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ORIGINAL ARTICLE

Evaluation of the potential exposure of butterflies to genetically modified maize pollen in protected areas in Italy

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Abstract

Environmental impacts of genetically modified crops are mandatorily assessed during their pre-market phase. One of the areas of concern is the possible impact on non-target organisms. Crops expressing Cry toxins might affect Lepidoptera larvae living outside cultivated fields, through pollen deposition on wild plants which constitute their food source. While pollen toxicity varies among different events, possible exposure of non-target species depends on the agro-environmental conditions. This study was conducted in two protected areas in Italy, characterized by different climatic conditions, where many Lepidoptera species thrive in proximity to maize cultivations. To estimate the possible exposure in absence of the actual stressor (e.g. Cry1-expressing maize plants), we conducted a two-year field survey of butterflies and weeds. Indicator species were selected - *Aglais (Inachis) io* in the Northern site and *Vanessa cardui* in the Southern site - and their phenology was investigated. Pollen dispersal from maize fields was measured by collection in Petri dishes. Duration and frequency of exposure was defined by the overlap between pollen emission and presence of larvae on host plants. Different risk scenarios are expected in the two regions: highest exposure is foreseen for *A. io* in the Northern site, while minimal exposure is estimated for *V. cardui* in the Southern site. In the latter case, locally grown maize cultivars flower in mid-summer in coincidence with an aestivation period for several butterfly species due to hot and dry conditions. Moreover, host plants of *V. cardui* are at the end of their life cycle thus limiting food availability.

Key words environmental risk assessment; exposure assessment; genetically modified plants; Lepidoptera; non-target organisms; receiving environment

Introduction

A critical first step for environmental risk assessment (ERA) is the problem formulation, which aims to identify (among other factors) the organisms of environmental concern and the determinants of their possible exposure to the stressors that may place these entities at risk (e.g. EFSA, 2009; Wolt *et al.*, 2010). Genetically modified (GM) maize resistant to lepidopteran pests via expression of *Bacillus thuringiensis* (Bt) derived toxins is largely cultivated worldwide, while in Europe only in Spain are significant areas cropped with cultivars including MON810 event (ISAAA, 2016). This GM maize express Cry1Ab toxins in most plant tissues, including pollen (Nguyen & Jehle, 2007). Maize is not an important food source for native European Lepidoptera (except a few pest species), however during flowering periods, the pollen of GM maize (e.g. MON 810, Bt11, 1507), expressing Cry1Ab or Cry1F toxins can be wind deposited on the leaves of wild plants within or in the surroundings of Bt-maize fields (e.g. Gathmann *et al.*, 2006; Felke *et al.* 2010). If larvae of non-target Lepidoptera susceptible to Cry toxins, including species of conservation concern found in protected natural habitats, are feeding on pollen-dusted plants in the period of pollen emission they could be affected by the ingestion of Cry toxins (Losey *et al.*, 1999; Lang & Otto, 2010). Several biological characteristics at local and landscape level may determine the actual exposure and the consequent probability of harm for non-target Lepidoptera (Perry *et al.*, 2010), including butterfly and moth species assemblage in the receiving environment, their phenology, the availability of host plants, their distance from pollen shedding maize plants, the pollen concentration at the emission source, the pollen deposition on leaves, etc. The exposure scenario was experimentally determined for the Monarch butterfly – *Danaus plexippus* (L.) – in the Midwest of the United States, by measuring maize pollen deposition on milkweeds in and around maize fields (Pleasants *et al.*, 2001), temporal and spatial overlap between larvae and pollen (Oberhauser *et al.*, 2001) and

modelling the possible exposure at the population level considering the proportion of the Monarch butterfly populations living in proximity of maize fields and the uptake of Bt-maize by farmers (Sears *et al.*, 2001). Such a concerted effort has not been undertaken so far in Europe. However, biological information relative to a few European butterfly species and the agro-environmental conditions under which maize is cultivated is becoming available.

Schuppener *et al.* (2012) conducted an ERA for *Aglais urticae* (L.) in two different landscapes in Germany, and estimated exposure by measuring pollen deposition on nettle leaves, mapping occurrence and abundance of the host plant and considering the phenology of local populations of *A. urticae*. In their conditions the distance of nettle plants from maize fields was variable (more than 50% of plants within 5 m in one case, while no plants were found within 25 m in a second case). Gathmann *et al.* (2016b) examined the larval distribution of the same species in a different area in Germany and found nettle plants to be present often in field margins, but of different cereal crops. Lang and Otto (2015) studied the feeding behavior of *A. urticae* larvae on nettles indicating that the possible exposure to Bt-maize pollen might be increased by the tendency of larvae to feed on the top parts of the plants and along leaf veins of nettles where pollen amounts are expected to be higher. Lang *et al.* (2015) examined the potential exposure of butterflies in protected habitats in Switzerland observing butterfly communities and maize pollen deposition on nettle leaves, and indicated that 49 species showed a partial overlap between the occurrence of active larvae and maize pollen shed, though nettle is a known host plant only for some of them.

While conducting an ERA for the application for commercialization of maize MON810 in Europe, the European Food Safety Authority (EFSA) used mathematical modelling (Perry *et al.*, 2010, 2012) to quantify risks associated with the ingestion of Bt-maize pollen deposited on

host plants (EFSA 2009, 2011, 2012a, b, c). Recent studies have contributed to further develop models of exposure scenarios for non-target Lepidoptera. To derive an estimated exposure to maize pollen, Holst *et al.* (2013) integrated models of the phenology of *Aglais (Inachis) io* (L.) and of maize crops. The authors indicated that even for the same species, under different environmental conditions the possible exposure differs drastically. Lang *et al.* (2015) described a new method for direct measurement of pollen grain densities on nettle leaves which improves the estimation of the amount of toxin which could be ingested by the larvae. Hofmann *et al.* (2014) based on a 10-years collection record, revised the relationships between pollen deposition and distance from the nearest pollen source and adopted a power function model which provided the best fit to the actual data collected in passive pollen samplers.

In the present study we investigated the role of the receiving environment in depicting risk scenarios, by defining possible exposure scenarios for butterflies (Papilionoidea and Hesperidae) occurring in protected areas in proximity of agricultural land, based on biological observations conducted in two Sites of Community Importance (SCIs) in Italy. Maize pollen emission, presence of larval host plants and phenology of locally selected butterfly species provided the biological data necessary to estimate exposure.

Materials and methods

Study sites

Biological observations were conducted in two SCIs of great natural interest that are included in the NATURA 2000 site network (European Commission Habitat Directive

92/43/EEC). The sites belong to different biogeographic regions; one (IT4050024) is located in Northern Italy (Emilia-Romagna region, in the Continental climatic zone) and the second one (IT9220090) in Southern Italy (Basilicata and Apulia regions, in the Mediterranean climatic zone). Sites of community importance are key target for environmental policy in the EU as they contribute to the maintenance or restoration of natural habitat types, in presence of other human activities.

The selected site in Northern Italy is located in the rural plain landscape of the Po valley in Bologna province. This area was once occupied by marshes, but since the seventeenth century the land has been progressively converted to cropland. Marsh birds are the main reason of conservation interest. The fragmented protected area (3224 ha) encompasses a high proportion of cultivated fields (59.1% of the total surface). Maize, wheat, sugar beet and sorghum are the dominant crops.

The Southern site is a 470 ha protected area located at the border of the provinces of Matera and Taranto along the coast of the Ionian Sea. Coastal dunes and scrubland, whose conservation is the main purpose of SCI protection, are the prevailing habitats; conifer forests are also present as a result of reforestation. The agricultural land represents approximately 30% of the protected area. Fruit orchards represent main cultivated crops in this area, maize is cultivated on small farms, mainly for silage. No genetically modified maize is currently grown in either of the two study areas.

Pollen collection

To study the maize pollen drifts in the receiving environments, pollen traps were placed around selected maize fields in 2011 and 2012. In the Northern site, an isolated

commercial maize field (~ 6 ha) was selected; in the Southern site, a field of approximately 0.5 ha was sampled. The first field was treated with herbicides (terbuthylazine 0.68 kg/ha, sulcotrione 0.43 kg/ha, S-metolachlor, 1.2 kg/ha), while no herbicides were used at the experimental field in the Southern site. In order to guarantee no bias in pollen sampling, it was verified that no other maize fields were present within a radius of at least 1 km.

In both sites, a transect was established on each of the four sides of the maize fields and five collection points at increasing distances from the field edges were fixed: 0.5, 2, 5, 10, 20 m. At each distance, three pollen samplers (1 m distance to each other) were installed, totalling 60 sampling points per fields. Pollen samplers were made by Petri dishes (55.4 cm²) filled with collagen gelatine. Petri dishes were fixed on top of plastic stakes at 1 m above the ground.

The exposure periods, each of 48 consecutive hours, were on June 28–30 and July 5–7 2011 for the Northern site; in the Southern site pollen samplings were conducted between August 8–10 in 2011, and between July 17–19 and July 25–27 in 2012. Pollen shed generally occurs over the course of a week, but may last from 2 to 14 days; a majority of the pollen will be shed around the third day of anthesis (Purseglove, 1972). The collections indicated that in both cases the traps were correctly placed in the temporal window of pollen shedding. At the end of the exposure periods, pollen samplers were taken back to laboratory. The gelatin was melted in a heated bath and pollen grains were recovered by centrifugation, mounted on slides and counted under a microscope.

Plant surveys

Sampling of weeds was carried out in both sites in 2011 and 2012 in two consecutive weeks around the anthesis of maize. The weed density (number of plants/m²) was assessed by repeated quadrat method. Measurements were performed both inside the maize fields (walking inside for about 5 m) and in the field margins within 5 m from the crop edge in order to analyze the most realistic risk scenario. A total of 48 randomly located quadrats (1 m² area each) were sampled at each field (12 per each side, 6 within the crop and 6 in the field margins).

Overall, three maize fields were sampled for weeds in the Northern site: one field in 2011 located in Bentivoglio and two fields in 2012 in San Pietro in Casale. In the Southern site, the weed surveys were carried around the maize experimental field in Metaponto and around two fields of silage maize located in the neighboring town of Pisticci and Bernalda. All fields were sampled both in 2011 and 2012.

Insect surveys

In 2011–2012 visual surveys of butterflies were conducted in the two SCIs. Surveys were carried out every two weeks, between March and September along four transects of 50 m of length per site. According to the methods of Pollard (1977) and Pollard and Yates (1993), butterflies were recorded when observed within 2.5 m on either side of the observer's path and ahead 5 m of the recorder. Only specimens that could not be recognized by sight were caught and returned to laboratory for identification.

The first aim of these surveys was to create, or update, faunistic list of butterfly species and to explore the range of possible non-target species. In addition, based on the observations

made in the area during 2010, some indicator species were selected to conduct specific surveys to verify the possible presence of larvae feeding on wild plants nearby the maize fields. To ensure a certain degree of homogeneity between sites and considering the specific literature in Europe, which mainly concerns species belonging to the family of Nymphalidae, we focused on this family of non-target Lepidoptera for further investigations. The presence and abundance of the species and their host plants in the area detected in preliminary surveys in 2010 (Table S1, S2. Supplementary Materials) or according to the existing literature (AA.VV. 2007) were the main criteria for the selection of indicator species.

A. io and *Vanessa atalanta* (L.) were selected as possible non-target species in the Northern site and surveys for larvae were carried out in 2011 and 2012 on nettle (*Urtica dioica* L.) their major host plant. Nettle stands were visually inspected along linear transects (approximately 100 × 1 m) established in maize field margins (5–10 m from crop edge) in areas with a high density of nettles (> 50 plants/m²). Two transects were sampled in 2011 and three in 2012. The surveys were carried out twice a week from the beginning of June to the end of July, a period that encompasses the anthesis of most maize cultivars cropped in Northern Italy.

In the Southern site, *Vanessa cardui* (L.) was commonly detected during preliminary surveys in 2010. This species is known to be rather polyphagous, and some of the weeds usually reported as preferred host plants, such as thistle (*Carduus* spp.) and mallow (*Malva* spp.), occurred close to cultivated fields. Therefore, *V. cardui* was selected as indicator species and thistle and mallow plants were visually searched for the presence of larvae. Surveys were conducted weekly on 100 plants of thistle or mallow and each plant was labeled. Five randomly selected leaves per plant were checked and the number of eggs and larvae was recorded. Plants were monitored throughout their growing season until the end of their cycle.

The data resulting from the surveys of adult Lepidoptera were stored in a digital database with labeled keys as tools to navigate. The database allows a sequential operational phase: compiling the database (phase I), data loading (phase II) and data analysis (phase III) (Santorsola *et al.*, 2013). With the use of the key “Graph” a visual display of presence/absence of a given Lepidoptera species over time is presented for each environment where the species was found.

Results

Northern Italian site

- *Pollen collection*

The samples from Petri dishes carried out in 2011, showed that pollen density declined rapidly at increasing distances from maize field margins (Fig. 1). Whatever field side was studied and whatever wind direction, the peak of densities were recorded in the first few meters from crop borders (Table 1). Log-linear regression (Pearson’s methods) provided a significant fit to the data ($y = 0.44 - 0.02x$; $R^2 = 0.20$, $P < 0.001$) however, due to the high variability of pollen grain abundance in Petri dishes, a low value of determination coefficient of log-linear regression was obtained.

- *Plant surveys*

In the margins of maize fields sampled for weeds, *U. dioica* was the dominant plant species followed by *Convolvulus arvensis* L. and *Phragmites australis* (Cav.) Trin. (Table 2). This last

species, due to its morphology (steep and elongated), does not represent a plausible substrate for pollen accumulation on leaves. Moreover, no butterfly larvae feeding externally on *Phragmites* is recorded in Italian fauna.

Few weeds were found inside the crop both in 2011 and 2012. Only *Abutilon theophrasti* Medicus, *Helianthus annuus* L. re-growth and *Cirsium arvense* L. (Scop.) were occasionally sampled.

- *Insect surveys*

The butterfly species observed in the in Northern site were *Polyommatus icarus* (Rottemburg), *A. io*, *Melitaea didyma* (Esper), *V. atalanta*, *Papilio machaon* L., *Iphiclides podalirius* (L.), *Colias croceus* (Fourcroy), *Pieris brassicae* (L.), *Pieris rapae* (L.), *Pieris napi* (L.) and *Coenonympha pamphilus* (L.). The complete list of the adult butterflies detected at each sampling date is presented in Table S1, supplementary materials.

In 2011, larvae of *A. io* were found in one of the two transects over a period of five consecutive dates between June 24 and July 8 and fully overlapped with flowering of maize (Masetti *et al.*, 2013). Caterpillars showed a very clumped distribution with most of nettles having no larvae and a few plants harbouring several tens of individuals. This is in agreement with the behaviour of the species, whose early instars live in web nests. Since it was not possible to count exactly the larvae occurring in the web nest, their number could only be estimated. Approximately 250 *A. io* larvae were overall found in 2011 between June 24 and July 8. In 2012, larvae were found in only one out of three transects, hence confirming their scattered distribution. A total of 14 caterpillars were counted: 12 larvae on June 26 and two larvae on June 29. Almost all larvae were in late instars and were found on plants growing on

the edge of a ditch separated from a maize field by an unpaved road ≈ 3 m wide. Early instars probably fed on nettles located inside the ditch, making them difficult to detect. It is likely that late instars migrated to the plants, where they were observed seeking suitable pupation sites. Also in 2012 the full temporal overlap between the occurrence of *A. io* caterpillars and flowering of maize was confirmed.

Although *V. atalanta* adults were often observed in Pollard walks, no larvae of this species were detected on the nettle transects.

Southern Italian site

- Pollen collection

In Figure 2A and B the average pollen density for the Southern site is reported. It can be noted that the pollen density declined rapidly at increasing distances from maize field margins.

The log-linear regression (Pearson's methods) of the pollen deposition ($\log_{10}(1 + \text{pollen grains/cm}^2/\text{day})$) as a function of the distance from the crop edge significantly fit the data distribution (2011: $y = 0.79 - 0.04x$; $R^2 = 0.39$, $P < 0.001$; 2012: $y = 0.27 - 0.01x$; $R^2 = 0.09$, $P < 0.001$) however, due to the high variability of pollen grain abundance in Petri dishes, a low value of determination coefficient of log-linear regression was obtained.

The amount of pollen detected in Petri dishes was higher in 2011 compared to the following season, while the decline over distance was steeper. In both years at 20 m

distance from the field margins, the amount of pollen collected was very limited (Table 1).

- *Plant surveys*

The results of weed diversity monitoring are synthesized in Table 3. The mean abundance of the most common weeds, considering both years and all field samples, indicates that three taxa (*Phragmites* spp., *Portulaca oleracea* L., *Beta* spp.) accounted for more than 50% of weeds found close to maize fields.

- *Insect surveys*

Butterflies found in 2010 in the Southern site were: *Gonepteryx rhamni* (L.), *Gonepteryx cleopatra* (L.), *Pontia edusa* (F.), *P. napi*, *Anthocharis cardamines* (L.), *C. croceus*, *Lasiommata megera* (L.), *Pararge aegeria* (L.), *V. cardui*, *V. atalanta*, *Maniola jurtina* (L.), *Polyommatus thersites* (Cantener). The complete list of the adult butterflies detected at each sampling date is presented in Table S2, supplementary materials.

The list of Lepidoptera identified in 2011 confirmed the presence of most of the species already identified during 2010. A few species that were sporadically detected during the first year (e.g., *P. thersites*) were not recorded during 2011. The second year of monitoring however enabled us to enrich the faunal list with the detection of: *C. pamphilus*, *Lycaena phlaeas* (L.), *P. brassicae*, *P. rapae*, and *P. icarus*.

In 2011 the presence of *V. cardui* larvae was only sporadic (2 individuals in total were recorded). In 2012, the population was slightly more abundant (9 eggs and 19 larvae in total were recorded). However, along with the thistle plants drying out during maize flowering also preimaginal stages of *V. cardui* were not detected anymore.

1. Exposure scenarios

The wild plant species observed around maize fields and a list of Lepidoptera known to feed on these plants are reported in Tables 2 and 3. It can be seen that several species of Lepidoptera were in fact present in these areas during our study period and they can be considered in the list of organisms potentially exposed to Cry 1 expressing pollen, if Bt-maize would be cultivated in the area where their larval host plants occur.

However, some differences were found in the composition of weeds on the edge of the maize fields in two sites. The three most abundant weed species around maize fields in the Northern site are host plants of several butterfly species which were detected during our surveys (Table 2). On the other hand, in the Southern site none of the butterflies recorded during surveys is known to feed on the most abundant weeds associated with maize cultivations (Table 3). These differences are particularly important because they may translate into different levels of risk for butterfly species and indicate the need of a regional consideration in selecting indicator species for ERA.

Once the potential butterfly species exposed to pollen are singled out, the next logical step is to estimate the possible overlap in the plant phenology and insect life cycles. Since young larvae of some Lepidoptera species are known to be more susceptible to Cry 1 toxins (e.g. Felke & Langenbruch, 2001), age-structure of populations is an important feature to consider when estimating possible risks consequent to exposure to pollen expressing Cry toxins. In the following examples, with the support of the database we estimated the possible exposure scenarios for the indicator species sampled in the areas.

- *A: Possible exposure of Aglais io larvae to GM maize pollen in the Northern site*

In Northern Italy, *A. io* is bivoltine (Chiavetta, 2000). The overwintering adults recover from hibernation in April and first generation caterpillars are found throughout May. The flights of the first generation adults occur until the end of July and it is likely that most of the butterflies detected from the beginning of June until the end of July (Fig. 3) belonged to the first generation. The caterpillars of the second generation are expected to thrive in the fields in June–July therefore in coincidence with maize pollen shed. This was confirmed by field observations in both years (Masetti *et al.*, 2013). Our field study seems to corroborate the prediction of increased potential exposure to maize pollen for larvae of *A. io* where this species is bivoltine (Holst *et al.*, 2013).

B: Possible exposure of Vanessa cardui to GM maize pollen in the Southern site

In Fig. 4 the temporal exposure scenario for *V. cardui* is presented. The species showed two flight periods, the first in April-May and the second in September. This is a common trend for many butterflies observed in the area, as several species suffer the extreme hot local conditions and typically only few species are seen actively flying in July-August (Santorsola *et al.*, 2012). In August, the typical flowering period for silage maize cultivars, wild thistles are almost completely dried, while mallow plants are still available. In our surveys, larvae of *V. cardui* were only detected either during spring or autumn, and exclusively on thistle plants. These plants were drying out during maize pollen shed, therefore no exposure to Bt-expressing pollen is expected with the use of current maize cultivars for this species.

Discussion

Availability of faunal lists is a fundamental pre-requisite for any biodiversity conservation plan. The survey conducted in the Southern site (SCI IT9220090) during this study represents the first ever record of butterflies in the area. The collection in the Northern site (SCI IT4050024) confirmed the presence of taxa that were previously reported in a survey of the butterfly species of aesthetic value carried out in 2007 by the Bologna Province authority (AA.VV. 2007). This report also mentioned the presence in the SCI of two species of conservation interest - *Zerynthia polyxena* (Denis & Schiffermüller) and *Lycaena dispar* (Haworth) - which were not detected in our investigations.

An environmental risk assessment is based on two main components, hazard and exposure, whose multiple interactions will define the estimates of environmental risks.

The preliminary step for an exposure analysis is constituted by the knowledge of the species that may be present in a given ecosystem and that might be exposed to a stressor. Among these, indicator species might be selected for further analyses, based on several possible criteria (see Arpaia, 2010). The typical components of the exposure, which is the aim of the present study, are the magnitude, the frequency and the duration of the expected exposure of the species/population to an environmental stressor. To proceed to the estimated exposure in absence of the actual stressor, we have identified two possible species of concern (*A. io* and *V. cardui*), the magnitude of the exposure (expected density of pollen grains over space), the duration of exposure defined as the time when pollen emission and larval activity on the host plants overlap. The frequency of exposure, for sake of simplicity, is assumed to be constant during the flowering period, though our data showed a decrease in pollen emission over time. Although in this study variable amounts of pollen were detected both between sites and

years, the exponential regression model showed a consistent decline in pollen grains densities with increasing distance from the field edge, in agreement with existing literature data (e.g. Jarosz *et al.*, 2004; Lang *et al.*, 2004).

The highest density of maize pollen detected in our samplings was 28.19 grains/cm²/day at 0.5 m from crop edge (Table 1). The relevance of pollen densities measured in pollen samplers compared to the actual deposition on leaves is an important point. Perry *et al.* (2010) included in their model a parameter for a reduction of the expected pollen concentration on leaves compared with measurement in pollen samplers, as allowance for a set of physical effects such as wind and rain removing pollen from leaves (Pleasant *et al.*, 2001). Hofmann *et al.* (2016) indicated that the accumulated pollen deposition over the whole flowering period is the best measure of the overall intensity of pollen deposition. However, to date experimental data collected simultaneously from samplers and on leaves at the same location and time are scarce.

Hofmann *et al.* (2014) provided a comprehensive EU dataset on the dispersal and deposition of maize pollen over long distances; this provides a unique source of data, particularly for pollen deposition at long distances. Due to the relevant differences in experimental protocols, it is difficult to compare data from our samplings and those of Hofmann *et al.* (2014). Different pollen samplers were used and we sampled pollen deposition during 48-hour intervals instead of the whole shedding period. Also different regression models to fitting field collected data were used. However, when distances from field edges are below 20 m as in our study, the exponential model (which was implemented in our analysis) gave results similar to the power model (Hofmann *et al.*, 2014). Taking into account all these uncertainties, our data are in the lower range of the 95% CI reported by Hofmann for single

observations. Inferred number of pollen grains from our data at 10 m from maize field edge range between 65000 (Southern site 2012) to 220000 (Southern site 2011)/m² when summarized for the whole period of anthesis. The median lethal concentration (LC₅₀) expressed in grains of pollen per cm² leaf measured in experimental conditions may also range widely because of the discrepancy among Bt events in the expression of *cry* genes in the pollen (Felke *et al.*, 2010) and because of the different susceptibility of different Lepidoptera species (Wolt *et al.*, 2005) Therefore the estimate of possible lethal or sublethal effects of cry-expressing pollen under field conditions is very difficult.

When considering potential impacts at population levels, for both species studied in our surveys there is no expected exposure for some of their annual generations. *Aglais io* in fact completes its first generation before maize pollen shed, and local populations of *V. cardui* (a migratory species) will be annually increased by immigrant adults. Therefore population dynamics curves should be included in spatially-explicit models to calculate more realistically the expected impacts (e.g. Dively *et al.*, 2014).

If the use of different regression models does not change expected outcomes at short distances from the field, uncertainties arise when comparing quantitatively maize pollen deposition data from different field studies, because of differences in sampling methods, sampling periods, geographic locations and maize cultivars. Pollen density found on host plant leaves as a function of distance from the source has been highly debated in the last few years (i.e. Hofmann *et al.*, 2014; EFSA, 2015; Kruse-Plass *et al.*, 2017; Perry *et al.*, 2017). Limitations in the calculation of pollen density on leaves and its possible accumulation were highlighted by the different authors and therefore no unambiguous estimates, even for a single species, exist to date.

The EFSA GMO Panel estimated the mortality of non-target lepidopteran larvae using both the Perry *et al.* (2010, 2011, 2012) and Hofmann *et al.* (2014) dose–distance relationships and concluded that under the most realistic and even conservative scenarios, the estimated mortality for all species considered is always very low, even within the first 20 m from the emission source (EFSA, 2015). However, if hypothetical species with greater sensitivities exist, then these exposure levels may be significant and larger isolation distances may be needed to ensure the required level of protection (EFSA, 2016).

Not surprisingly, in our study the characteristics of the butterfly fauna varied considerably between sites both in terms of species diversity and phenology. In addition, the use of maize cultivars with different flowering periods in the two areas influenced the outcomes of our scenarios. The maize in Northern Italy is typically grown for grain production (84% of the surface invested), while silage is the principal use for maize in the Southern area (73% of the maize cultivated surface). (Data source: Italian national census of agriculture 2010, available at <http://www.istat.it/it/censimento-agricoltura/agricoltura-2010>). The cultivars most commonly adopted by growers for the two commercial uses are different in phenology, and therefore their pollen emission is not coincident.

In our two case studies, when the recorded butterfly life cycles were individually overlaid with the anthesis of maize, different exposure scenarios were obtained. In Northern Italy a complete overlap between maize pollen emission and presence of *A. io* larvae on nettles growing in maize field margins (worst case scenario) was detected. On the other hand, in the Southern site, there was no overlap (minimum risk scenario) between the presence of *V. cardui* susceptible instars and pollen emission. In this area, maize flowering is typically recorded in July-August when most lepidopteran species suspend their activity (Santorsola *et*

al., 2012). In addition, thistles (the preferred host plants of *V. cardui*) dry out due to the climatic conditions, therefore, first generation larvae must complete their immature development before maize flowering starts. However, there are additional factors that contribute to accounting for the expected exposure to maize pollen (Perry *et al.*, 2010). Among these, the actual deposition of pollen (and its persistence) on different host plant leaves may change sensibly the outcomes of modelling estimates. The phenology of lepidopteran populations and their host plants are largely regulated by abiotic factors of the environment as the accumulation of degrees-day after winter, and rainfall or moisture conditions during spring. Moreover, different farmers can sow maize on different dates within its sowing window, thus producing a large phenological variation in each region. To further reduce uncertainties, exposure scenarios need to be evaluated on a case-by-case basis during ERA. For example, the number of species potentially exposed in the Southern site would definitely increase if the ERA would focus for instance on different GM plants expressing Cry 1 toxins. In fact, six butterfly species were detected during maize pollen emission (Table S2. Supplementary Materials). With the exception of the Satyridae *L. megera* and *P. aegeria* whose larvae feed on grasses (Poaceae), all the species belong to the Pieridae family and have a rather narrow range of suitable food plant. Unlike maize, other crop species flower normally in April-May when almost all Lepidoptera in the area are actively reproducing (21 species were recorded in our collections) and the availability of weed host plants is very abundant due to weather conditions. If the wind-drifted dispersal of cry-expressing pollen would be relevant these cropping systems could constitute a more likely source of exposure for non-target Lepidoptera. *V. cardui*, could become exposed in its larval stages on thistle leaves at this time and therefore the risk scenario could be rather different.

Differences in vegetation were also evident between our study areas, with the Southern site being generally poorer in weed densities, and the most common plant species detected represent poor food source for local butterflies.

The ecological database built during our study has proved to be a useful tool to support assessment of potential risk scenarios from environmental "stressors" related to GM plants cultivated near the protected areas. In fact, besides allowing the characterization of Lepidoptera fauna of the study sites, the database can support the definition of temporal exposure scenarios based on the presence-absence of the species during the year.

The outcomes of the present study reinforce the idea that a thorough consideration of the receiving environment is paramount for any ERA. In the specific case study of ERA for non-target butterflies and cry-expressing maize, it is clear that the differences detected in butterfly assemblage, in the availability of wild host plants and in the Lepidoptera life cycles can point to very different exposure scenarios. When the differences in species sensitivity to the investigated stressor – in this case Cry toxins (van Frankenhuyzen, 2009) – is also considered, the hazard component of the risk equation creates additional variation in the case studies.

The possible effects of Bt-expressing maize pollen still represent a controversial issue in the debate on the environmental safety of GM crops (e.g. Hofmann *et al.*, 2016; EFSA, 2016). The use of mathematical models might support the estimate of the additional effects due to cry-expressing pollen on butterfly populations which are often already declining due to major environmental threats such as habitat fragmentation, pesticide use, change in agricultural management, land drainage, etc. (Van Swaay *et al.*, 2006). Some knowledge gaps still need to be filled, especially regarding the actual amount of pollen that could be ingested by non-target Lepidoptera larvae feeding on wild plants and the susceptibility to Cry toxins for a number of European butterfly species. We claim here that the collection of ecological data in the receiving

environments can offer important support in current ERA frameworks. While an ERA valid at European level can only be realistically conducted on specific case studies and with the support of mathematical modelling, knowledge of local agro-ecological conditions needs to be promoted to support national and regional risk managers to decide upon risks and management options. This would ensure the adoption of reliable systems for all new, or incoming, technologies in agriculture, whose sustainability has become the main priority in a scenario of growing world population and need of sustainable food provision.

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Disclosure

The authors declare that they have no conflict of interest in connection with the work submitted

References

- Arpaia, S. (2010) Genetically modified plants and “non-target” organisms: analysing the functioning of the agro-ecosystem. *Collection of Biosafety Reviews*, 5, 12–80.
- AA.VV. (2007) Studio sullo stato di conservazione e gestione del patrimonio naturale nelle Aree di Riequilibrio Ecologico e nei siti Rete Natura 2000 della pianura bolognese. Commessa 06- 054 Provincia di Bologna, Servizio Pianificazione Paesistica.
- Chiavetta, M. (2000) Le Farfalle d'Italia. Atlante biogeografico. Nuova Editoriale Grasso, Bologna Italy. 112 p.
- EFSA Panel on Genetically Modified Organisms (GMO) (20 (2009). Scientific Opinion of the Panel on Genetically Modified Organisms on applications (EFSA-GMO-RX-MON810) for the renewal of authorisation for the continued marketing of (1) existing food and food ingredients produced from genetically modified insect resistant maize MON810; (2) feed consisting of and/or containing maize MON810, and maize MON810 for feed use (including cultivation); and of (3) food additives and feed materials produced from maize MON810, all under Regulation (EC) No 1829/2003 from Monsanto. *EFSA Journal*, 1149, 1–84. doi:10.2903/j.efsa.2009.1149.
- EFSA Panel on Genetically Modified Organisms (GMO) (2010) Guidance on the environmental risk assessment of genetically modified plants. *EFSA Journal*, 8(11): 1879.
- EFSA Panel on Genetically Modified Organisms (GMO) (2011) Scientific Opinion updating the evaluation of the environmental risk assessment and risk management recommendations on insect resistant genetically modified maize 1507 for cultivation. *EFSA Journal*, 9(11): 2429, 73 pp. doi:10.2903/j.efsa.2429.
- EFSA Panel on Genetically Modified Organisms (GMO) (2012a) Scientific Opinion updating the risk assessment conclusions and risk management recommendations on the genetically modified insect resistant maize 1507. *EFSA Journal*, 10(10): 2933, 46 pp. doi:10.2903/j.efsa.2933.
- EFSA Panel on Genetically Modified Organisms (GMO) (2012b) Scientific Opinion updating the risk assessment conclusions and risk management recommendations on the genetically

modified insect resistant maize Bt11. *EFSA Journal*, 10(12): 3018, 104 pp. doi:10.2903/j.efsa.3018.

EFSA Panel on Genetically Modified Organisms (GMO) (2012c) Scientific Opinion updating the risk assessment conclusions and risk management recommendations on the genetically modified insect resistant maize MON 810. *EFSA Journal*, 10(12): 3017, 98 pp. doi:10.2903/j.efsa.3017.

EFSA Panel on Genetically Modified Organisms (GMO) (2015) Updating risk management recommendations to limit exposure of non-target Lepidoptera of conservation concern in protected habitats to Bt-maize pollen. *EFSA Journal*, 13(7): 4127 doi:10.2903/j.efsa.2015.4127.

EFSA GMO Panel (EFSA Panel on Genetically Modified Organisms) (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(7): 4127, 31 pp. doi:10.2903/j.efsa.2015.4127.

EFSA (2016) Relevance of a new scientific publication (Hofmann *et al.*, 2016) for previous environmental risk assessment conclusions and risk management recommendations on the cultivation of Bt-maize events MON810, Bt11 and 1507. *EFSA Supporting Publication*, 13(7): EN-1070. 13 pp. doi: 10.2903/sp.efsa.2016.EN-1070.

Falci *et al.* (1995) *Parco delle Madonie, le Farfalle: conoscerle per proteggerle* - Paruzzo Editore.

Felke, M. and Langenbruch G.A. (2001) Gefährdet Bt-Pollen Schmetterlinge? *Gesunde Pflanzen*, 53, 24–28.

Felke, M., Langenbruch, G.A., Feiert, 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.

Gathmann, A., Wirooks, L., Hothorn, L.A., Bartsch, D. and Schuphan, I. (2006) Impact of Bt maize pollen (MON810) on lepidopteran larvae living on accompanying weeds. *Molecular Ecology*, 15, 2677–2685.

Gathmann, A., Wirooks, L., Eckert, J. and Schuphan, I. (2006) 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. *Environmental Biosafety Research*, 5, 27–36.

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, 24–37.

Hofmann, F., Kruse-Plass, M., Kuhn, U., Otto, M., Schlechtriemen, U., Schröder, B., Vögel, R. and Wosniok, W. (2016) Accumulation and variability of maize pollen deposition on leaves of European Lepidoptera host plants and relation to release rates and deposition determined by standardised technical sampling. *Environmental Sciences Europe*, 28, 14, doi:10.1186/s12302-016-0082-9.

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.

ISAAA (2016) Global Status of Commercialized Biotech/GM Crops: 2016. ISAAA Brief No. 52. ISAAA: Ithaca, NY.

Jarosz, N., Loubet, B. and Huber, L. (2004) Modelling airborne concentration and deposition rate of maize pollen. *Atmospheric Environment*, 38, 5555–5566.

- Jõgar, K., Metspalu, L., Hiisaar, K., Ploomi, A., Svilponis, E., Kuusik, A. *et al.* (2009) Influence of white cabbage cultivars on oviposition preference of the *Pieris rapae* L. (Lepidoptera: Pieridae). *Agronomy Research*, 7, 283–288.
- Kruse-Plass, M., Hofmann, F., Kuhn, U., Otto, M., Schlechtriemen, U., Schröder, B., Vögel, R. and Wosniok, W. (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 deposition of Bt maize pollen in field margins / Pollenflug von Bt-Mais in angrenzende Feldränder. *Zeitschrift für Pflanzenkrankheiten und Pflanzenschutz / Journal of Plant Diseases and Protection*, 111(5), 417–428.
- Lang, A. and Otto, M. (2010) A synthesis of laboratory and field studies on the effects of transgenic *Bacillus thuringiensis* (Bt) maize on non-target Lepidoptera. *Entomologia Experimentalis et Applicata*, 135, 121–134.
- 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. (2015) Feeding behaviour on host plants may influence potential exposure to Bt maize pollen of *Aglaia urticae* larvae (Lepidoptera, Nymphalidae). *Insects*, 6(3), 760–771.

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

Masetti, A., Perry, J.N., Dinelli, G. and Burgio, G. (2013) Phenology of *Inachis io* larvae and maize pollen deposition on nettles in Northern Italy field margins. *IOBC-WPRS Bulletin*, 97, 73–79.

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.

Oberhauser, K.S., Prysby, M.D., Mattila, H.R., Stanley-Horn, D.E., Sears, M.K., Dively, G., Olson, E., Pleasants, J.M., Lami Wai-Ki, F. and Hellmich, R.L. (2001) Temporal and spatial overlap between monarch larvae and corn pollen. *Proceedings of the National Academy of Sciences USA*, 98, 11913–11918.

Perry, J.N., Devos, Y., Arpaia, S., Bartsch, D., Gathmann, A., Hails, R.S., Kiss, J., Lheureux, K., Manachini, B., Mestdagh, S., Neemann, G., Ortego, F., Schiemann, J. and Sweet, J.B. (2010) A mathematical model of exposure of nontarget Lepidoptera to Bt-maize pollen expressing Cry1Ab within Europe. *Proceedings of the Royal Society B*, 277, 1417–1425.

Perry, J.N., Devos, Y., Arpaia, S., Bartsch, D., Gathmann, A., Hails, R.S., Kiss, J., Lheureux, K., Manachini, B., Mestdagh, S., Neemann, G., Ortego, F., Schiemann, J. and Sweet, J.B. (2011) The usefulness of a mathematical model of exposure for environmental risk assessment. *Proceedings of the Royal Society B: Biological Sciences*, 278, 982–984.

Perry, J.N., Devos, Y., Arpaia, S., Bartsch, D., Ehlert, C., Gathmann, A., Hails, R.S., Hendriksen, N., Kiss, J., Messean, A., Mestdagh, S., Neeman, G., Nuti, M., Sweet, J.B. and Tebbe, C.

- (2012) Estimating the effects of Cry1F Bt-maize pollen on nontarget Lepidoptera using a mathematical model of exposure. *Journal of Applied Ecology*, 49, 29–37.
- Perry, J.N., Barberi, P., Bartsch, D., Birch, A.N.E., Gathmann, A., Kiss, J. et al. (2017) Response to Kruse-Plass *et al.* (2017) regarding the risk to non-target lepidopteran larvae exposed to pollen from one or more of three Bt maize events (MON810, Bt11 and 1507). *Environmental Sciences Europe*, 29, 21–23.
- 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 cornfields. *Proceedings of the National Academy of Sciences USA*, 98, 11919–11924.
- Pollard, E. (1977) A method for assessing changes in the abundance of butterflies. *Biological Conservation*, 12, 115–134.
- Pollard, E. and Yates, T. (1993) *Monitoring Butterflies for Ecology and Conservation*. Chapman & Hall, London, UK.
- Purseglove, J.W. (1972) *Tropical crops. Monocotyledons. 1 & 2*. London, Longman.
- Robinson *et al.* (2012) *HOSTS, A Database of the World's Lepidopteran Hostplants*. Natural History Museum, London. Available at <http://www.nhm.ac.uk/hosts>.
- Santorsola, S., Baldacchino, F., Errico, S., Magarelli, A. and Arpaia, S. (2012) Lepidotteri del SIC “Costa Ionica – Foce Bradano”, ed. ENEA, 26 pp.
- Santorsola, S., Baldacchino, F., Magarelli, R.A. and Arpaia, S. (2013) Un database a supporto della stima della biodiversità della Lepidotterofauna. *Atti del IX Convegno Nazionale sulla Biodiversità*, 1, 283–289.

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. doi:10.1111/j.1365-294X.2012.05716.x.

Sears, M.K., Hellmich, R.L., Stanley-Horn, D.E., Oberhauser, K.S., Pleasants, J.M., Mattila, H.R., Siegfriedi, B.D. and Dively, G.P. (2001) Impact of Bt corn pollen on monarch butterfly populations: A risk assessment. *Proceedings of the National Academy of Sciences USA*, 98, 11937–11942.

Tolman, T. and Lewington, R. (2009) *The Most Complete Guide to the Butterflies of Britain and Europe* - Collins Butterfly Guide.

Tscharntke, T. (1999) Insects on common reed (*Phragmites australis*) community structure and the impact of herbivory on shoot growth. *Aquatic Botany*, 64(3–4), 399–410.

van Frankenhuyzen, K. (2009) Insecticidal activity of *Bacillus thuringiensis* crystal proteins. *Journal of Invertebrate Pathology*, 101, 1–16.

van Swaay, C.A.M., Warren, M.S. and Lois, G. (2006) Biotope use and trends of European butterflies. *Journal of Insect Conservation*, 10, 189–209.

Villa *et al.* (2009) *Farfalle d'Italia*. Istituto per i beni artistici, culturali e naturali della regione Emilia-Romagna - Editrice Compositori.

Wolt, J.D., Conlan, C.A. and Majima, K. (2005) An ecological risk assessment of Cry1F maize pollen impact to pale grass blue butterfly. *Environmental Biosafety Research*, 4, 243–251.

Wolt, J.D., Keese, P., Raybould, A., Fitzpatrick, J.W., Burachik, M., Gray, A. *et al.* (2009) Problem formulation in the environmental risk assessment for genetically modified plants. *Transgenic Research*, 19, 425–436.

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Tables

Table 1 Maximum and minimum density of maize pollen grains detected in Petri dish pollen samplers at five distances from maize field edge.

Site (year)	Distance from maize edge (m)	Maximum maize pollen density (no. grains/cm ² /day)	Minimum maize pollen density (no. grains/cm ² /day)
Northern site (SCI IT4050024) 2011	0.5	18.58	0.17
	2	6.30	0
	5	11.74	0
	10	2.45	0
	20	2.77	0
Southern site (SCI IT9220090)	0.5	28.19	1.38
	2	16.90	1.06

2011	5	9.59	0.09
	10	3.99	0
	20	3.13	0
Southern site (SCI IT9220090)	0.5	6.25	0
	2	26.63	0
2012	5	15.93	0
	10	5.42	0
	20	11.94	0

Table 2 List of wild plant species in the Northern site (SCI IT4050024), their mean abundance (average of two years of samplings) and the list of Lepidoptera species for which these are known to be food plants for larvae.[†] In bold the butterfly species detected during the samplings in the area.

Weed plant species	Mean No. of plants/m ²	Lepidoptera for which the species is a known host plant
<i>Urtica dioica</i>	14.21	<i>Aglais urticae</i> , <i>Aglais io</i> , <i>Polygonia c-album</i> , <i>Vanessa atalanta</i> , <i>Vanessa cardui</i> , <i>Noctuidae</i>
<i>Convolvulus arvensis</i>	4.92	<i>Acontia trabealis</i> , <i>Tyta luctuosa</i> , <i>Agrius convolvuli</i>
<i>Phragmites australis</i>	3.72	<i>Noctuidae</i> , <i>Phragmataeca castaneae</i> , <i>Euthrix potatoria</i> , <i>Chilo phragmitellus</i> , <i>Calamochrous acutellus</i>
<i>Conyza albida</i>	2.54	
<i>Onobrychis viciifolia</i>	2.54	<i>Cupido osiris</i> , <i>Lycaeides abetonica</i> , <i>Polyommatus thersites</i>
<i>Trifolium pratense</i>	2.48	<i>Ematurga atomaria</i> , <i>Eupithecia centaureata</i> , <i>Horisme tersata</i> , <i>Callophrys rubi</i> , <i>Polyommatus icarus</i> ,

		<i>Colias croceus</i> , <i>Colias hyale</i> , <i>Cupido argiades</i> , <i>Cyaniris semiargus</i> , <i>Lasiocampa quercus</i> <i>Diacrisia sannio</i> , <i>Zygaena lonicerae</i>
<i>Cirsium arvensis</i>	2.34	<i>Eupithecia centaureata</i> , <i>Diacrisia sannio</i> , <i>Vanessa cardui</i>
<i>Amaranthus spp.</i>	1.67	<i>Noctuidae</i>
<i>Galega officinalis</i>	1.29	<i>Cupido alcetas</i>

[†]Table 2 was compiled based on the following sources: <http://www.ukbutterflies.co.uk/>, www.leps.it accessed on September 15, 2017. Robinson *et al.* (2012); Tolman and Lewington (2009); Falci *et al.* (1995); Tschardtke (1999); Jögar *et al.* (2009); Villa *et al.* (2009).

Table 3 List of wild plant species in the Southern site (SCI IT9220090), their mean abundance (average of two years of samplings) and the list of Lepidoptera species for which these are known to be food plants for larvae.[†] In bold the butterfly species detected during the samplings in the area.

Weed plant species	No. plants/m ²	Lepidoptera for which the species is a known host plant
<i>Phragmites spp.</i>	1.37	<i>Photedes spp.</i> , <i>Phragmatiphila nexa</i> , <i>Rhizedra lutosa</i> , <i>Sesamia nonagrioides</i> , <i>Laelia coenosa</i> , <i>Phragmataeca castaneae</i>
<i>Portulaca oleracea</i>	1.36	<i>Discestra trifolii</i> , <i>Hadula trifolii</i>
<i>Beta spp.</i>	1.20	<i>Noctuidae</i> , <i>Phthorimaea ocellatella</i>
<i>Polygonum spp.</i>	0.60	<i>Diacrisia sannio</i> , <i>Parasenia plantaginis</i>
<i>Ecballium elaterium</i>	0.56	
<i>Picris spp.</i>	0.53	

<i>Sonchus</i> spp.	0.49	<i>Cuculia umbratica</i> , <i>Naenia typical</i> , <i>Polia bombycina</i>
<i>Convolvulus arvensis</i>	0.42	<i>Acontia trabealis</i> , <i>Agrius convolvuli</i>
<i>Cirsium</i> spp.	0.40	<i>Eupithecia centaureata</i> , <i>Vanessa cardui</i> , <i>Diacrisia sannio</i>
<i>Rumex</i> spp.	0.24	<i>Idaea aversata</i> , <i>Rhodostrophia calabra</i> , <i>Lycaena phlaeas</i> , <i>Lycaena dispar</i> , <i>Parasenia plantaginis</i>
<i>Chenopodium album</i>	0.22	<i>Coleophora terinella</i> , <i>Scythris sinensis</i>
<i>Solanum nigrum</i>	0.22	
<i>Amaranthus albus</i>	0.20	<i>Noctuidae</i>
<i>Amaranthus retroflexus</i>	0.20	<i>Noctuidae</i>
<i>Chrozophora tinctoria</i>	0.13	
<i>Aster squamatus</i>	0.11	
<i>Chrysanthemum</i>	0.07	
<i>Fallopia convolvulus</i>	0.07	<i>Coleophora terinella</i> , <i>Spilosoma lubricipeda</i> , <i>Mamestra brassicae</i>
<i>Heliotropium europaeum</i>	0.04	
<i>Brassica napus</i>	0.02	<i>Pieris</i> spp. , <i>Pontia edusa</i> , <i>Anthocharis cardamines</i> , <i>Pieris rapae</i> , <i>Pieris brassicae</i> ,
<i>Trifolium</i> spp.	0.02	<i>Ematurga atomaria</i> , <i>Eupithecia centaureata</i> , <i>Horisme tersata</i> , <i>Callophrys rubi</i> , <i>Cupido alcetas</i> , <i>Polyommatus icarus</i> , <i>Colias croceus</i> , <i>Lasiocampa quercus</i> , <i>Diacrisia sannio</i>
<i>Xanthium</i> spp.	0.02	

[†]Table 3 was compiled based on the following sources: <http://www.ukbutterflies.co.uk/>, www.leps.it accessed on September 15, 2017. Robinson *et al.* (2012); Tolman and Lewington (2009); Falci *et al.* (1995); Tschardtke (1999); Jögar *et al.* (2009); Villa *et al.* (2009).

Figure Legends

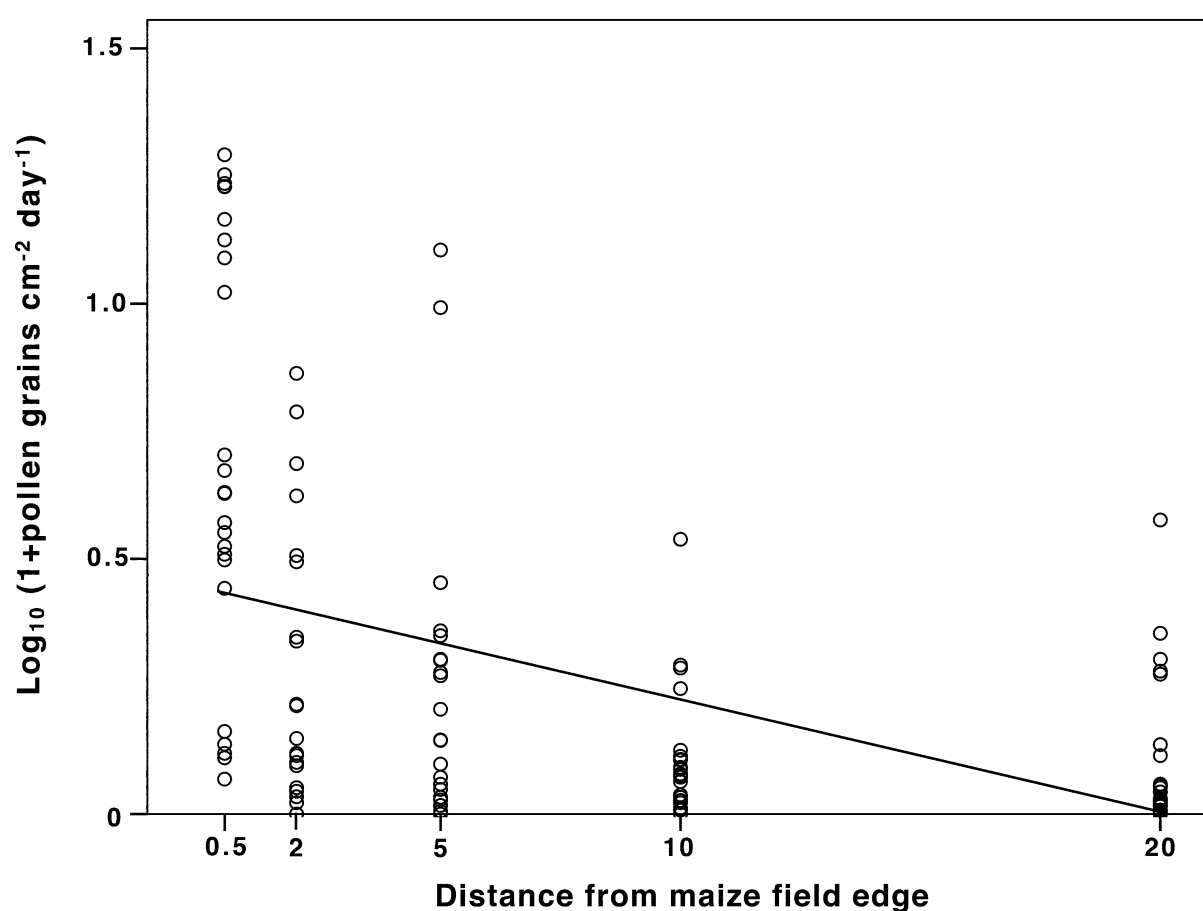


Fig. 1 Log-linear regression of the maize pollen deposition (1+number of pollen grains/cm²/day) as a function of distance from field edge (m) in the Northern site. Data include pollen counts that were collected along the 4 field sides in 2011 over two periods of exposure during maize anthesis (redrawn from data by Masetti *et al.*, 2013).

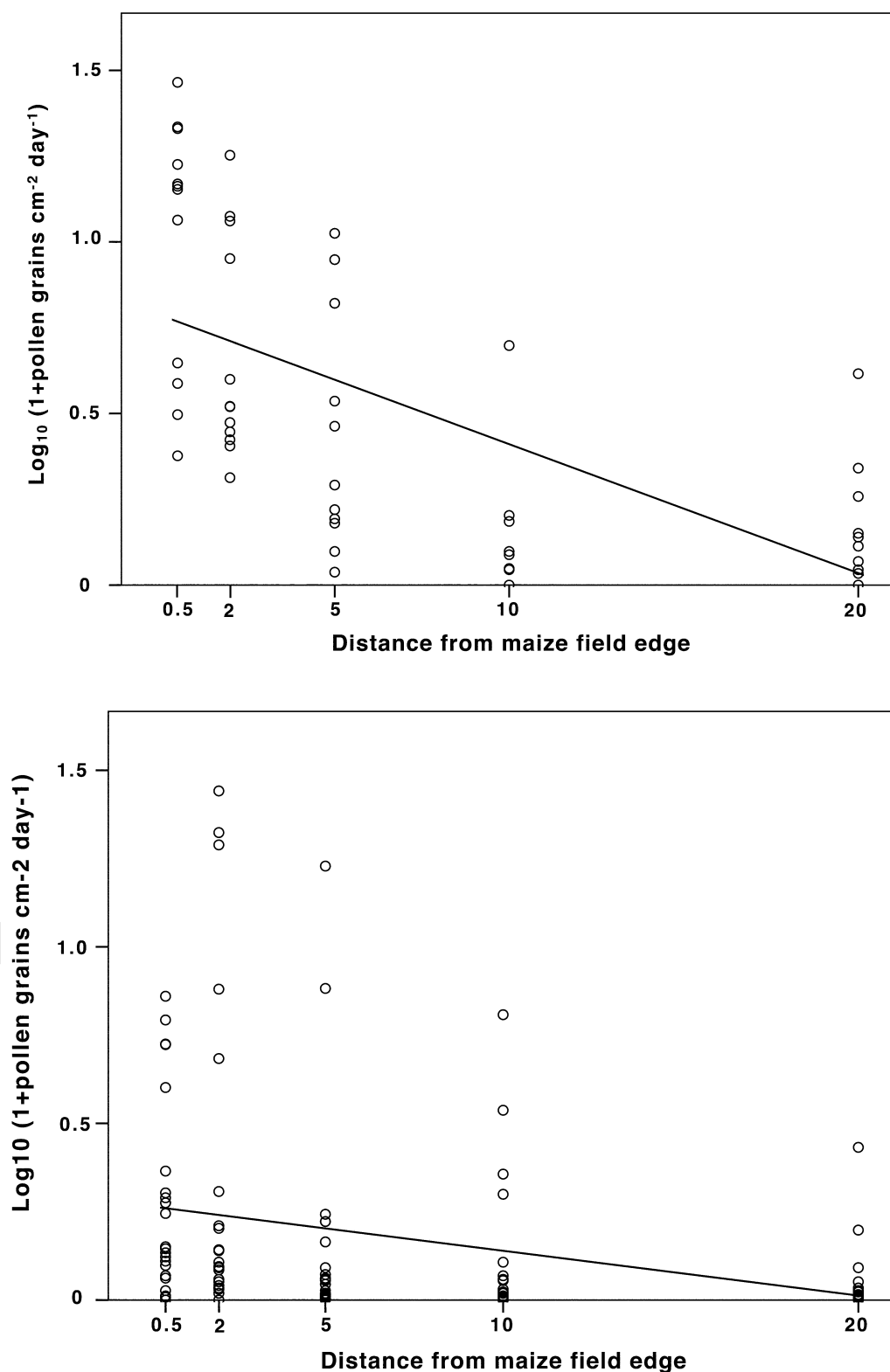


Fig. 2 Log-linear regression of the maize pollen deposition (1+number of pollen grains/cm²/day) as a function of distance from field edge (m) in the Southern site. Data

include pollen counts that were collected along the 4 field sides. In 2012 data were obtained during two exposure periods. Panel A = 2011, Panel B = 2012.

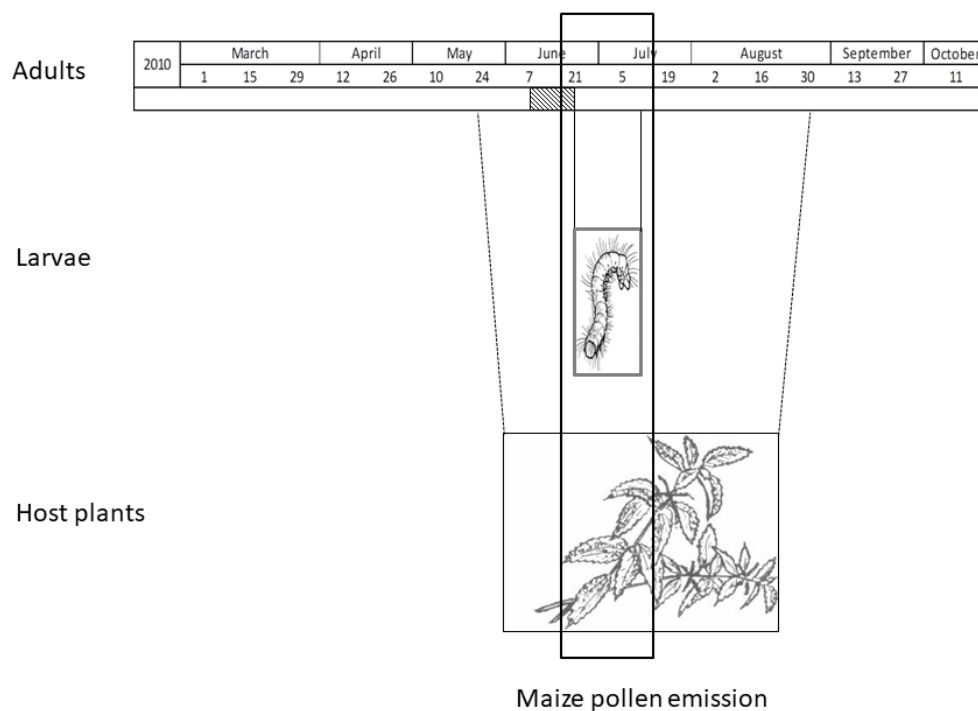


Fig. 3 Exposure scenario for *Aglais io* to maize pollen in the Northern Italian site. The horizontal bar indicates the flying period of second-generation adults, the vertical rectangle indicates the pollen shedding period and the width of the picture of larvae and host plants covers the respective presence in the field.

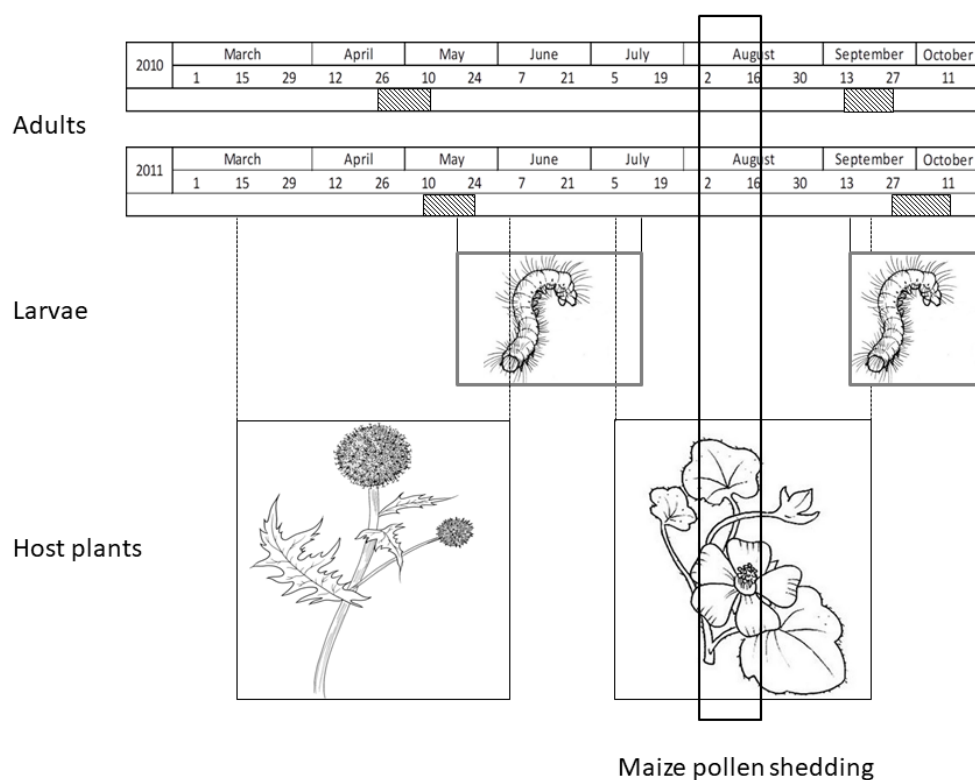


Fig. 4 Exposure scenario for *Vanessa cardui* to maize pollen in the Southern Italian site. The horizontal bars indicate the flying period of the first generation of locally developed adults, the vertical rectangle indicates the pollen shedding period and the width of the picture of larvae and host plants covers the respective presence in the field.