four countries considered, 17 in three of them, 18 in two, and an additional 22 in one (Table S2). In the four countries, 369 protected Lepidoptera species were identified (204 spp. in Germany, 185 spp. in Hungary, 59 spp. in Romania, and 43 spp. in Spain). Of these protected species, 140 were found to feed on one or more of the common weeds growing in maize crops, in one or more of the above countries (Table S3), and may be therefore indirectly endangered by the potential elimination of their food plants. The majority of those species were Nymphalidae (53 species), followed by Noctuidae (31 spp.), Erebidae (16 spp.), Geometridae (11 spp.), and Hesperiidae (10 spp.), with Lemoniidae, Lycaenidae, Pieridae, Zygenidae, Sphingidae, and the saturniid *Saturnia pavoniella* making up the rest (Table S3). The majority of species were only protected in a single country within the area investigated, and only seven species were protected in three countries; and the nymphalid *Lopinga achine* in all four (Table S3). Most of the species at risk were polyphagous; 39 of them (27.9%) feed on various Gramineae while 15 (10.7%) include at least one of the weed species (i.e., *Convolvulus arvensis*, *Datura stramonium*, *Elymus repens*, *Poa annua*, *Polygonum aviculare*, *Stellaria media*, and *Taraxacum officinale*) among its host plants (Table S3). In addition, host plants of a further 14 species in 8 lepidopteran families include one or more species that are congeneric with maize weeds.

Forty-six species appeared on both lists: they have larvae during pollen shedding, and also have one or more host plants that were listed as weeds in maize. These species include eight lycaenids, five hesperids, and the erebid moth *Euplagia quadripunctaria*; the rest are all nymphalids (Tables S1 and S3). Considering the overlap between larval activity and pollen shedding, several of these have overlap with pollen shedding in at least half of their larval activity period, *and* feed on maize weeds, too. Five of these (*Coenonympha hero*, *C. oedippus*, *Lopinga achine*, *Lycaena dispar*, and *Melitaea diamina*) are also listed on Annexes of the Habitat's Directive.

Discussion

The first publication that wind-dispersed GM maize pollen can harm the larvae of the monarch butterfly *D. plexippus*, by coating leaves of its host plant, the milkweed *Asclepias syriaca* (Losey *et al*., 1999), generated heated debate and further studies (Hansen Jesse & Obrycki, 2000; Pleasants *et al*., 2001; Stanley-Horn *et al*., 2001; Zangerl *et al*., 2001). After more precise measurements, it turned out that pollen densities causing such toxicity can be deposited on host plant leaves in the field (Dively *et al*., 2004). This turned attention to similar potential, unwanted negative effects on butterflies elsewhere (Shirai & Takahashi, 2005; Schuppener *et al*., 2012). For example, the occurrence of the larvae of the European peacock butterfly, *Inachis io* overlaps with maize pollen shed at least in some parts of Europe (Holst *et al*., 2013), and if GM maize were grown in Europe, the species could be exposed to GM maize pollen during its larval development in those parts of the European continent (Lang *et al*., 2015). In Austria, Traxler *et al*. (2005) estimated that of 152 butterfly species occurring in arable land, 95% show an overlap of the larval periods with pollen shedding of maize (with an overlap ranging from 8% to 100%). In Saxonia, Germany, Musche *et al*. (2009) predict that the larvae of 74% of the butterflies and moths occurring in the state are potentially exposed to *Bt* maize cultivation, including six species that are listed in the Annexes of the EU Habitats and Species Directive (EC, 1992). A recent report from the Netherlands (Wallis de Vries *et al*., 2017) identified 19 butterfly and 28 macromoth species whose larvae may be exposed to maize pollen. Most dependent on maize field margins is the Dusky Large Blue (*Phengaris nausithous*) that has only one population in the country. This species, however, spends most of its larval period inside ant nests, where it is not exposed to maize pollen (Fiedler, 1990). As shown in this study, protected and endangered lepidopteran species would be exposed as well, if their habitats are neighboring to maize fields and/or if their larval host plants grow in field margins adjacent to maize (Lang *et al*., 2015).

In general, we found that species with more than one generation/season were more exposed to pollen dispersal than species with one generation per year. It has to be kept in mind, however, that the presented results are coarse estimates, because the larval periods and number of generations as well as the period of maize pollen shedding would change with region, weather conditions, maize cultivars and maize sowing dates (e.g., Abrecht & Carberry, 1993; Traore *et al*., 2000; Nguyen & Jehle, 2007). Such regional differences can lead to a different exposure and impact of *Bt* maize cultivation, even for populations of the same butterfly species (Holst *et al*., 2013). Also, we have not considered spatial exposure, that is, to what extent the occurrence of species and their habitats would overlap with maize cultivation areas (cf. Dolezel & Heisenberger, 2018).

Reported pollen densities on host plants in Europe are within a range for which adverse effects of *Bt* maize pollen have been demonstrated (Lang *et al*., 2004; Lauber, 2011; Schuppener *et al*., 2012), but the generality and reliability of these measurements is a matter of continuing debate (Emberlin *et al*., 1999; Hofmann *et al*., 2014; EFSA, 2015; Kruse-Plasse *et al*., 2017). Available data about pollen densities on host plants are not always comparable, because methods and study designs are too diverse, especially the exposure time of the sampling devices. This shortcoming can possibly be removed by systematic surveys. There is also a lack of data for maize pollen numbers deposited on host plants at distances >10 m of field edges (Lang *et al*., 2015).

Apart from phenological overlap and larval sensitivity, several other factors influence the risk butterfly larvae will have to face, so it is not possible to translate a given degree of overlap to a certain level of hazard. The effect of IR maize pollen depends on the actual construct. Even existing lines have different expression patterns, and the amount of *Bt* toxin in the pollen varies (Lang *et al*., 2004; Nguyen & Jehle, 2007; Székács *et al*., 2010). Precipitation and wind patterns have a profound influence on the dynamics of pollen deposition, as well as the amount of maize in the landscape, within which the amount of GM maize will also be clearly important. The landscape context will also be influential, as the size of fields, their arrangement, cultivation traditions and constraints all modify butterfly behavior, which very likely will have an influence on the distribution of the larvae that are less mobile than the egg-laying adults. On the larval side, sensitivity varies with age (Losey *et al*., 1999; Lauber, 2011), physiological condition, and other kinds of environmental stress (Lauber, 2011), which adds to the complexity of the eventual effect of GM pollen on them.

Even though monarch larvae may be directly threatened by GM maize pollen, the fact that most of its host plant was removed from maize fields makes this hazard currently minuscule (Stenoien *et al*., 2018). A more serious harm, leading to an alarming decrease in monarch populations happened because the increased use of broad-spectrum herbicides in herbicide-resistant GM crops greatly diminished the food resource for larvae at the landscape scale (Stenoien *et al*., 2018). Other species may face similar hazards. In the United States, Losey *et al*. (2003) reported 132 plant species in 33 families associated with maize, and 229 lepidopteran species in 21 families that feed on these plants. For the Netherlands, Wallis De Vries *et al*. (2017) compiled a list of 198 plant species occurring within or at the margin of maize fields serving as potential larval host plants and/or nectar plants for adult butterflies and moths.

We identified a number of protected European Lepidoptera that could face a similar threat if HR transgenic maize were widely planted in Europe. Published information on plant species associated with maize cultivation mainly focuses on weeds within maize fields due to the implicated economic relevance (Heard *et al*., 2003; Zalai *et al*., 2014), and less so on herbs occurring in field edges and margins. This applies to the information used in this study, too. In edges and margins of fields, however, more herb species are growing, and the microclimate is more suitable for lepidopteran larvae, thus these habitats harbor more species. A further note of caution is warranted due to the uncertainty of the host plant use over the landscape. Several species are not expected to search for host plants inside maize stands (although they may do so at the edges or in field defects), but spray drift can impact their host plants also outside maize fields, for example, at landscape level. Additionally, as HR growing substantially changes weed composition, and weed seed dynamics, there could be landscape-scale consequences of these for weed populations. For the above reasons, our results are probably conservative in terms of possibly affected species.

The two hazards, the one due to the deposition of potentially toxic maize pollen on host plants, and the lower density of host plants due to the more effective weed control by increased use of herbicides may actually have opposite effects on butterfly populations. If the host plant density in and near maize fields were lower, the butterflies would be less exposed to the first risk, because they will have to find suitable host plants further away from maize fields where pollen densities will also be lower. However, in-field and off-field hazards may add up, that is, butterflies may not only lose their host plants within fields but will be exposed to toxic Bt maize pollen off-field at the same time, which would make the situation even more risky. Much depends on host plant finding behavior, female egg laying strategies, and the flexibility of host plant choice. Modeling and detailed field studies would still be needed to understand why GM maize in North America threatens the monarch butterfly (Stenoien *et al*., 2018)—similar studies are sorely lacking in Europe (but see Holst *et al*., 2013). Conditions in the current European cultivated landscapes are rarely favorable for butterflies (Bubova *et al*., 2015), and additional changes in landscapes and cultivation practices, both of which will be unavoidable under widespread GM crop cultivation (Bøhn & Lövei, 2017), will be unlikely to improve the current suboptimal situation for butterflies. Butterfly diversity is still high in some agricultural settings in Europe, for example, in Romania (Loos *et al*., 2014, 2015), and GM crop cultivation could affect this high level of species richness.

Finally, we should not forget that butterflies and macromoths are more charismatic species than small, dull-colored, and nocturnal Lepidoptera and as a consequence, only a minimal part of the lepidopteran diversity is well-studied and subject to conservation measures (Braby, 2018). The introduction of GM maize in Europe could potentially affect many species that live within or near to agricultural areas (Masetti *et al*., 2017), with large consequences on ecosystem services such as pollination, on which agriculture ultimately depends (New, 2004; Kovács-Hostyánszki *et al*., 2017). Our results clearly show the importance of regionally protected species which gives EU Member States a clear mandate to consider possible risks of GM maize given their responsibility for national biodiversity conservation. In addition, the responsibility of individual Member States for the protection for FFH species is highly relevant in the context of Bt maize cultivation, particularly for Annex IV species that are to be protected throughout their range within a country.

Acknowledgements

This material was first presented at the ICCB-ECCB conference in Montpellier, France, 2015. We thank the organisers for giving us the possibility to present and discuss our ideas. We would like to register our gratitude to the administrators of the consulted websites that are an important source for any such analysis. M. Ferrante was supported by a postdoctoral fellowship from Aarhus University, and V. Bacle by an Erasmus Mobility grant. This is a scientific output of the AMIGA Project, supported by the EC (grant agreement no. 289706). We thank M. Wiemers and L. Peregovits for information, N. Holst and two anonymous reviewers for comments on the manuscript.

Disclosure

The authors disclose no conflicts of interest of any kind associated with this manuscript.

References

Andow, D.A. and Zwahlen, C. (2006) Assessing environmental risks of transgenic plants. *Ecology Letters*, 9, 196–214.

Arbeitsgruppe Schmetterlinge Deutschlands (2017) *Schmetterlinge Deutschlands*. http://www.schmetterlinge-d.de. Accessed 17 Oct 2017.

Anghiera, P.M. (1907) *De Orbe Novo* (1st edn. 1530). Leroux, Paris, France.

Abrecht, D.G. and Carberry, P.S. (1993) The influence of water deficit prior to tassel initiation on maize growth, development and yield. *Field Crops Research*, 31, 55–69.

Beadle, G.W. (1939) Teosinte and the origin of maize. *Journal of Heredity*, 30, 245–247.

Benbrook, C.M. (2016) Trends in glyphosate herbicide use in the United States and globally. *Environmental Sciences Europe*, 28, 1–15.

Binot-Hafke, M., Balzer, S., Becker, N., Gruttke, H., Haupt, H., Hofbauer, N. *et al*. (eds.) (2011) *Rote Liste gefährdeter Tiere, Pflanzen und Pilze Deutschlands Band 3: Wirbellose Tiere (Teil 1)*, p. 70, Vol. 3. Bundesamt für Naturschutz, Bonn-Bad Godesberg, Naturschutz und Biologische Vielfalt.

Bøhn, T. and Lövei, G.L. (2017) Complex outcomes from insect and weed control with transgenic plants: ecological surprises? *Frontiers in Environmental Science*, 5, 60. https://doi.org/10.3389/fenvs.2017.00060

Braby, M.F. (2018) Threatened species conservation of invertebrates in Australia: an overview. *Austral Entomology*, 57, 173–181.

Bräu, M., Bolz, R., Kolbeck, H., Nummer, A., Voith, J. and Wolf, W. (2013) *Tagfalter in Bayern*. Verlag Eugen Ulmer, Stuttgart, Germany.

Bubova, T., Vrabec, V., Kulma, M. and Nowicki, P. (2015) Land management impacts on European butterflies of conservation concern: a review. *Journal of Insect Conservation*, 19, 805–821.

Dammak, M., Khedher S.B., Boukedi, H., Chaib, I., Laarif, A. and Tounsi, S. (2016) Involvement of the processing step in the susceptibility/tolerance of two lepidopteran larvae to *Bacillus thuringiensis* Cry1Aa toxin. *Pesticide Biochemistry and Physiology*, 127, 46–50.

Dewar, A.M. (2009) Weed control in glypohsate-tolerant maize in Europe. *Pest Management Science*, 67, 1045–1058.

Dively, G.P., Rose, R., Sears, M.K., Hellmich, R.L., Stanley-Horn, D.E., Calvin, D.D. *et al*. (2004) Effects on monarch butterfly larvae (Lepidoptera Danaidae) after continuous exposure to Cry1Ab-expressing corn during anthesis. *Environmental Entomology*, 33, 1116–1125.

Dolezel, M., Bartel, A. and Heissenberger, A. (2018) Spatial analysis of the occurrence of protected butterflies in six European biogeographic regions as a tool for the environmental risk assessment of *Bt* maize. *BioRisk*, 13, 31–52.

Dutton, A., Klein, H., Romeis, J. and Bigler, F. (2002) Uptake of Bt-toxin by herbivores feeding on transgenic maize and consequences for the predator *Chrysoperla carnea*. *Ecological Entomology*, 27, 441–447.

Ebert, G. (1991) *Die Schmetterlinge Baden-Württembergs*, Band 2: Tagfalter II. Verlag Eugen Ulmer, Stuttgart, Germany.

Ebert, G., Hofmann, A., Karbiener, O., Meineke, J.U., Steiner, A. and Trusch, R. (2008) *Rote Liste und Artenverzeichnis der Großschmetterlinge Baden-Württembergs* (Stand: 2004) unter Mitarbeit von Bartsch D., Bläsius R., Geissler-Strobel S., Hafner S., Hermann G., Meier M., Nunner A., Ratzel U., Schanowski A. und Steiner R., LUBW. www.lubw.baden-wuerttemberg.de/servlet/is/29039/rl_av_schmetterlinge_bw_2004_281108.xls?command=downloadContent&filename=rl_av_schmetterlinge_bw_2004_281108.xls

Ebert, G. and Rennwald, E. (1991) *Die Schmetterlinge Baden-Württembergs*, Band 1: Tagfalter I. Verlag Eugen Ulmer, Stuttgart, Germany.

EFSA (2015) Scientific opinion updating risk management recommendations to limit exposure of non-target Lepidoptera of conservation concern in protected habitats to *Bt*-maize pollen. *EFSA Journal*, 13, 4127.

Emberlin, J., Adams-Groom, B. and Tidmarsh, J. (1999) *The dispersal of maize (Zea mays) pollen*. A report commissioned by the Soil Association: National Pollen Research Unit, University College Worcester, England.

European Council (1992) EC Directive 92/43/EEC of 21 May 1992 on the conservation of natural habitats and of wild fauna and flora. *Official Journal*, 206, 7.

FAO (2018) FAOSTAT. Online Statistical Database. http://faostat.fao.org. Accessed 30 Apr 2018.

Feil, B. and Schmid, J.E. (2001) *Pollenflug bei Mais, Weizen und Roggen*. Shaker Verlag, Aachen, Germany.

Felke, M., Langenbruch, G.A., Feiertag, S. and Kassa, A. (2010) Effect of Bt-176 maize pollen on first instar larvae of the peacock butterfly (*Inachis io*) (Lepidoptera; Nymphalidae). *Environmental Biosafety Research*, 9, 5–12.

Fiedler, K. (1990) New information on the biology of *Maculinea nausithous* and *M. teleius* (Lepidoptera: Lycanidae). *Nota Lepidopterologica*, 12, 246–256.

Firbank, L.G., Heard, M.S., Woiwod, I.P., Hawes, C., Haughton, A., Champion, G.T. *et al*. (2003) An introduction to the farm-scale evaluations of genetically modified herbicide-tolerant crops. *Journal of Applied Ecology*, 40, 2–16.

Gaedike, R., Nuss, M., Steiner, A. and Trusch, R. (2017) Verzeichnis der Schmetterlinge Deutschlands (Lepidoptera). *Entomologische Nachrichten Berichte (Dresden)*, Beiheft 21, 1–362.

Hansen Jesse, L.C. and Obrycki, J.J. (2000) Field deposition of *Bt* transgenic corn pollen: lethal effects on the monarch butterfly. *Oecologia*, 125, 241–248.

Hayes, M.P. (2015) The biology and ecology of the large blue butterfly *Phengaris (Maculinea) arion*: a review. *Journal of Insect Conservation*, 19, 1037–1051.

Heard, M.S., Hawes, C., Champion, G.T., Clark, S.J., Firbank, L.G., Haughton, A.J. *et al*. (2003) Weeds in fields with contrasting conventional and genetically modified herbicide-tolerant crops. 1. Effects on abundance and diversity. *Philosophical Transactions of the Royal Society B*, 358, 1819–1832.

Hilbeck, A., Baumgartner, M., Fried, P.M. and Bigler, F. (1998) Effects of transgenic *Bacillus thuringiensis* corn-fed prey on mortality and development time of immature *Chrysoperla carnea* (Neuroptera: Chrysopidae) *Environmental Entomology*, 27, 480–487.

Hofmann, F., Otto, M. and Wosniok, W. (2014) Maize pollen deposition in relation to the distance from the nearest pollen source under common cultivation—results of 10 years of monitoring (2001–2010). *Environmental Sciences Europe*, 26, 24.

Holst, N., Lang, A., Lövei, G. and Otto, M. (2013) Increased mortality is predicted of *Inachis io* larvae caused by *Bt*-maize pollen in European farmland. *Ecological Modelling*, 250, 126–133.

Janick, J. and Caneva, G. (2005) The first images of maize in Europe. *Maydica*, 50, 71.

Jensen, P.K., Bibard, V., Czembor, E., Dumitru, S., Foucart, G., Froud-Williams, R.J. *et al*. (2011) Survey of weeds in maize crops in Europe. *DJF Report Agricultural Science*, 149, 1–44.

Jonko, C. (2018) *Lepidoptera mundi*. Online database. https://lepidoptera.eu. Accessed 25 Nov 2019.

Kovács-Hostyánszki, A., Espindola, A., Vanbergen, A.J., Settele, J., Kremen, C. and Dicks, L.V. (2017). Ecological intensification to mitigate impacts of conventional intensive land use on pollinators and pollination. *Ecology Letters*, 20, 673–689.

Kruse-Plass, M., Hofmann, F., Kuhn, U., Otto, M., Schlechtriemen, U., Schröder, B. *et al*. (2017) Reply to the EFSA (2016) on the relevance of recent publications (Hofmann et al. 2014, 2016) on environmental risk assessment and management of Bt-maize events (MON810, Bt11 and 1507). *Environmental Sciences Europe*, 29, 12.

Lang, A., Ludy, C. and Vojtech, E. (2004) Dispersion and distribution of *Bt* maize pollen in field margins. *Journal of Plant Diseases and Protection*, 111, 417–428.

Lang, A., Oehen, B., Ross, J.H., Bieri, K. and Steinbrich, A. (2015) Potential exposure of butterflies in protected habitats by *Bt* maize cultivation: a case study in Switzerland. *Biological Conservation*, 192, 369–377.

Lang, A. and Otto, M. (2010) A synthesis of laboratory and field studies on the effects of transgenic Bt-maize on non-target Lepidoptera. *Entomologia Experimentalis et Applicata*, 135, 121–134.

Lauber, E. (2011) *Cry1 toxin content of MON 810 Bt-corn and the effect of its pollen on protected butterfly species in Hungary*. PhD dissertation, University of Horticulture, Budapest, Hungary.

Loos, J., Dorresteijn, I., Hanspach, J., Fus, P., Rakosy, L. and Fischer, J. (2014) Low-intensity agricultural landscapes in Transylvania support high butterfly diversity: implications for conservation. *PLoS ONE*, 9, e103256.

Loos, J., Kuussaari, M., Ekroos, J., Hanspach, J., Fust, P., Jackson, L. and Fischer, J. (2015). Changes in butterfly movements along a gradient of land use in farmlands of Transylvania (Romania). *Landscape Ecology*, 30, 625–635.

Losey, J.E., Hufbauer, R.A. and Hartzler, R.G. (2003) Enumerating lepidopteran species associated with maize as a first step in risk assessment in the USA. *Environmental Biosafety Research*, 2, 247–261.

Losey, J.E., Rayor, L.S. and Carter, M.E. (1999) Transgenic pollen harms monarch larvae. *Nature*, 399, 214.

Masetti, A., Arpaia, S., Ghesini, S., Magagnoli, S., Magarelli, R.A., Neri, U. *et al*. (2017) Macro-moths as possible assessment endpoints for non-target effects of *Bt*-maize pollen: a faunistic study in three Italian protected areas. *Bulletin of Insectology*, 70, 307–314.

Mazzei, P., Morel, D. and Panfili, R. (2017) *Moths and butterflies of Europe and North Africa*. www.leps.it. Accessed 15 Oct 2017.

Musche, M., Settele, J. and Durka, W. (2009) Basisstudie zur Wechselwirkung von gentechnisch verändertem MON810-Mais mit spezifischen Schmetterlingsarten. Report to the Sächsisches Staatsministerium für Umwelt und Landwirtschaft, Ref. 55/Bio- und Gentechnik, Chemikalien. 73 pp. www.umwelt.sachsen.de/umwelt/download/luft/studie_mon810_schmetterlinge29042008%281%29.pdf. Accessed 6 February 2020.

New, T.R. (2004) Moths (Insecta: Lepidoptera) and conservation: background and perspective. *Journal of Insect Conservation*, 8, 79–94.

Nguyen, H.T. and Jehle, J.A. (2007) Quantitative analysis of the seasonal and tissue-specific expression of Cry1Ab in transgenic maize Mon810. *Journal of Plant Diseases and Protection*, 114, 82–87.

Obrist, L.B., Dutton, A., Klein, H., Romeis, J. and Bigler, F. (2006) Biological activity of Cry1Ab toxin expressed by *Bt* maize following ingestion by herbivorous arthropods and exposure of the predator *Chrysoperla carnea*. *Biocontrol*, 51, 31–48.

Parisi, C., Tillie, P. and Rodriguez-Cerezo, E. (2016) The global pipeline of GM crops out to 2020. *Nature Biotechnology*, 34, 31–36.

Pearse, I.S. and Altermatt, F. (2013) Extinction cascades partially estimate herbivore losses in a complete Lepidoptera–plant food web. *Ecology*, 94, 1785–1794.

Pleasants, J.M., Hellmich, R.L., Dively, G.P., Sears, M.K., Stanley-Horn, D.E., Mattila, H.R. *et al*. (2001) Corn pollen deposition on milkweeds in and near corn fields. *Proceedings of the National Academy of Sciences USA*, 98, 11919–11924.

Rákosy, L. (2005) EU legislation and the protection of Lepidoptera species in Romania. *Bulletin de Informare Entomologica*, 16, 89–96.

Rennwald, E. and Rodeland, J. (2004) *Lepiforum: Bestimmung von Schmetterlingen (Lepidoptera) und ihren Präimaginalstadien*. http://www.lepiforum.de/. Accessed 2 Feb 2019.

Rostoks, N., Grantiņa-Ieviņa, L., Ieviņa, B., Evelone, V., Valciņa, O. and Aleksejeva, I. (2019) Genetically modified seeds and plant propagating material in Europe: potential routes of entrance and current status. *Heliyon*, 5, e01242.

SBN (1994) *Tagfalter und ihre Lebensräume*. Band 1. Schweizerischer Bund für Naturschutz, Basel, Switzerland.

Schuppener, M., Mühlhause, J., Müller, A.K. and Rauschen, S. (2012) Environmental risk assessment for the small tortoiseshell *Aglais urticae* and a stacked *Bt*-maize with combined resistances against Lepidoptera and Chrysomelidae in central European agrarian landscapes. *Molecular Ecology*, 21, 4646–4662.

Settele, J., Steiner, R., Reinhardt, R., Feldmann, R. and Hermann, G. (2009) *Schmetterlinge. Die Tagfalter Deutschlands*. Verlag Eugen Ulmer, Stuttgart, Germany.

Shirai, Y. and Takahashi, M. (2005) Effects of transgenic *Bt* corn pollen on a non-target lycaenid butterfly, *Pseucozizeeria maha. Applied Entomology and Zoology*, 40, 151–159.

Stenoien, C., Nail, K.R., Zalucki, J.M., Parry, H., Oberhauser, K.S. and Zalucki, M.P. (2018) Monarchs in decline: a collateral landscape-level effect of modern agriculture. *Insect Science*, 25, 528–541.

Stanley-Horn, D.E., Dively, G.P., Hellmich, R.L., Mattila, H.R., Sears, M.K., Rose, R. *et al*. (2001) Assessing the impact of Cry1Ab-expressing corn pollen on monarch butterfly larvae in field studies. *Proceedings of the National Academy of Sciences USA*, 98, 11931–11936.

Szekacs, A., Lauber, E., Juracsek, J. and Darvas, B. (2010). Cry1ab toxin production of MON 810 transgenic maize. *Environmental and Toxicological Chemistry*, 29, 182–190.

Thogmartin, W.E., Wiederholt, R., Oberhauser, K., Drum, R.G., Diffendorfer, J.E., Altizer, S. *et al*. (2017) Monarch butterfly population decline in North America: identifying the threatening processes. *Royal Society Open Science*, 4, 170760.

Traore, S.B., Carlson, R.E., Pilcher, C.D. and Rice, M.E. (2000) Bt and non-Bt maize growth and development as affected by temperature and drought stress. *Agronomy Journal*, 92, 1027–1035.

Traxler, A., Minarz, E., Höttinger, H., Pennerstorfer, J., Schmatzberger, A., Bank, G. *et al*. (2005). *Biodiversitäts-Hotspots der Agrarlandschaft als Eckpfeiler für Risikoabschätzung und Monitoring von GVO*. Bundesministerium für Gesundheit und Frauen, Forschungsberichte der Sektion IV, Band 5, Wien, Austria. 85 pp.

Treu, R.J. and Emberlin, J. (2000) Pollen dispersal in the crops maize (*Zea mays*), oilseed rape (*Brassica napus* spp. *olifera*), potatoes (*Solanum tuberosum*), sugar beet (*Beta vulgaris* ssp. *vulgaris*) and wheat (*Triticum aestivum*). Report for the Soil Association, National Pollen Research Unit, University College, Worcester, UK.

Van Swaay, C., Cuttelod, A., Collins, S., Maes, D., López Munguira, M., Šašić, M. *et al*. (2010) *European Red List of Butterflies*. Publications Office of the European Union, Luxembourg.

Van Swaay, C.A.M. and Warren, M.S. (1999) *Red Data book of European Butterflies (Rhopalocera)*. Nature and Environment, No. 99, Council of Europe Publishing, Strasbourg, France.

Wagner, W. (2018) *Lepidoptera and their ecology*. http://www.pyrgus.de/ Accessed 31 Jan 2019.

Wallis de Vries, M.F., van Deijk, J. and van Alebeek, F. (2017) *The Importance of Maize and Oilseed Rape Field Margins for Lepidoptera*. Report VS2017.005/CGM 2017-03, De Vlinderstichting/Dutch Butterfly Conservation, Wageningen.

Zalai, M., Dorner, Z. and Keresztes, Z. (2014) Seasonal weed structure of maize in the light of farming systems. *Applied Ecology and Environmental Research*, 12, 765–776.

Zangerl, A.R., McKenna, D., Wraight, C.L., Carroll, M., Ficarello, P., Warner, R. and Berembaum, M.R. (2001) Effects of exposure to event 176 *Bacillus thuringiensis* corn pollen on monarch and black swallowtail caterpillars under field conditions. *Proceedings of the National Academy of Sciences USA*, 98, 11908–11912.

Ziegler, H. (2018) *Butterflies & moths of palaearctic regions*. http://www.euroleps.ch. Accessed 25 Oct 2019.

Manuscript received June 9, 2020
Final version received July 2, 2020
Accepted July 5, 2020

Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Table S1. The list of the analyzed "Red List" Lepidoptera (protected butterfly and moth species from Germany: Papilionoidea, and moth species only from the

Habitat's Directive Annexes), and the potential Temporal exposure of their larvae to pollen shedding of maize.

Table S2. Common maize weeds in Germany, Hungary, Romania, and Spain.

Table S3. Protected Lepidoptera species in Germany, Hungary, Romania, and Spain that have host plants which are maize weeds in the respective countries.